

FUMONISIN B<sub>1</sub> LEVELS IN CEREALS AND FEEDS FROM SOUTHERN BRAZILC.A. Mallmann<sup>1</sup>, J.M. Santurio<sup>1</sup>, C.A.A. Almeida<sup>1</sup>, P. Dilkin<sup>2</sup><sup>1</sup>Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil, CEP 97105-900. E-mail: mallmann@ccr.ufsm.br. <sup>2</sup>Bolsista do CNPq.

## ABSTRACT

A survey was conducted to evaluate fumonisin B<sub>1</sub> (FB<sub>1</sub>) contamination in cereals and feeds of southern Brazil. A total of 407 samples was obtained from different local warehouses and feed industries between January 1996 and June 1998. Extraction of FB<sub>1</sub> was performed with acetonitrile-water (50+50, v/v) and cleanup with end-capped C<sub>18</sub> silica columns; the extracted mycotoxin was resolved by liquid chromatography with o-phthalaldehyde and 2-mercaptoethanol derivatization, and identified by fluorescence. Positive results were found in 32.2% of the samples assayed. FB<sub>1</sub> concentrations varied from 0.086 to 78.92 µg/g, and the highest levels recorded were: 14.21 µg/g in rice; 68.33 µg/g in feed; 78.92 µg/g in corn; 24.35 µg/g in wheat; 0.17 µg/g in oat; and 2.43 µg/g in barley. No detectable levels of this mycotoxin were found in soybean meal samples. The results demonstrate that cereals harvested in southern Brazil were contaminated with FB<sub>1</sub>. Considering that these products are consumed in large amounts either directly or as components of foods and feeds, the levels of contamination reported herein indicate a potential threat to animal and public health.

KEY WORDS: Fumonisin, mycotoxin, cereals, feeds.

## RESUMO

NÍVEIS DE FUMONISINA B<sub>1</sub> EM CEREAIS E ALIMENTOS DO SUL DO BRASIL. Avaliou-se a ocorrência de fumonisin B<sub>1</sub> em cereais e rações do sul do Brasil. Foram analisadas 407 amostras provenientes de diferentes granjas e indústrias agropecuárias, coletadas entre os meses de janeiro de 1996 e junho de 1998. A extração da fumonisin foi realizada com acetonitrila-água (50+50, v/v) e clarificação com colunas de sílica C<sub>18</sub> encapada. A fumonisin foi submetida a derivatização com o-ftalaldeído e 2-mercaptoetanol e quantificada por cromatografia líquida com detecção por fluorescência. Das amostras analisadas, 32% apresentaram positividade. As concentrações foram de 0,086 até 78,92 mg/g. As maiores concentrações foram: arroz, 14,21 µg/g; rações, 68,33 µg/g; milho, 78,92 µg/g; trigo, 24,35 µg/g; aveia, 0,17 µg/g e cevada, 2,43 µg/g. Não se detectou presença de fumonisin em farelo de soja. Os resultados obtidos indicam a ocorrência da fumonisin B<sub>1</sub> em alimentos colhidos no sul do Brasil. Considerando que os cereais e rações analisadas são amplamente empregados na composição da dieta alimentar, os níveis de contaminação detectados no presente trabalho representam uma ameaça à saúde humana e animal.

PALAVRAS-CHAVE: Fumonisin, micotoxina, cereais, alimentos.

