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Fumonisins in brewers grain (barley) used as dairy cattle feed in the State of Bahia, Brazil

M.J.M. Batatinha [a,*], M.M.S. Simas [b], M.B. Botura [a], T.C. Bitencourt [a], T.A. Reis [c], B. Correa [c]

[a] *Escola de Medicina Veterinária, Universidade Federal da Bahia, Av. Ademar de Barros, 500, Salvador, Bahia 40170-110, Brazil*
[b] *Instituto de Ciências da Saúde, Universidade Federal da Bahia, Av. Reitor Miguel Calmon, Salvador, Bahia 40110-100, Brazil*
[c] *Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, São Paulo, São Paulo 05508-900, Brazil*

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Abstract

Fumonisins are mycotoxins produced by the genus *Fusarium* that may induce toxic effects in several animal species and may be found in several kinds of foods and feed. In the State of Bahia, Brazil, brewers grain, which are a brewery by-product, have been largely used in the feeding of animals, specially dairy cattle, due to their nutritional value and low cost of transportation. The aim of this study was to establish the presence of fumonisins in brewers grain used as dairy cattle feed in the State of Bahia. Twenty samples of brewers grain were collected every three months during a whole year, for a total of 80 samples, in five properties located in the "reconcavo baiano". These samples were analyzed for the presence of fumonisins using high efficiency liquid chromatography (HPLC). Results showed contamination of 58 (72.5%) samples, with contamination mean level equal to 226.5 μg/kg, with 50.30 and 908.47 μg/kg as the minimum and maximum levels, respectively. This is the first report of the occurrence of this mycotoxin in the State of Bahia.

Keywords: Fumonisins; Cattle; Barley

1. Introduction

The use of agroindustrial residues in animal feeding has become a constant practice in rural properties, as an alternative to minimize feeding costs without reducing the productivity of the herd. In the State of Bahia, brewers grain are the most widely used feeding products in dairy cattle breeding, due to the high nutritional value and low cost of transportation, as farms and breweries are close to each other. Scientific studies indicate that the inclusion of brewers grain in the diet as 16–30% dry matter results in an increase in milk production (Belibasakis & Tsirgogianni, 1996; Cardoso, Silva, Mello, & Motta, 1982).

Climatic conditions of the microregions where the main dairy production units of the state are located, together with the form of storage of brewers grain in the rural properties and the composition of this substrate, provide an ideal environment for the development of fungi, including those that produce mycotoxins. These toxins are chemically diverse secondary metabolites that may induce a series of harmful effects in human and animal health (CAST, 2003).

Among the various known mycotoxins, fumonisins, produced by the fungi *Fusarium verticillioides* (Sheldon) and *Fusarium proliferatum* (Matsushima), may be emphasized. These toxins were isolated in 1988 by Bezuidenhout et al., and up to now, 18 molecules have already been characterized (Ah-Seo & Lee, 1999). Fumonisin B_1 (FB_1) is considered to be the most toxic and most frequently of these toxins found in nature (Gelderblom, Marasas, Vleggaar, Thiel, & Cawood, 1992), corresponding to approximately 70% of all fumonisins (Pittet, 1998).

Fumonisins are believed to produce their toxic effects by interrupting the biosynthesis of sphingolipids through the

* Corresponding author. Tel.: +71 3235 8770; fax: +71 3245 2813.
E-mail address: mjmb@ufba.br (M.J.M. Batatinha).

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inhibition of an enzyme called ceramide synthetase (Norred, Wang, Yoo, Riley, & Merrill, 1992; Wang, Norred, Bacon, Riley, & Merrill, 1991). These toxins have been related to different toxic effects in animals, such as leukoencephalomalacia in horses (Mallmann, Santurio, & Dilkin, 1999), pulmonary edema in pigs (Casteel, Turk, & Rottinghaus, 1994), renal and hepatic alterations in cattle (Mathur et al., 2001), and neoplasms in rats (Gelderblom et al., 2001). In humans, the consumption of corn products contaminated with fumonisins have been epidemiological related to the development of esophageal cancer (Chu & Li, 1994; Franceschi, Bidoli, Baron, & La Vacchia, 1995).

