

JOURNAL OF ANIMAL SCIENCE

The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Effects of fumonisin B1-contaminated feeds on weanling angora goats

N. K. Gurung, D. L. Rankins, Jr, R. A. Shelby and S. Goel

J Anim Sci 1998. 76:2863-2870.

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jas.fass.org>



American Society of Animal Science

www.asas.org

Effects of Fumonisin B₁-Contaminated Feeds on Weanling Angora Goats¹

N. K. Gurung*, D. L. Rankins, Jr.*², R. A. Shelby†, and S. Goel‡

Departments of *Animal and Dairy Sciences and †Plant Pathology, Auburn University, Auburn, AL 36849 and ‡Toxicology Program, School of Public Health, University of Michigan, Ann Arbor 48109

ABSTRACT: Two diets containing no (< 1.0 mg/kg) or 95 mg of fumonisin B₁ (FB₁)/kg were fed to eight weanling Angora goats for 112 d. Dry matter intake, apparent nutrient digestibilities, serum chemistry profiles, sphingolipid concentrations, and persistency of FB₁ in tissues were evaluated. No differences ($P > .10$) were found between control and treated goats in terms of DMI, apparent nutrient digestibilities, or ADG. Elevated concentrations ($P < .10$) of blood-borne enzymes such as aspartate aminotransferase, lactate dehydrogenase, and gamma glutamyl transpeptidase and increased concentrations of cholesterol and triglycerides indicated mild liver damage and kidney dysfunction in treated goats. Linear relationships ($P < .10$) were observed between

these serum constituents and duration of FB₁ exposure. The sphingolipid analysis of liver, kidney, and heart tissues showed elevated free sphinganine:free sphingosine ratios in the treated group. The elevated sphingolipid ratios were mainly due to increased concentrations of free sphinganine in tissues. However, without serum profile and sphingolipid analyses, fumonisin toxicosis would not have been recognized because treated animals showed no clinical signs of toxicosis throughout the trial. No measurable FB₁ was present in liver, kidney, and heart tissues (detection limit of 1 ppm). However, further research is needed to analyze tissues for FB₁ or its metabolites with a lower detection limit. In conclusion, goats can be fed for up to 112 d with diets containing 95 mg FB₁/kg of diet without any overt signs of toxicosis and also without any effect on live weight gain.

Key Words: Fumonisin, Goats, Liver, Mycotoxins, Sphingolipids

©1998 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 1998. 76:2863–2870

Introduction

Fumonisin is a family of mycotoxins produced by *Fusarium moniliforme* (Sheldon) and *F. proliferatum* that frequently infect corn crops around the world (Marasas et al., 1988). Fumonisin has been associated with equine leukoencephalomalacia (**ELEM**) in horses (Marasas et al., 1988; Ross et al., 1991) and porcine pulmonary edema (**PPE**) in pigs (Harrison et al., 1990). Experimentally, high doses of fumonisin (i.e., > 225 mg/kg) caused adverse effects in poultry (Weibking et al., 1993) and cattle (Osweiler et al., 1993) and increased incidence of hepatic cancer in rats (Gelderblom et al., 1991). Human consumption of fumonisin-contaminated corn has been correlated with increased incidence of esophageal cancer in certain

parts of the world (Sydenham et al., 1991; Chu and Li, 1994).

Very few studies have focused on the effects of fumonisin on ruminants. Osweiler et al. (1993) found no treatment-related effects on feed intake or weight gain; however, significant changes in liver and immune function were reported in cattle fed 148 ppm of fumonisin B₁ (FB₁) for 30 d. Edrington et al. (1995) found that culture material containing fumonisin was acutely toxic to sheep when dosed orally. Conversely, Smith and Thakur (1996b) reported that cattle seem to tolerate high levels of fumonisin exposure (400 ppm of FB₁ and 130 ppm of FB₂ for up to 30 d) without developing any clinical signs of toxicosis, although liver functionality tests revealed some hepatobiliary compromise. Ruminal metabolism of fumonisin was minimal because more than 80% of FB₁ and FB₂ were detected in feces and only trace amounts were in urine.

We are not aware of any information on the effects of long-term exposure of fumonisin on ruminants. This experiment was conducted to determine the effects of long-term exposure of ruminants to fumonisin-contaminated feeds.

¹Journal article 4-985906 of the Alabama Agric. Exp. Sta., Auburn. Appreciation is expressed to B. W. Kemppainen for lending her laboratory and equipment.

²To whom correspondence should be addressed.

Received March 24, 1998.

Accepted July 13, 1998.

Table 1. Ingredient and chemical composition of the basal diet fed to weanling goats

Item	Percentage of diet ^a
Ingredient	
Corn	62.4
Cottonseed hulls	22.1
Soybean meal	9.3
Molasses	4.2
Dicalcium phosphate	1.0
Limestone	.4
Trace-mineralized salt	.6
Vitamin A	.025
Chemical composition	
DM	89.3
OM	93.4
CP	10.7
NDF	19.6
ADF	10.4

^aDM basis.

