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Short communication

Efficacy of AdiDetox™ in reducing the toxicity of fumonisin B_1 in rats

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ABSTRACT

The objective of this study is to evaluate the efficacy of a new mycotoxin inactivator (AdiDetox™) in reducing the toxic effects of fumonisin B_1 (FB_1) in the diet of rats. Sixty-four male Sprague-Dawley growing rats (125 g ± 1 g BW) were assigned to eight dietary treatments for seven days. The experiment had a 2 × 4 factorial arrangement with two levels of FB_1 (0 mg and 15 mg of FB_1/kg feed) and four levels of AdiDetox™ (0 g, 1 g, 2 g and 5 g /kg feed) in the diet. No significant differences were observed in the growth performance among treatments ($P > 0.05$), though low levels of sphingosine (So) and sphinganine (Sa) were detected in the liver. However, So and Sa and the Sa/So ratio in kidneys were higher in rats receiving the FB_1 diets ($P < 0.0001$) than in those fed the Control diet. Supplementation of AdiDetox™ to the diet significantly reduced the toxic effects of FB_1, leading to a significant decrease in the Sa content and in the Sa/So ratio in kidneys. In conclusion, the results suggest that AdiDetox™ can effectively reduce toxicity of FB_1 in growing rats.

1. Introduction

Fumonisins are secondary toxic metabolites produced by the *Fusarium* species, such as *Fusarium moniliforme* and *Fusarium verticillioides*, which are common contaminants of corn and several foods and feeds worldwide (Marasas, 2001). Fumonisin B_1 (FB_1) inhibits the ceramide synthase enzyme and disrupts overall sphingolipid metabolism (Voss et al., 2013), which may result in a toxic dose-dependent accumulation of sphinganine (dihydrosphingosine-Sa) and sphingosine (So) (Riley et al., 1993) in the kidney and liver of animals (Gelderblom et al., 1991). The increase in the Sa/So ratio in urine or serum has been proposed as a biomarker to evaluate exposure to fumonisins. Through this physiopathological mechanism, fumonisin consumption has been associated with animal diseases such as equine leucoencephalomalacia and porcine pulmonary edema (Diaz and Boermans, 1994), as well as kidney and liver toxicity in rats (Riley and Voss, 2006; Voss et al., 1993). Fumonisin B_1 has also been classified by the International Agency for Research on Cancer (IARC) as a possible carcinogenic in humans (Class 2B) (IARC, 2002).

A practical approach to mycotoxin inactivation involves the addition of selective adsorbents in the diet that tightly bind mycotoxins, reducing their bioavailability in the gastrointestinal tract of animals. Several sequestering agents were tested for their capacity to absorb FB_1 *in vitro* and *in vivo*. Cholestyramine, activated carbon, celite and bentonite showed an adsorbtion capacity to FB_1 *in vitro*; however, the results were ineffective when tested *in vivo* (Solfrizzo et al., 2000, 2001a).

The aim of this study is to investigate both diets containing FB_1 and the ability of an adsorbent to prevent toxic effects of FB_1 on male rats. The hypothesis tested whether or not the supplementation of AdiDetox™ at 1 g, 2 g, and 5 g/kg in contaminated diets would reduce the toxic effects associated with FB_1 on internal organ weights, serum biochemistry, and the sphinganine/sphingosine (Sa/So) ratio in the liver and kidneys of rats.

2. Materials and methods

The experiment was performed at the Animal Facility Research Center of the Universitat Autònoma de Barcelona (UAB) and received prior approval from the Animal Protocol Review Committee of this institution. The treatment, management, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines (ETS No 123, ETS No 170).

2.1. Chemicals and feed contamination

Pure FB_1 (Sigma-Aldrich Chimie S.a.r.l; France) was dissolved in absolute ethanol (mg/ml) and the solution was sprayed out on 100 g of ground Control diet to prepare a contaminated feed premix. The treated feed was left overnight at room temperature

Abbreviations: FB_1, fumonisin B_1; So, sphingosine; Sa, sphinganine; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; TP, total protein; UA, albuminuric acid; HPLC, high performance liquid chromatography.

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for the solvent to evaporate (min 12 hours). After this time, it was mixed into the basal diet to obtain the desired level of 15 mg of FB_1/kg. AdiDetox™ (ADIVETER, S.L. Pol. Ind. Agro-Reus; Tarragona, Reus, Spain) is a new additive designed to have high efficacy in binding mycotoxins (Zearalenone and FB_1) at several dosages in feed, which may allow for its use as a preventive agent. AdiDetox™ has been obtained from the modification and activation of a diatomaceous soil, which is a naturally-ocurring material extracted from a quarry, with a maximum of 70% silicium dioxide. AdiDetox™ presents a highly porous surface and adequate polar micro environment that yields a high *in vitro* adsorption capacity against Zearalenone and FB_1.

2.2. Animals and experimental procedures

Rats were used as experimental animals due to their greater sensitivity to fumonisin toxicity (Riley et al., 1994). Sixty-four young male Sprague-Dawley rats (125 g ± 1 g BW) were allocated in 32 cages (two rats per cage) and assigned to eight treatments (four cages, eight rats per treatment). The eight experimental treatments resulted from a 2 × 4 factorial arrangement with two levels of FB_1 (0 mg and 15 mg of FB_1/kg feed) and four levels of the adsorbent AdiDetox™ (0 g, 1 g, 2 g and 5 g /kg feed). The experimental diets were prepared by the incorporation of AdiDetox™ and FB_1, as described above, into a Standard Certified Rodent Chow diet (SAFE-Scientific Animal Food Engineering; France). Rats were housed in wire cages with filter tops at a temperature of 24ºC, 55%–60% humidity and a 12h-light/12h-dark cycle. The experiment lasted for seven days, during which diets were offered *ad libitum*. Individual average daily gain, feed intake and feed conversion rate per cage were recorded at the end of the experiment.