Fumonisins have been identified in various agricultural products to be used as animal feeds, including corn, sorghum and barley (Almeida et al., 2002; Park, Kim, Shon, & Kim, 2002; Silva, Pozzi, Mallozzi, Ortega, & Corrêa, 2000; Sydenham, Marasas, Shepard, Thiel, & Hirooka, 1992). In Brazil, most studies on the contamination of grains with fumonisins refer to the south and south-east regions of the country (Salay & Mercadante, 2002). In the State of Bahia, investigations regarding the contamination of food products with mycotoxins are scarce. Only Bautista, Oliveira, Miranda, and Sales (1989) and Batatinha et al. (2003) reported the presence of aflatoxins in corn and peanuts, but there is no report regarding the contamination of grains or other food products with fumonisins. Therefore, the objective of the present study was to determine the presence of fumonisins in brewers grain used as dairy cattle feed in this state.

2. Material and methods

2.1. Sample collection

Brewers grain samples were collected in five farms (A, B, C, D and E) located in cities of the "reconcavo baiano" (exceptionally fertile region on the coast of the State of Bahia). These properties were selected because they have the largest concentration of dairy cattle and constantly use this material as animal feed. In each property, four samples were collected every three months over a period of one year, in a total of 80 samples. The mean period the product remained in the storage tanks was considered to be the determining factor for sample collection.

A questionnaire regarding sanitary and nutritional management of the herd, as well as storage conditions and the form of use of brewers grain, was applied during the visits to the farms.

2.2. Moisture determination

The moisture content of the brewers grain samples was determined according to the method by Silva (1991). The samples were weighed and then dried at 105 °C until they reached constant weight. Moisture content of each sample was determined based on the difference between dry weight and initial weight.

2.3. Fumonisin determination

Toxins were extracted as described by Sydenham et al. (1996). Briefly, 50 g of each brewers grain sample was added to a 100 mL of a 3:1 solution of methanol:water and stirred for 45 min. The extract was then filtered through filter paper (Whatmann no. 1) and the pH was corrected to 5.8–6.5 with 0.1 M sodium hydroxide solution, if necessary. A 10-mL aliquot was passed through a 500-mg strong-anion-exchange silica cartridge (Varian, Bond Elut) previously calibrated with 5 mL methanol and 5 mL methanol:water (3:1), and the column was washed with 5 mL methanol:water (3:1) and 3 mL methanol. Fumonisins were eluted with 15 mL methanol:acetic acid (99:1) at a flow rate kept at 1 mL/min in all steps. The eluate was then dry evaporated in a water bath (60 °C).

Fumonisins were quantified as described by Stack and Eppley (1992) and Sydenham et al. (1996), with some modifications. The residue obtained by extraction of each sample was resuspended in 500 μL acetonitrile:water (1:1) and filtered through a 0.2-μm GV membrane in PVDF. A 100-μL aliquot was placed in an test tube and 200 μL *O*-ophthaldialdehyde solution (OPA) (40 mg of OPA in 1 mL methanol, 5 mL of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol) was added. After 2 min, 20 μL of this mixture was applied to a chromatograph (Shimadzu LC-10AD, pump and RF-10AXL fluorescence detector), using a 5O ODS-20 C_{18} column (150 × 4.6 mm, Phenomenex, Ultracarb) maintained in an oven at 30 °C. The mobile phase consisted of acetonitrile:water:acetic acid (50:50:1) at a flow rate of 1 mL/min. Fumonisin derivatives were detected by fluorescence at excitation and emission wavelengths of 335 and 440 nm, respectively, and detection times were 8.5 min for FB_1 and 22.5 min for FB_2.