Materials and Methods

Production of Fumonisin-Containing Culture Material. Lyophilized cultures of *Fusarium moniliforme* M-1325 were obtained from the Fusarium Research Center, The Pennsylvania State University. The fumonisin-containing culture material (**FCCM**) was produced with the previously described method of Weibking et al. (1993) using corn as a substrate. The FCCM contained 3,851 ppm of FB₁.

Animals and Diets. Eight weanling Angora female goats (15 ± 2.1 kg BW) were assigned randomly to two diets with four goats per diet. The control diet contained less than 1 ppm of FB₁ and the treated diet contained 95 mg of FB₁/kg diet. The diets contained trace amounts of aflatoxin B₁ (.5 ppm); other common mycotoxins (zearalenone, deoxynivalenol, ochratoxin, and T-2 toxin) could not be detected by the analysis. The diet was formulated to meet NRC (1981) requirements for weanling goats (Table 1). The FCCM made up approximately 2% of the treated diet. The experiment was conducted at the USDA Regional Parasite Laboratory, Auburn, AL. All procedures conformed to the Auburn University Institutional Animal Care and Use Committee.

Goats were acclimated to the experimental diet (without addition of FCCM) for 4 wk and weighed prior to initiation of the study. Goats were housed in a common drylot with free access to water. Each day, all goats were caged (.71 × 1.0 m) individually for 7 to 8 h for feeding of the diet. All goats were drenched with Valbazen[®] before the study began. The goats were shorn at the beginning and end of the 112-d study.

The dry, powdered FCCM was mixed with the basal diet every week, and the mixed diet was stored under dry conditions at ambient temperature. Feed and orts were weighed daily and sampled (10%) to estimate

FB₁ intake during the feeding period based on HPLC analysis of feed and orts. Approximately 10 mL of blood was collected via jugular venipuncture on d 0, 22, 56, 84, and 112 into sterile serum separator tubes and allowed to clot for at least 30 min, after which serum was harvested by centrifugation at 900 × *g* for 20 min and stored frozen (-20°C) until serum chemistry analyses were performed.

Digestibility Trial. Eight days before the termination of the experiment (after 104 d of feeding treated diets), goats were placed in individual metabolism stalls to determine nutrient digestibility, FB₁ and water intake, and nitrogen and energy balance. Total orts, feces, and urine were collected, weighed, and sampled daily (10%) for 7 d. In addition, daily water consumption was recorded for each goat. Similarly, urine was collected daily into bottles containing .2 mL of toluene, and composite collections were stored at -20°C until analyzed. Similarly, feed, orts, and fecal samples were stored at -20°C until analyzed.

Proximate Analysis. Feed, orts, and feces were dried at 55°C to a constant DM of approximately 90% and then ground to pass a 2-mm screen in a Wiley mill and analyzed for DM, ash, N (AOAC, 1990), NDF, and ADF (Goering and Van Soest, 1970) adapted to the Ankom^{200/220} Fiber Analyzer (ANKOM Co., Fairport, NY). Gross energy was determined by isoperibol bomb calorimetry (Parr Instrument Company, Moline, IL). Urine samples were analyzed for N and GE only. For urine GE analysis, approximately .75 g of cellulose powder was pelleted, and 1 mL of urine was allowed to soak into the pellet for bomb analysis.

Visceral Organs. At the end of the experiment, all goats were killed (Beuthanasia-D special euthanasia solution, Schering-Plough Animal Health, Kenilworth, NJ). At necropsy, organ weights were recorded for each goat, and sections of liver, kidney, and heart were frozen. The samples were stored at -20°C for later fumonisin and sphingolipid analysis.

Fumonisin Analysis of Feed, Orts, Feces, and Urine. The composite samples of feed, orts, and feces were ground to pass a 1-mm screen in a Wiley mill. Fumonisin B₁ analysis was performed according to a modified procedure of Shephard et al. (1990). Ten grams of each sample was extracted with 100 mL of distilled water by shaking for 1 h in a Wrist Action Shaker (Burrell Corp., Pittsburgh, PA). Samples were sequentially filtered through Whatman No. 4 filter paper and .45-μm PTFE leuc filter (Fisher Scientific, Pittsburgh, PA) and centrifuged at 2,000 × *g* in a microcentrifuge (Denver Instrument, Denver, CO) for 15 min prior to derivatization with *o*-phthalaldehyde (**OPA**) and injection into the HPLC. Urine samples were filtered through Whatman No. 4 and then through a .45-μm PTFE filter before derivatization and injection into the HPLC.