## INTRODUCTION

Fumonisin belongs to a large group of mycotoxins produced by fungi of the genera *Fusarium* (ROSS et al., 1990; NELSON, 1991; THIEL et al., 1992) and *Alternaria* (CHEN et al., 1992) but especially by *Fusarium moniliforme* (VISCANTI & DOKO, 1994; MUSSER & PLATTNER, 1997). These species are natural contaminants of cereals worldwide and are mostly found in corn and its derived products (SHEPHARD et al., 1996). The occurrence of fumonisin B<sub>1</sub> (FB<sub>1</sub>) in Brazilian feeds was demonstrated by several investigations (SYDENHAM et al., 1992; HIROOKA et al., 1996; MALLMANN

et al., 1999; DIAS et al., 1999; ORSI et al., 2000). The contamination of foods and feeds usually reflects the incidence of fungal infection on the original crops, which is affected by factors such as origin, drought-stress, and insect damage (LEW et al., 1991; MEIRELES et al., 1994). Several naturally occurring fumonisins are known. FB<sub>1</sub> is always the most abundant and toxic metabolite of this group of mycotoxins, representing ca. 70% of the total concentration in naturally contaminated foods and feeds, followed by fumonisins B<sub>2</sub> and B<sub>3</sub> (MURPHY et al., 1993; NORRED, 1993; PIÑEIRO et al., 1997). FB<sub>1</sub> acts by inhibiting the enzyme N-acetyltransferase and leads to a reduced production of

sphingolipids (SCHROEDER et al., 1994). FB<sub>1</sub> is known to be toxic to domestic animals and to induce leukoencephalomalacia in horses (KELLERMAN et al., 1990) and porcine pulmonary edema (OSWEILER et al., 1992). Liver hyperplastic nodules and lesions in the distal esophageal mucosa of weaning pigs fed with fumonisins have also been reported (CASTEEL et al., 1993). Body weight and average daily weight gain decreased in chicks in parallel with increasing dietary FB<sub>1</sub>, whereas liver, proventriculus and gizzard weights increased (LEDoux et al., 1992). Additionally, the occurrence of FB<sub>1</sub> has been statistically associated with a high incidence of human esophageal cancer (RHEEDER et al., 1992). On the basis of available toxicological evidence, the International Agency for Research on Cancer (IARC) has declared *Fusarium moniliforme* toxins as possibly carcinogenic (class 2B carcinogen) to humans (IARC, 1993). The natural occurrence of FB<sub>1</sub> in cereals and corn based-foods and feeds from southern Brazil had not been studied to date, so the present survey was conducted in order to evaluate FB<sub>1</sub> contamination of corn and others cereals from that region.

**Safety note:** Fumonisin B<sub>1</sub> is declared a possibly carcinogenic for human by the IARC (1993). Therefore, should be handled with care.

## MATERIALS AND METHODS

**Sample collection:** A total of 407 samples of corn and other cereals were obtained from different warehouses in southern Brazil between January 1996 and June 1998. Wheat and rice were destined for human consumption and the other products for feeding of poultry, swine and horses. Samples of approximately 1 kg were collected, being 14 of barley, 267 of unprocessed corn kernels, 8 of oat, 13 of wheat, 92 of corn-based ration, 5 of rice, and 8 of soy meal. All samples were ground to 0.2 mm flour and individually stored at -18°C prior to analysis.

**Extraction and clean-up:** The method was adapted from a previously described procedure (BINKERD et al., 1993). First, each sample was thoroughly mixed. A 10g sub-sample obtained for final analysis was then mixed with 50 mL acetonitrile-water (50+50, v/v) for five minutes in a blender and the suspension was filtered through a Whatman filter paper in a Buchner funnel. Next, a 2 mL aliquot of the filtered extract containing 0.4 g of sample was diluted in 6 mL of water and applied to a solid-phase extraction (3 mL) end-capped C<sub>18</sub> silica (500 mg) column previously conditioned with 3 mL of acetonitrile and 3 mL of water. The column was washed with 2 mL of water

and FB<sub>1</sub> was then eluted with 2 mL of acetonitrile-water (70+30, v/v). Washing and elution were accomplished by gravity flow. The eluates were dried at 60°C and kept in a freezer, at temperatures below -18°C. Prior to derivatization, the eluates were redissolved in 200 mL acetonitrile-water (50+50, v/v) for liquid chromatography analysis.