Recoveries were performed with brewers samples ($n = 3$) spiked with 0.1, 0.5, 1.0, 5.0 and 10 μg/g. Average recoveries for brewers was 106.6 with relative standard deviation (RSD) at 7.04%, for fumonisin B1, and 87.2%, RSD 7.88% for fumonisin B2. Detection limit (signal-to-noise ratio of 3) was approximately 50 μg/kg for each toxin. Confirmation of the identity of the peaks assigned as FB_1 and FB_2 was made by comparing test chromatograms with standards, with attention to retention, start and end time of peak elution. Samples that presented a peak at the fumonisin retention time were confirmed by addition of standard and reprocessing.

3. Results and discussion

Analysis of the information obtained from the questionnaires applied to the farms studied revealed a semi-intensive animal breeding system with closely similar feeding regimen, consisting of bulky feed and brewers grain, although other agroindustrial residues, such as sugarcane, were also added to the diet when available.

Brewers grain were stored in cement tanks with a capacity of approximately 20 tons in all properties, except for

farm C, where this material was kept on a cemented surface covered with a black plastic sheet. The storage period varied as a function of the number of lactating cows, generally ranging from 8 to 15 days. As a conservation procedure, common salt was added to the barley as it arrived in the farms.

Analysis of the meteorological factors of the microregions where the farms studied were located demonstrated that the mean maximum temperature was significantly lower in farm E than in the other farms (Table 1). The highest mean, minimum and maximum temperatures were observed during the fourth collection period (Table 1).

Rainfall levels and relative humidity varied significantly between properties; the highest levels were observed during the first and second collection periods, in farm E (Table 1). Moisture content of the samples was significantly lower in farm C (Table 1), possibly because of the form of storage of brewers grain in this property.

Analysis of fumonisins in brewers grain revealed contamination in 58(72.5%) samples (Table 2), with levels ranging from 50.30 to 908.47 μg/kg. This is a pioneer finding in the State of Bahia and suggests the presence of the main fungi that produce these toxins, considered to be highly adapted to tropical climate. Similar results have also been reported by Camargos et al. (2000) who analyzed the presence of FB_1 in sorghum grains in the State of São Paulo. The highest incidence of contamination (88.5%) was observed in corn grains collected in the same state (Almeida et al., 2002).

Concentrations observed in the samples obtained in the different properties varied according to collection period. The highest concentrations were observed in samples obtained from farm B during the fourth collection period and from farms D and E during the third collection (Table 3), although these periods were characterized by the lowest rainfall, relative humidity and moisture content of the sample (Table 1), factors that influence the development of mycotoxin-producing fungi (Lillehoj et al., 1980; Mallozzi & Correa, 1998).

Although no significant correlation was observed between fumonisin levels and climatic factors, these results corroborate those reported by Pozzi et al. (1995) and Silva et al. (2000), who found a negative correlation between rainfall and the presence of fumonisins, with greater levels of these toxins being observed in samples collected during periods characterized by low rainfall. Fumonisin-producing fungi have also been detected in South African regions with predominantly dry climate (Marasas, Van Rensburg, & Mirocha, 1979).

Mean, minimum and maximum temperatures were significantly higher during the fourth collection period (Table 1), although mean temperatures observed during all periods were close to those considered favorable for the growth of *Fusarium*. The ideal temperature for fumonisin production ranges from 20 to 25 °C, but production of this mycotoxin may also occur at 13–28 °C (Alberts et al., 1990; Le Bars, Le Bars, Dupuy, & Boudra, 1994).

The highest mean contamination levels were obtained for farm C and for samples collected during the third and fourth periods. No significant difference in contamination levels of samples derived from farm A was observed between the different collection periods (Table 3).