Fumonisin Analysis in Tissues. The levels of FB₁ in liver, kidney, and heart were determined by HPLC according to the procedure of Smith and Thakur

(1996a) with some modifications. For each tissue, 5 g was blended with 37.5 mL of acetonitrile:water (50:50, vol/vol) in a blender at high speed for 5 min. The homogenate substrate was filtered through a Whatman No. 4 filter paper. Residues were re-extracted with 25 mL of acetonitrile:water (50:50, vol/vol). Filtrates were combined, and a 5-mL aliquot was applied to a bond-Elut SAX cartridge (Varian Associates, Walnut, CA) that was conditioned with methanol (8 mL), followed by methanol:water (3:1, 8 mL). Subsequently, the cartridge was washed successively with methanol:water (3:1; 8 mL) and methanol (3 mL), and the toxins were eluted with .5% acetic acid in methanol (14 mL). The eluate was evaporated to dryness under a stream of air at room temperature, the residue was suspended in .1 M sodium borate (200 μ L), and aliquots (50 μ L) of this solution were used for derivatization with 200 μ L of OPA. Ten microliters of the derivatized solution were injected into the HPLC (Waters Associates, Milford, MA) exactly 1 min after derivatization.

Serum Chemistry Analysis. Serum samples were analyzed for 20 serum constituents using a clinical chemistry analyzer at the Clinical Pathology Laboratory, Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL. Serum constituents measured included glucose, urea nitrogen, creatinine, sodium, potassium, chloride, total carbon dioxide, anion gap, calcium, phosphorus, albumin, globulin, total protein, triglycerides, cholesterol, total bilirubin, creatine phosphokinase, lactate dehydrogenase, aspartate aminotransferase, and gamma glutamyl transpeptidase.

Sphingolipid Analysis. Free sphinganine and free sphingosine concentrations were determined in liver, kidney, and heart with the HPLC method of Merrill et al. (1988).

Statistical Analysis. Data were subject to analysis of variance using the GLM procedure of SAS (1985). All data were analyzed as a completely randomized design. Orthogonal contrasts were used to describe the relationships of serum constituents in control and fumonisin-exposed goats (i.e., linear and/or quadratic effects). These relationships within each sampling were compared across treatments to determine whether relationships were similar.

Results

The average daily intake of FB₁ by treated goats was 45.3 \pm 3.70 mg for the 112-d feeding trial. The average daily recovery of unmetabolized FB₁ in the feces measured during the last 7 d of the trial was 21.1 \pm 4.30 mg, which constituted 46.6% of the average intake (Table 2).

Feed intake between controls and the treated group did not differ ($P > .10$; Table 3) over the entire study. However, during the 7-d digestibility trial, treated

Table 2. Mean daily fumonisin B₁ (FB₁) intake (mg·goat⁻¹·d⁻¹) by individual goats during the experimental period (112 d)

Animal number	FB ₁ consumed	mg/d	
		FB ₁ in feces	FB ₁ absorbed
602	85.3	32.4	52.9
615	66.3	18.9	47.4
611	66.7	21.0	45.7
603	47.2	12.0	35.2
Avg	66.4	21.1	45.3

goats tended to eat less than control goats (422 vs 563 g/d; $P = .16$). Likewise, no differences were detected when expressed as percentage of BW. Daily water consumption was measured only during the 7-d digestibility trial and was not affected by FB₁ consumption ($P > .10$). No differences ($P > .10$) were determined for apparent digestibilities. Similarly, energy (Mcal/d) and N retention (g/d) were not different ($P > .10$).

Goats in the control group tended to have faster weight gains than treated goats (48.7 vs 29.4 g/d; SE = 8.05; $P = .14$) during the 112-d trial. The relative weights of liver, kidney, and heart were not altered with the addition of FB₁ to the diet (Table 4). Goats consuming fumonisin appeared clinically normal throughout the trial, except one goat in the treated group that had a very pale liver and kidney and an enlarged gallbladder at necropsy.

Concentrations of free sphingosine (**So**), free sphinganine (**Sa**), and their ratios (**Sa/So**) in liver, kidney, and heart are shown in Table 5. The amount of So in liver was increased almost threefold ($P < .10$) in treated goats relative to control goats. Similarly, Sa was increased eightfold by FB₁ (30,448 vs 3,729 pmol/g). Consequently, the Sa/So ratio was threefold higher in fumonisin-dosed goats ($P < .10$). In kidney, free So, free Sa, and the Sa/So ratio were elevated ($P < .10$). A similar pattern was observed in heart tissue with regard to free So and free Sa, but the Sa/So ratio was not different ($P > .10$). The concentration of free So was highest for kidney, followed by liver and heart tissues in goats fed control diets. But if total sphingoid bases (Sa + So) are considered, liver (38,631 pmol/g) had the highest amount, followed by kidney (15,315 pmol/g) and heart (1,780 pmol/g) tissues.