2.3. Serum biochemistry

Rats were anesthetized by an intramuscular injection of ketamine-xylazine (80 mg/kg), and 2-mL blood samples per animal were collected from all animals in each treatment by heart puncture, with samples being processed within 1 h for hematological and biochemical analyses. The serum was obtained by centrifugation (2,500 × *g* for 15 min) and stored at –80 °C until further analysis. Serum biochemical parameters were measured by using Olympus System Reagents (Olympus; Clare, Ireland) and an automatic clinical chemistry analyzer (Olympus AU 400; Hamburg, Germany). The concentration of total protein (TP) was measured by following the biuret method, albuminuric acid (UA) by following the Uricase method, cholesterol by following the cholesterol esterase-peroxidase method, triglyceride by following the glycerol phosphate oxidase method, and the enzymatic activities of alkaline phosphatase (ALP) and aspartate aminotransferase (AST, without pyridoxal phosphate addition) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods.

2.4. Internal organ weights, and sphinganine (Sa) and sphingosine (So) in liver and kidney tissue

After taking blood samples, rats were euthanized by intravenous injection of sodium pentobarbital. The abdominal cavity was exposed by middleline laparotomy, and the weight of the excised visceral organs (liver, kidneys, spleen, and the intestinal tract) was determined. Data were expressed as relative organ weight (grams of organ per 100 g BW). Liver and kidney samples were kept at –80°C until the analysis of sphinganine and sphingosine by UPLC-TOF. Tissue (100 mg) was homogenized in PBS, and sphingolipid extracts, fortified with C17-sphinganine (0.5 nmol), were prepared as described by Merrill et al. (2005). The extracts were analyzed by liquid chromatography–mass spectrometry, as described by Canals et al. (2009), using a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal, accelerated time of flight mass spectrometer (Waters; Millford, MA), which was used in the positive electrospray ionization mode.

2.5. Statistical analysis

Data were analyzed by ANOVA using the GLIMMIX procedure of SAS Institute (1996) 9.2 (Cary, NC, USA). The main factors used in the model were FB_1 contamination and adsorbent level, and their interaction was also included. Multiple mean comparisons were made using Tukey's correction. The experimental unit was the pen. The alpha level used for the determination of significance was 0.05.

3. Results

3.1. Effects of FB1 and AdiDetox™ on growth performance and organ weight parameters

No differences were observed in the growth performance among treatments ($P > 0.05$), averaging 13.4 g/d for the feed intake, 3.72 g/d for the body-weight gain and 3.60 for the feed conversion rate (data not shown). As shown in Table 1, the relative weights of the kidneys, spleen and intestinal tract were not affected by treatments ($P > 0.05$). However, dietary FB_1 increased ($P < 0.05$) the relative weight of the liver. Supplementation of AdiDetox™ to the diet contaminated with FB_1 decreased its toxic effect, reducing the relative weight of the liver ($P < 0.05$) to that of rats of the Control group.

3.2. Clinical biochemical parameters

Serum total protein, uric acid, triglyceride, and ALP enzyme activity were not affected by the dietary addition of FB_1 or AdiDetox™ ($P > 0.05$, Table 2). Nevertheless, serum activity of AST decreased in the groups of rats fed the FB_1 treatments. A significant interaction between FB_1 and AdiDetox™ in cholesterol concentration was observed. The presence of FB_1 in the diet increased the concentration of cholesterol in serum, while the addition of AdiDetox™ to the FB_1-contaminated diets reduced the cholesterol concentration to values not significantly different from those of the Control group.

3.3. The effect on sphingosine (So) and sphinganine (Sa) in the liver and kidney

Low levels of sphingosine (So) and sphinganine (Sa) were detected in the liver (data not shown), while larger amounts were observed in kidney tissue (Table 3). A significant interaction between FB_1 and AdiDetox™ was observed in the Sa and So content and the Sa/So ratio in kidney tissue samples. While the group of rats which

Table 1
Effects of dietary Fumonisin B_1 and AdiDetox™ on internal organ weight in growing rats (n = 8).

Treatments		Liver (g/BW, %)	Kidneys (g/BW, %)	Spleen (g/BW, %)	Intestinal tract (g/BW, %)
FB_1 (mg/kg)	AdiDetox™ (g/kg)				
0	0	4.24[b]	0.98	0.36	9.7
0	1	4.26[b]	1.06	0.32	9.8
0	2	4.53[b]	0.90	0.44	9.2
0	5	4.50[b]	1.00	0.42	10.5
15	0	5.40[a]	0.96	0.44	9.9
15	1	4.96[ab]	1.02	0.44	9.2
15	2	4.42[b]	1.00	0.41	9.4
15	5	5.02[ab]	0.92	0.43	9.9
Sem[1]		0.182	0.042	0.038	0.409
Main effect		Probability			
FB_1		**	NS	NS	NS
AdiDetox™		NS	NS	NS	NS
FB_1 × AdiDetox™		*	NS	NS	NS

[ab] Means within the same line with different superscripts differ significantly ($P < 0.05$).
[1] Standard error of the mean.
* $P < 0.05$, ** $P < 0.001$.
NS, not significant.