**HPLC Analysis:** The purified extract was analysed by high performance liquid chromatography (HPLC) as described (STACK & EPPLEY, 1992; BINKERD et al., 1993), with some modifications. One hundred microliters of extract residues were mixed with 100 µL of borate buffer (0.1 M pH 8-9), after which 100 µL of OPA reagent (30 mg of o-phthalaldehyde dissolved in 9.5 mL of acetonitrile containing 0.5 mL of 2-mercaptoethanol) were added and allowed to react for 10 minutes. Two hundred microliters of 0.01 M boric acid were then added and an aliquot of 20 µL of the sample were injected into the LC system. FB<sub>1</sub> working standards (10 µg/mL) were similarly treated. Quantification of FB<sub>1</sub> was performed by HPLC (GBC Scientific Equipment Pty Ltd. - Victoria, Australia), using acetonitrile-water-acetic acid (50+50+1, v/v) as mobile phase. A C<sub>18</sub> ODS reversed-phase column (150 x 4.6 mm, 5 µm particle size - Phenomenex, Torrance, USA) and on-line corresponding guard column were employed under oven temperature of 35°C. Flow rate of the mobile phase was set at 1 mL/min. Detection was based on elicitation of the FB<sub>1</sub> derivative fluorescence, with wavelength set of 335 nm and 440 nm for excitation and emission, respectively. The chromatogram retention time for FB<sub>1</sub> derivative was approximately nine minutes (Fig. 1). Quantification was based on peak area measurement and comparison with FB<sub>1</sub> standard.

Method recoveries from five samples from each matrix spiked with 0.1 to 3 µg FB<sub>1</sub>/g ranged from 88.6 to 103% for corn, 83 to 97.8% for rations, 78 to 94.5% for rice, 82.4 to 94.8% for wheat, 86.3 to 97.5% for oat, 82 to 102.3% for barley, and 68,2 to 92% for soy meal. The detection limit was approximately 0.04 µg/g. Samples of rice, wheat and barley that presented a peak within the FB<sub>1</sub> retention area were confirmed by addition of the internal standard in cleaned sample at 1 and 3 µg FB<sub>1</sub>/g and processed again. Samples that presented concentrations above 20 mg/g were diluted again and re-analysed.

## RESULTS AND DISCUSSION

Of the 407 samples examined, 32.2% (131/407) were positive and presented contamination levels of FB<sub>1</sub> that varied from 0.086 µg/g to 78.92 µg/g (Table 1). Corn was the most frequently analysed, corresponding to

Table 1 - Occurrence of fumonisin B<sub>1</sub> in cereals and feeds from southern Brazil.

	Samples		Levels of fumonisin B <sub>1</sub> (µg/g)		
	Number	Positive	Minimum	Maximum	Average
Corn	267	94 (35.2%)	0.086	78.92	8.86
Ration	92	28 (30.4%)	0.64	68.33	15.39
Rice	5	4 (80.0%)	1.14	14.21	4.95
Wheat	13	1 (7.7%)	24.35	24.35	24.35
Oat	8	2 (25.0%)	0.045	0.17	0.11
Barley	14	2 (14.3%)	1.97	2.43	2.20
Soya meal	8	0 (0.0%)	—	—	—
Total	407	131 (32.2%)	—	—	—

65.6% of the total number of samples. The highest contamination levels were found in this category, and were associated with outbreaks of equine leukoencephalomalacia (MALLMANN et al., 1999). Ration fed to poultry, swine and horses presented the largest average (15.39 µg/g) concentration of FB<sub>1</sub> in positive samples. Although, overall, lesser amounts of rice, wheat, oat, barley and soya meal were assayed, contaminated samples were found in each category except the latter. This suggests that FB<sub>1</sub> could be present in a wider variety of products from southern Brazil. Therefore, larger numbers of samples should be tested in order to obtain precise statistical information on the occurrence of FB<sub>1</sub> in these cereals. The data on positive samples (percent values) and the minimum, maximum, and average FB<sub>1</sub> concentrations recorded are shown in Table 1.