The blood serum constituents of experimental animals at d 0, 22, 56, 84, and 112 are given in Table 6. On d 0, none of the serum constituents was different ($P > .10$) between control and treated goats. No differences were observed for sodium, potassium, chloride, total CO₂, phosphorus, anion gap, or creatine phosphokinase with the addition of FB₁ at the level of 95 mg/kg of feed fed for 112 d. By d 22, serum concentrations of cholesterol, lactate dehydrogenase (**LDH**), and aspartate aminotransferase (**AST**) were elevated ($P < .10$) in goats fed fumonisin compared

Table 3. Dry matter intake for the entire study (112 d) and during the digestibility trial (last 7 d) and nutrient digestibilities by weanling goats fed control and fumonisin (FB₁)-containing diets

Item	FB ₁ , mg/kg		SE ^a	P-value
	0	95		
Experimental period				
DMI, g/d	527	478	37.4	.39
DMI, % BW	3.0	2.9	.16	.69
Digestibility trial				
DMI, g/d	563	422	61.2	.16
DMI, % BW	2.8	2.3	.29	.31
Nutrient digestibility, %				
DM	76	77	2.5	.64
OM	76	78	2.5	.60
GE	75	77	2.6	.61
NDF	23	39	8.1	.23
ADF	7	24	9.3	.24
N retained, g/d	2.5	2.4	.29	.91
Energy retained, Mcal/d ^b	1.21	1.00	.100	.20

^aSE = Standard error of mean, n = 4/group.

^bDoes not account for gaseous losses.

with control goats. By d 56, blood urea nitrogen (BUN) and creatinine were elevated ($P < .10$) in treated goats. In addition, cholesterol, triglycerides, LDH, and AST remained elevated ($P < .10$). Following 84 d of fumonisin exposure, gamma glutamyl transpeptidase (GGTP) was increased ($P < .10$) but AST was not ($P > .10$). Linear relationships ($P < .10$) were observed between cholesterol, triglycerides, creatinine, LDH, GGTP, and AST and duration of fumonisin B₁ exposure.

With a limit of 1 ppm, no fumonisin residues were detected in liver, kidney, or heart tissues of goats exposed to fumonisin B₁.

Discussion

Goats fed fumonisin-containing diets for 112 d exhibited no signs of overt toxicosis. These results are in agreement with Osweiler et al. (1993), who reported no differences in the clinical appearance of calves fed FCCM with 15, 31, and 148 mg FB₁/kg of diet, although their feeding period lasted only 31 d. Similarly, Smith and Thakur (1996b) found no signs of toxicosis in cattle fed 530 mg FB₁/kg supplied by FCCM for 30 d. In contrast, Edrington et al. (1995) observed clinical signs such as diarrhea, lethargy, and, ultimately, death in fumonisin-treated lambs dosed intraruminally with 11.1, 22.2, and 45.5 mg of total fumonisins/kg of BW for 4 d. The actual amounts of total fumonisin intake were determined to be 355.2, 710.4, and 1,456 mg·lamb⁻¹·d⁻¹ in their experiment. Daily FB₁ intake in the present study averaged 66.4 ± 15.56 mg·goat⁻¹·d⁻¹, which is approximately 19% of the lowest amount used by Edrington et al. (1995). The toxicosis reported by Edrington et al. (1995)

seemed to be due to excessively high amounts of fumonisins. Such concentrations have not been reported to occur under normal feeding conditions. During 1989, which was a bad year for fumonisin contamination, feeds associated with ELEM contained FB₁ concentrations of less than 1 to 126 mg/kg of feed, whereas feeds associated with PPE contained less than 1 to 330 mg FB₁/kg (Ross et al., 1991).

Fumonisin has been reported to be poorly absorbed, rapidly excreted, and persistent in small amounts in liver and kidney (Norred et al., 1996), but results of the present experiment showed that only 32% of FB₁ was excreted in the feces. The remaining 68% was either hydrolyzed and the metabolites were absorbed or hydrolyzed and the metabolites were excreted. Even though hydrolyzed metabolites were not measured in the present experiment, the absence of FB₁ in the tissues indicated that the parent compound was not absorbed in sufficient quantity to be detected with the HPLC method used in this study. The fecal recovery observed in the present study was lower than that reported elsewhere in the literature.

Table 4. Effects of feeding control and fumonisin (FB₁)-containing diets for 112 d to weanling goats on relative organ weights (g/100 g BW)

Organ	FB ₁ , mg/kg		SE ^a
	0	95	
Liver	2.1	2.0	.16
Kidney	.34	.35	.02
Heart	.40	.41	.01

^aSE = Standard error of mean, n = 4/group.

Table 5. Effect of fumonisin on sphingolipid concentrations in liver, kidney, and heart tissues of weanling goats

Item	FB ₁ , mg/kg		SE ^a
	0	95	
Liver			
Sphingosine, pmol/g	2,488 ^b	8,185 ^c	1,314.6
Sphinganine, pmol/g	3,729 ^b	30,446 ^c	2,754.9
Sa/So ^d	1.43 ^b	4.33 ^c	.315
Kidney			
Sphingosine, pmol/g	3,557 ^b	5,682 ^c	297.8
Sphinganine, pmol/g	3,511 ^b	9,633 ^c	616.4
Sa/So ^d	.94 ^b	1.70 ^c	.139
Heart			
Sphingosine, pmol/g	428 ^b	1,069 ^c	95.4
Sphinganine, pmol/g	206 ^b	711 ^c	60.9
Sa/So ^d	.54	.67	.092

^aSE = Standard error of mean, n = 4/group.

^{b,c}Row values with different superscripts differ ($P < .10$).

^dSa/So = ratio of sphinganine to sphingosine.