Table 1
Mean climatic conditions in the properties and during each sample collection

Climatic conditions	Property					Collection			
	A	B	C	D	E	First	Second	Third	Fourth
Minimum temperature (°C)	20.9	20.9	20.9	20.4	19.9	20.4^{A}	19.4^{B}	20.3^{A}	22.9^{C}
Mean temperature (°C)	25.1	25.1	25.1	24.8	23.9	24.9^{A}	22.5^{B}	25^{A}	27.8^{C}
Maximum temperature (°C)	30.9^{a}	30.9^{a}	30.8^{a}	30.9^{a}	28.5^{b}	30.5^{A}	27^{B}	31.3^{A}	34.7^{C}
Mean rainfall (mm^3)	40.9^{a}	40.9^{a}	59.8^{ab}	79.5^{bc}	230.4^{c}	136.6^{A}	93.4^{B}	5.6^{C}	31.6^{BC}
Relative air humidity (%)	75^{a}	75^{a}	75.8^{ab}	76.8^{ab}	83.5^{b}	80.4^{A}	87.6^{B}	70.8^{C}	63.5^{D}
Moisture content (%)	73.3^{a}	74.6^{a}	69.3^{b}	75.4^{a}	74.5^{a}	75.3^{A}	73.6^{AB}	71.8^{B}	72.9^{AB}

Uppercase shows the comparison of values in the rows between properties, and lowercase shows the comparison of values in the collection of samples ($p < 0.05$).

Table 2
Number of positive samples for the presence of fumonisins ($B_1 + B_2$) collected from March/2002 to May/2003

Property	Collection				Total of positive samples (%)
	First	Second	Third	Fourth	
A	3	4	3	4	14 (87.5)
B	1	1	4	4	10 (62.5)
C	3	4	2	4	13 (81.25)
D	0	1	4	4	9 (56.25)
E	1	3	4	4	12 (75)
Total of positive samples (%)	8 (55)	13 (65)	17 (95)	20 (100)	58 (72.5)

Table 3
Mean levels of fumonisin B_1 (μg/kg) on barley samples collected from March/2002 to May/2003

Property	Collection				Mean
	1	2	3	4	
A	139.16[a]	166.35[a]	166.07[a]	193.76[a]	198.01[A]
B	ND[a]	ND[a]	345.64[a]	507.84[b]	213.37[A]
C	43.65[a1]	399.22[b]	131.9[b]	384.20[b]	294.83[A]
D	ND[a]	90.65[a]	446.57[b]	345.65[b]	220.72[A]
E	131.98[a]	94.13[a]	431.73[b]	306.07[b]	240.98[A]
Mean	91.96[a]	153.51[a]	348.45[b]	347.5[b]	

Uppercase shows the comparison of values in the rows, and lowercase shows the comparison of values in the columns ($p < 0.05$).
ND – not detected.
[1] Below detection limit.

FB_1 concentrations found in brewers grain samples were lower than those reported for other substrates such as corn and sorghum (Almeida et al., 2002; Camargos et al., 2000; Orsi et al., 2000; Silva et al., 2000). FB_2 was only detected in samples obtained during the second collection period in farm C, with mean concentration of 83.37 μg/kg.

No legal regulation regarding the maximum levels of fumonisins in feeds is available. The Committee on Mycotoxins of the American Association of Veterinarians recommended maximum levels of 5, 10 and 50 μg/kg for horses, pigs and cattle, respectively (Riley et al., 1993).

Results of the present study represent a pioneer finding for the State of Bahia, where scientific information regarding the contamination of food products with mycotoxins is scarce. Only Bautista et al. (1989) and Batatinha et al. (2003) reported the presence of aflatoxins in corn and peanuts, respectively. The present results also suggest the presence of fumonisin-producing fungi in foods in this state, and enabled the identification of a quality profile of brewers grain used as dairy cattle feed in relation to mycotoxin contamination. Based on this information, there is a need for implantation of a constant monitoring program, not only for barley husks, but also for other agricultural products used as animal feed. The importance of this kind of program should be emphasized in order to guarantee the quality of animal feed, which consequently reflects on cattle production, the internal market and exports of products from this state.

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