Previous research work from Brazil reported a greater number of FB<sub>1</sub> positive corn samples and lower mean concentrations. In a survey conducted in the State of Paraná, Brazil, HIROOKA et al. (1996) detected positivity for FB<sub>1</sub> in over 97% of 48 corn samples. Similarly, ORSI et al. (2000) found a percentage of positive samples above those presently reported. They studied the incidence of fumonisins in freshly-harvested and stored corn from the State of São Paulo, Brazil, and obtained positive results in over 90% of the 195 samples tested, with FB<sub>1</sub> concentrations ranging from 0.87 to 49.31 µg/g.

The percentage of FB<sub>1</sub> positive corn samples obtained in our study is also lower than that found in neighbouring countries. RAMIREZ et al. (1996) and PIÑEIRO et al. (1997) described contamination frequencies of 100 and 50% associated with average contamination levels of 2.16 and 1.74 µg/g, respectively. However, in our evaluation, the average FB<sub>1</sub> concentration in positive corn samples was greater, being 8.86 µg/g. This is probably due to the fact that samples related to outbreaks of leukoencephalomalacia in horses were assayed, which were highly contaminated with FB<sub>1</sub> and raised the average detectable level of the mycotoxin.

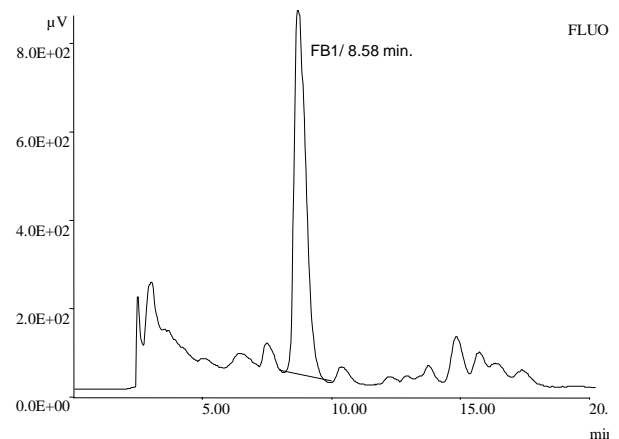


Fig. 1 - Liquid chromatogram of o-phthalaldehyde derivatives of fumonisin B<sub>1</sub> (8.58 min, 78.92 µg/g) extracted from naturally contaminated corn.

The analysis of our data demonstrates a high incidence and high contamination levels of FB<sub>1</sub> in rations from southern Brazil. Research work carried out by several authors (ROSS et al., 1991; THIEL et al., 1992) shows that significant productivity losses occurred among animals exposed to dietary FB<sub>1</sub> at concentrations that were even lower than those presently reported. Elevated concentrations of FB<sub>1</sub> in human foods have been associated with a high incidence of oesophageal cancer in South Africa (RHEEDER et al., 1992; SYDENHAM et al., 1990), Italy (FRANCESCHI et al., 1990), and China (CHU & LI, 1994). In southern Brazil, oesophageal cancer has been typically linked to the tradition of drinking “chimarrão” (an infusion of hot water and herbs), although such association has never been demonstrated. On the other hand, local populations could easily be exposed to FB<sub>1</sub>, either through a marked consumption of contaminated corn-based foods, rice and wheat-based foods or, indirectly, through the ingestion of mycotoxin-containing meat from animals that had been exposed to contaminated feeds, as proposed by PRELUSKY et al. (1996). Therefore, we raise the possibility that FB<sub>1</sub> may be partially responsible for the incidence of human oesophageal cancer in southern Brazil and propose a reevaluation of the aetiology of the disease in that region. As there are no detailed statistical data available on the incidence of FB<sub>1</sub>, or compounds generated through its biotransformation, in meat derived from breeds of southern Brazil, novel research work on meat from that region must be carried out in order to assess the true risk of human contamination with FB<sub>1</sub> due to consumption of local animal products.

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