Norred et al. (1993) reported that up to 80% of an oral dose of radiolabeled FB₁ was eliminated in the feces, indicating that after it was absorbed FB₁ was rapidly eliminated. Similarly, Shephard et al. (1992) showed that 24 h after i.p. injection, 66% of the radioactive fumonisin was recovered in feces, 32% in urine, 1% in liver, and < 1% in kidney and red blood cells in rats, but, when it was dosed by gavage, nearly 100% of the detectable radioactivity was recovered in feces and only trace amounts were found in urine, liver, kidney, and red blood cells. Such discrepancies

in percentage recovery of FB₁ could be in part due to differences in the methods of fumonisin detection and sources of FB₁ used. In the present study, FB₁ was supplied as crude culture material, whereas results from Norred et al. (1993) and Shephard et al. (1992) were obtained using purified FB₁. No other long-term feeding reports in ruminants are available for comparison. In summary, approximately one-third of the fumonisin ingested was excreted in unmetabolized form by goats consuming fumonisin over a 112-d period.

Table 6. Serum constituents of goats fed control and treated diet containing 95 mg of fumonisin B₁/kg diet

Analyte	Day					SE	0 vs others ^a	L ^b ,Q
	0	22	56	84	112			
Cholesterol, mg/dL								
C	45	38	42	46	54	11.65	.97	—
T	45	60 ^c	100 ^c	93 ^c	109 ^c	11.65	.0001	L
Triglycerides, mg/dL								
C	17	16	17	20	28	8.28	.69	—
T	29	27	37 ^c	60 ^c	61 ^c	8.28	.05	L
Creatinine, mg/dL								
C	.28	.70	.60	.60	.90	.077	.0001	L
T	.33	.80	1.0 ^c	1.0 ^c	1.3 ^c	.077	.0001	L ^d
Lactate dehydrogenase, U/L								
C	43	25	25	30	49	20.53	.59	—
T	21	120 ^c	137 ^c	124 ^c	110 ^c	20.53	.0001	Q
Gammaglutamyl transpeptidase								
C	41	42	49	36	56	14.65	.76	—
T	35	70	94	99 ^c	131 ^c	14.65	.0002	L
Aspartate aminotransferase, U/L								
C	50	47	51	45	50	11.81	.85	—
T	59	74 ^c	93 ^c	64	98 ^c	11.81	.06	L

^a P -value for comparing d 0 to other d.

^bLinear (L) effect or quadratic (Q) effect ($P < .10$).

^cValues different between treatments ($P < .10$), C = control (< 1 mg FB₁/kg), T = treated (95 mg FB₁/kg).

^dSlopes different between control and treated group ($P < .10$).

Inclusion of FB₁ into the diet tended to decrease DMI by goats. Osweiler et al. (1993) reported no significant differences in feed intake of calves fed fumonisin-contaminated corn screenings at FB₁ concentration of 148 ppm for 30 d. Similarly, dose-dependent decreases in feed intake have been reported in several other animals fed fumonisins: turkeys (Weibking et al., 1995; Kubena et al., 1997); swine (Haschek et al., 1992; Colvin and Harrison, 1992; Casteel et al., 1993; Becker et al., 1995); broiler chicks (Ledoux et al., 1992); Sprague-Dawley rats (Voss et al., 1992); channel catfish (Lumertdacha et al., 1995); and horses (Ross et al., 1993). Apparent nutrient digestibilities were unaffected by addition of FB₁, indicating that ability of ruminal microbes to digest feedstuffs was not compromised.

Average daily gains were similar between treatments, but treated goats tended to grow slower ($P = .14$). No differences in ADG were reported by Osweiler et al. (1993) or Smith and Thakur (1996b) for cattle. However, reduced weight gains have been reported in swine (Haschek et al., 1992), broiler chicks (Ledoux et al., 1992), channel catfish (Lumertdacha et al., 1995), and turkey poults (Kubena et al., 1995) resulting from ingestion of fumonisin supplied by FCCM.

The relative weights of liver, kidney, and heart were not altered in fumonisin-fed goats relative to control goats, and this conflicts with other results. Edrington et al. (1995) with high concentration of FB₁ found increased liver and kidney weights in lambs fed fumonisin-containing culture material, but no differences in heart weights were reported by these workers. Increased liver and kidney weights have been reported in other animals: poultry (Wu et al., 1995) and turkeys (Weibking et al., 1993; Bermudez et al., 1996). It seems likely that changes in relative organ weights are species-specific and dependent on level of fumonisin in the diet as well as duration of exposure to fumonisins.

Hepatic injury is apparently the primary manifestation of fumonisin toxicosis in goats. One of the treated goats had a very pale yellow liver and very enlarged gall bladder. Hepatotoxicosis has been previously reported in rats (Voss, 1990), channel catfish (Lumertdacha et al., 1995), calves (Osweiler et al., 1993), swine (Colvin and Harrison, 1992; Casteel et al., 1993), poultry (Weibking et al., 1993), and horses (Ross et al., 1993).

Alterations in sphingolipid metabolism caused by fumonisins were consistent with results reported for pigs (Riley et al., 1993), ponies (Wang et al., 1992), rats (Riley et al., 1994), channel catfish (Goel et al., 1994), and chickens (Weibking et al., 1993). The ratio of Sa/So for heart tissues was not different between treatments. By far the most affected tissue was the liver. The ratio of Sa/So in liver increased 2.5- to 6.5-fold more than in kidney and heart. This is

reasonable because the liver is the major site of sphingolipid biosynthesis (Merrill and Jones, 1990).

The increased accumulation of sphinganine (the immediate precursor in the biosynthetic pathway of dihydroceramide) is due to inhibition of ceramide synthase (N-acyltransferase) by fumonisins, and the presence of free sphingosine is believed to be primarily derived from turnover of more complex sphingolipids or from dietary sources (Merrill et al., 1997).

The elevated sphingoid bases in goats exposed to fumonisin in this experiment are consistent with earlier results that have demonstrated that elevation in sphingoid bases, particularly in liver, is a useful biomarker for fumonisin exposure. To the best of our knowledge, this was the first study that has attempted to relate the effects of fumonisin B₁ on *de novo* sphingolipid biosynthesis in ruminants. The use of this biomarker has been proposed as a diagnostic tool in catfish (Goel et al., 1994); pigs (Riley et al., 1993); rats, and ponies (Riley et al., 1994).

Even though goats appeared normal during the feeding period, serum metabolite changes reflected fumonisin toxicosis. Serum LDH and AST concentrations were elevated within 22 d in treated goats relative to controls. The measured AST activity increased linearly ($P < .10$) during the period of exposure (22 to 112 d), and the LDH increase was quadratic. An increase in serum AST is indicative of altered hepatocyte membrane integrity with the leakage of the enzyme or hepatic necrosis (Duncan and Prasse, 1986). Increased AST activity due to fumonisin has previously been observed in lambs (Edrington et al., 1995) and calves (Osweiler et al., 1993). Increased serum LDH indicates myocardial infarction, skeletal muscle disease, liver damage, and some anemias (Murray et al., 1990). There was a linear correlation ($P < .10$) between serum GGTP and duration of fumonisin exposure over the experimental period, but significant differences between control and treated animals occurred between d 84 and 112. Increased GGTP levels along with elevated AST and LDH activities reflect a mild toxicosis characterized by leakage of hepatocellular enzymes (Tiez, 1976).

Serum cholesterol concentrations were the same (45 mg/dL) in control and treated goats, respectively, prior to feeding fumonisins. The level did not change in the control group, but goats receiving fumonisins had increased concentrations of serum cholesterol on d 22, 56, 84, and 112. Similar elevations have been reported in calves (Osweiler et al., 1993), lambs (Edrington et al., 1995), and cattle (Smith and Thakur, 1996b). Elevated serum cholesterol levels reflect fatty infiltration of the hepatocytes and biliary obstruction (Evans, 1988).

Creatinine levels increased linearly in control and treated goats, but, when these relationships were compared, the rate of increase in treated goats was higher than the increase in control animals, indicating

adverse effect of fumonisins. Serum creatinine is a more specific and sensitive indicator of renal dysfunction than is BUN, but the use of simultaneous BUN and creatinine determinations provides more information (Murray et al., 1990).

Considering these changes individually would have little diagnostic value or toxicological importance, but combined changes in the serum constituents discussed above allow some conclusions to be drawn. Significant changes in AST, GGTP, LDH, triglycerides, and cholesterol following exposure to fumonisins in the present experiment indicate an effect on liver organelles, cell membranes, and(or) biliary excretion. Similarly, significant elevations in serum BUN and creatinine indicate effects on kidney function. However, goats did not exhibit overt signs of fumonisin toxicosis. This lack of overt toxicosis may be due to the fact that the serum changes were significant but still within normal biological limits. The carry-over effects of fumonisins or their metabolites into animal-derived products intended for human consumption have been investigated by several researchers with various animals (Shephard et al., 1992 in rats; Prelusky et al., 1994 in pigs; and Vudathala et al., 1994 in laying hens). In the present experiment, no residues were found in liver, kidney, and heart tissues after 112 d of fumonisin exposure. However, results from this experiment must be viewed with caution, because the detection limit of FB₁ was 1 ppm and fumonisin metabolites were not measured.

Implications

Fumonisin B₁-contaminated feed (95 mg/kg) fed for 112 d caused no treatment-related effects on feed intake, nutrient digestibilities, or weight gain in weanling Angora goats. However, sphingolipid disruption and mild liver and kidney damage were observed in weanling Angora goats. Thus, fumonisin B₁ disrupted sphingolipid biosynthesis in ruminants. The absence of fumonisin B₁ in liver, kidney, and heart tissues at a 1 ppm level of detection indicates the need for further study with lower detection limits.

Literature Cited

- AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.
- Becker, B. A., L. Pace, G. E. Rottinghaus, R. Shelby, M. Misfeldt, and P. F. Ross. 1995. Effects of feeding fumonisin B₁ in lactating sows and their suckling pigs. *Am. J. Vet. Res.* 56: 1253–1258.
- 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.
- Casteel, S. W., J. R. Turk, R. P. Cowart, and G. E. Rottinghaus. 1993. Chronic toxicity of fumonisin in weanling pigs. *J. Vet. Diagn. Invest.* 5:413–417.
- Chu, F. S., and G. Y. Li. 1994. Simultaneous occurrence of fumonisin B₁ and other mycotoxins in moldy corn collected from the People's Republic of China in regions high in esophageal cancer. *Appl. Environ. Microbiol.* 60:847–852.
- Colvin, B. M., and L. R. Harrison. 1992. Fumonisin-induced pulmonary edema and hydrothorax in swine. *Mycopathologia* 117: 79–82.
- Duncan, J. R., and K. W. Prasse. 1986. *Veterinary Laboratory Medicine: Clinical Pathology* (2nd Ed.). Iowa State University Press, Ames.
- Edrington, T. S., C. A. Kamps-Holtzapfle, R. B. Harvey, L. F. Kubena, M. H. Elissalde, and G. E. Rottinghaus. 1995. Acute hepatic and renal toxicity in lambs dosed with fumonisin-containing culture material. *J. Anim. Sci.* 73:508–515.
- Evans, R. J. 1988. Hepatobiliary damage and dysfunction: A critical overview. In: D. J. Blackmore (Ed.) *Animal Biochemistry in the Future*. Cambridge University Press, Cambridge, U.K.
- Gelderblom, W.C.A., N.P.J. Kriek, W.F.O. Marasas, and P. G. Thiel. 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B₁, in rats. *Carcinogenesis* 12: 1247–1251.
- Goel, S., S. D. Lenz, S. Lumlertdacha, R. T. Lovell, R. A. Shelby, M. Li, R. T. Riley, and B. W. Kempainen. 1994. Sphingolipid levels in catfish consuming *Fusarium moniliforme* corn culture material containing fumonisins. *Aquat. Toxicol.* 30:285–294.
- Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). *Agric. Handbook No. 379*. ARS, USDA, Washington, DC.
- Harrison, L. R., B. M. Colvin, J. T. Green, L. E. Newman, and J. R. Cole. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* 2:217–221.
- Haschek, W. M., G. Motelin, D. K. Ness, K. S. Harlin, W. F. Hall, R. F. Vesonder, R. E. Peterson, and V. R. Beasley. 1992. Characterization of fumonisin toxicity in orally and intravenously dosed swine. *Mycopathologia* 177:83–96.
- Kubena, L. F., T. S. Edrington, R. B. Harvey, T. D. Phillips, A. B. Sarr, and G. E. Rottinghaus. 1997. Individual and combined effects of fumonisin B₁ present in *Fusarium moniliforme* culture material and diacetoxyscirpenol or ochratoxin A in turkey poults. *Poult. Sci.* 76:256–270.
- Kubena, L. F., T. S. Edrington, C. Kamps-Holtzapfle, R. B. Harvey, M. H. Elissalde, and G. E. Rottinghaus. 1995. 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.
- 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.
- Lumlertdacha, S., R. T. Lovell, R. A. Shelby, S. D. Lenz, and B. W. Kempainen. 1995. Growth, hematology, and histopathology of channel catfish, *Ictalurus punctatus*, fed toxins from *Fusarium moniliforme*. *Aquaculture* 103:201–218.
- Marasas, W.F.O., T. S. Kellerman, W.C.A. Gelderblom, J.A.W. 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.
- Merrill, A. H., Jr., and D. D. Jones. 1990. An update of the enzymology and regulation of sphingomyelin metabolism. *Biochem. Biophys. Acta* 1044:1–12.
- Merrill, A. H., Jr., E.-M. Schmeilz, D. L. Dillehay, S. Spiegel, J. A. Shayman, J. J. Schroeder, R. T. Riley, K. A. Voss, and E. Wang. 1997. Sphingolipids—The enigmatic lipid class: Biochemistry, physiology, and pathophysiology. *Toxicol. Appl. Pharmacol.* 142:208–225.
- Merrill, A. H., Jr., E. Wang, R. E. Mullins, W. Charles, L. Jamison, S. Nimkar, and D. C. Liotta. 1988. Quantitation of free sphingosine in liver by high-performance liquid chromatography. *Anal. Biochem.* 171:373–381.

- Murray, R. K., D. K. Granner, P. A. Mayes, and V. W. Rodwell. 1990. Harper's Biochemistry (22nd Ed.). Appleton and Lange, East Norwalk, CT.
- Norred, W. P., R. D. Plattner, and W. J. Chamberlain. 1993. Distribution and excretion of [^{14}C] fumonisin B₁ in male Sprague-Dawley rats. *Nat. Toxins* 1:341–346.
- Norred, W. P., K. A. Voss, R. T. Riley, and R. D. Plattner. 1996. Fumonisin toxicity and metabolism studies at the USDA. In: L. S. Jackson, J. W. DeVries, and L. B. Bullerman (Ed.) *Fumonisin in Food*. pp 225–236. Plenum Press, New York.
- NRC. 1981. Nutrient Requirements of Goats: Angora, Dairy, and Meat Goats in Temperate and Tropical Countries. National Academy Press, Washington, DC.
- Oswiler, G. D., M. E. Kehrl, J. R. Stabel, J. R. Thurston, P. F. Ross, and T. M. Wilson. 1993. Effects of fumonisin-contaminated corn screenings on growth and health of feeder calves. *J. Anim. Sci.* 71:459–466.
- Prelusky, D. B., H. L. Trenholm, and M. E. Savard. 1994. Pharmacokinetic fate of ^{14}C -labelled fumonisin B₁ in swine. *Nat. Toxins* 2:73–80.
- Riley, R. T., N.-H. An, J. L. Shoowker, H.-S. Yoo, W. P. Norred, W. J. Chamberlain, E. Wang, A. H. Merrill, Jr., G. Motelin, V. R. Beasley, and W. M. Haschek. 1993. Alteration of tissue and serum sphinganine to sphingosine ratio: An early biomarker of exposure to fumonisin-containing feeds in pigs. *Toxicol. Appl. Pharmacol.* 118:105–112.
- Riley, R. T., E. Wang, and A. H. Merrill, Jr. 1994. Liquid chromatographic determination of sphinganine and sphingosine: use of the free sphinganine-to-sphingosine ratio as a biomarker for consumption of fumonisins. *J. Assoc. Off. Anal. Chem. Int.* 77: 533–540.
- Ross, P. F., A. E. Ledet, D. L. Owens, L. G. Rice, H. A. Nelson, G. D. Oswiler, and T. M. Wilson. 1993. Experimental equine leukoencephalomalacia, toxic hepatosis, and encephalopathy caused by corn naturally contaminated with fumonisins. *J. Vet. Diagn. Invest.* 5:69–74.
- Ross, P. F., L. G. Rice, R. D. Plattner, G. D. Oswiler, T. M. Wilson, H. A. Nelson, and J. L. Richard. 1991. Concentrations of fumonisin B₁ in feeds associated with animal health problems. *Mycopathologia* 114:129–135.
- SAS. 1985. SAS User's Guide: Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.
- Shephard, G. S., E. W. Sydenham, P. G. Thiel, and W.C.A. Gelderblom. 1990. Quantitative determination of fumonisin B₁ and B₂ by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* 13:2077–2087.
- Shephard, G. S., P. G. Thiel, E. W. Sydenham, J. F. Alberts, and W.C.A. Gelderblom. 1992. Fate of a single dose of the ^{14}C -labelled mycotoxin, fumonisin B₁, in rats. *Toxicol* 30: 768–770.
- Smith, J. S., and R. A. Thakur. 1996a. Determination of fumonisin B₁ and B₂ and their hydrolyzed products in corn, feed and meat, using HPLC. *J. Agric. Food Chem.* 44:1047–1052.
- Smith, J. S., and R. A. Thakur. 1996b. Occurrence and fate of fumonisins in beef. In: L. S. Jackson, J. W. DeVries, and L. B. Bullerman (Ed.) *Fumonisin in Food*. pp 39–55. Plenum Press, New York.
- Sydenham, E. W., G. S. Shephard, P. G. Thiel, W.F.O. Marasas, and S. Stockenstrom. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* 39: 2014–2018.
- Tiez, N. 1976. *Fundamentals of Clinical Chemistry*. W. B. Saunders, Philadelphia, PA.
- Voss, K. A. 1990. Toxins from *Fusarium moniliforme*, a common fungus in corn. *Vet. Human Toxicol.* 32(Suppl.):57.
- Voss, K. A., W. P. Norred, and C. W. Bacon. 1992. Subchronic toxicological investigations of *Fusarium moniliforme* contaminated corn, culture material, and ammoniated culture material. *Mycopathologia* 117:97–104.
- Vudathala, D. K., D. B. Prelusky, M. Ayroud, H. L. Trenholm, and J. D. Miller. 1994. Pharmacokinetic fate and pathological effects of ^{14}C -fumonisin B₁ in laying hens. *Nat. Toxins* 2:81–88.
- Wang, E., P. F. Ross, T. M. Wilson, R. T. Riley, and A. H. Merrill, Jr. 1992. Alteration of serum sphingolipids upon dietary exposure of ponies to fumonisins, mycotoxins produced by *Fusarium moniliforme*. *J. Nutr.* 122:1706–1716.
- Weibking, T. S., D. R. Ledoux, A. J. Bermudez, J. R. Turk, and G. E. Rottinghaus. 1993. Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B₁ on young broiler chicks. *Poult. Sci.* 72:456–466.
- Weibking, T. S., 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.
- Wu, W., T. Liu, and R. F. Vesonder. 1995. Comparative cytotoxicity of fumonisin B₁ and moniliformin in chicken primary cultures. *Mycopathologia* 132:111–116.

Citations

This article has been cited by 1 HighWire-hosted articles:
<http://jas.fass.org#otherarticles>