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Prevalence of *Fusarium* species of the *Liseola* section on selected Colombian animal feedstuffs and their ability to produce fumonisins

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Abstract

A total of 57 samples of feedstuffs commonly used for animal nutrition in Colombia (corn, soybean, sorghum, cottonseed meal, sunflower seed meal, wheat middlings and rice) were analyzed for *Fusarium* contamination. *Fusarium* fungi were identified at species level by means of conventional methods and the ability to produce fumonisins of the most prevailing species was determined. A total of 41 of the feedstuffs analyzed (71.9%) were found to contain *Fusarium* spp. Most contaminated substrates were corn (100%), cottonseed meal (100%), sorghum (80%), and soybean (80%). Wheat middlings and rice showed lower levels of contamination (40% and 20%, respectively), while no *Fusarium* spp. could be isolated from sunflower seed meal. The most prevalent species of *Fusarium* isolated were *F. verticilliodes* (70.8%), *F. proliferatum* (25.0%), and *F. subglutinans* (4.2%). All of them correspond to section *Liseola*. Production of fumonisins on corn by the isolated *Fusarium* was screened through liquid chromatography. Almost all strains of *F. verticilliodes* (97.1%) produced FB1 (5.6–25,846.4 mg/kg) and FB2 (3.4–7507.5 mg/kg). Similarly, almost all strains of *F. proliferatum* (91.7%) produced fumonisins but at lower levels than *F. verticilliodes* (FB1 from 6.9 to 3885.0 mg/kg, and FB2 from 34.3 to 373.8 mg/kg), while *F. subglutinans* did not produce these toxins. This is the first study in Colombia describing toxigenic *Fusarium* isolates from animal feedstuffs.

Key words: *Fusarium* spp., fumonisins, feedstuffs

Introduction

Mycotoxins are secondary toxic metabolites produced during plant growth or during storage of post-harvest crops [1]. *Fusarium* fungi have worldwide distribution and are important pathogens of cereal plants in all stages of their development, from the first hours of kernel germination to harvest time, including post-harvest decay of grains [2]. When cereal grains are colonised by *Fusarium* there is a significant risk of contamination with mycotoxins [3].

Fusarium species produce a wide range of mycotoxins of diverse structure and chemistry. Important *Fusarium*-produced mycotoxins capable of affecting animal health and performance include the trichothecenes, zearalenone and the fumonisins B1 (FB1) and B2 (FB2) [3]. Fumonisins have been implicated in equine leukoencephalomalacia and porcine pulmonary oedema. Besides, fumonisins may have adverse effects on performance parameters on poultry and may cause mild changes on the liver and immune function of cattle [2].

Production of particular mycotoxins by *Fusarium* fungi depends upon the fungal species involved. For example, *F. tricinctum* and *F. sambucinum* are capable of producing type A trichothecenes, particularly diacetoxyscirpenol (DAS),

T-2 toxin and deoxynivalenol; zearalenone is produced mainly by *F. graminearum* and fumonisins by *F. verticillioides* (formerly *F. moniliforme)* and *F. proliferatum* [4].

Very little is known about the toxigenic species of *Fusarium* occurring in feeds and feedstuffs used for animal nutrition in Colombia. Previous studies, however, have shown the occurrence of zearalenone [5] and fumonisins [6], indicating the presence of toxigenic *Fusarium* fungi in Colombian feeds and feedstuffs. The aim of the present study was to isolate and identify at species level by conventional methods [7] the *Fusarium* fungi present in the major feedstuffs used in Colombia for animal nutrition. Additionally, the ability of the more prevalent isolates to produce FB1 and FB2 was also investigated.

Materials and methods

Samples

A total of 57 samples of the feedstuffs most commonly used in Colombia were obtained from local feed mills: 17 corn samples, 10 soybean samples, 10 sorghum samples, 5 cottonseed meal samples, 5 sunflower seed meal samples, 5 wheat middlings samples, and 5 rice samples.

Isolation, identification and enumeration of the mycoflora

Ten grams of finely ground sample was mixed with 90 ml of sterile distilled water, followed by ten-fold serial dilutions up to 10^{-4}. Duplicate 1 ml volumes of each dilution were added to Petri dishes containing 10–15 ml of Potato Dextrose Agar (PDA). All plates were incubated at 25 °C for 5–7 days and observed daily. *Fusarium* fungi were classified by their macroscopic characteristics and CFU were determined. Those that contained 15–150 CFU were used for counting and the results were expressed as CFU per gram of sample. Individual species were subcultured in Komada (Nash and Snyder) medium until pure cultures were obtained. They were then observed under light microscope and identified using previously described keys [7].

Fumonisin production

One ml spore suspension of each *Fusarium* strain cultured in PDA at 25 °C was inoculated into Erlenmeyer flasks containing 50 g of sterilized ground corn and 20 ml of distilled water. The Erlenmeyer flasks were incubated at 18 °C for 12 days, time required for FB1 and FB2 production. After incubation the cultures were homogenized and 25 g were extracted with 100 ml acetonitrile + water (50 + 50) for 1 h using a wrist-action shaker at high speed. The extracts were then filtered using qualitative high speed filter paper and purified with SAX (strong anion exchange) cleanup columns. Separation and identification of fumonisins was done using HPLC according to the method of Perilla and Diaz, with some modifications [6]. Briefly, the dried residue was dissolved with 1 ml of methanol and then derivatized with 0.5 ml *o*-phthaldialdehyde (OPA) solution (20 mg of OPA dissolved in 1 ml methanol and diluted with 19 ml water) plus 0.5 ml of 0.5% $NaHCO_3$, pH 9.0, containing 2 μl of mercaptoethanol/ml. The derivatization reaction was carried out at room temperature for exactly 10 min and then 20 μl of the mixture was injected into the HPLC system. The analysis was done using an LC-9A liquid cromatograph (Shimadzu, Kyoto, Japan), with an analytical reversed phase C18 column. An isocratic mobile phase was used (0.1 M sodium phosphate buffer pH 3.3 and methanol, 30 + 70) at a flow rate of 1 ml/min. Signal was monitored by a fluorescence detector (excitation and emission wavelengths set a 335 and 440 nm, respectively).

Results

A total of 41 samples of the 57 tested were found to be contaminated with *Fusarium* fungi. *Fusarium* was isolated from all corn and cottonseed meal samples, 80% (8/10) of the sorghum and soybean samples, 40% (2/5) of the wheat middlings samples, and 20% (1/5) of the rice samples. No *Fusarium* fungi could be isolated from sunflower seed meal.

The total number of *Fusarium* isolates found in the 41 contaminated samples was 53, from which 48 belong to section *Liseola* (90.6%). This section was represented by *F. verticilliodes* (32 isolates),

F. proliferatum (14 isolates) and *F. subglutinans* (2 isolates). These fungi were isolated from different substrates. *F. verticilliodes* was present on corn, sorghum, soybean, cottonseed meal, and wheat middlings, whereas *F. proliferatum* was found on corn, sorghum, and cottonseed meal. *F. subglutinans* was isolated from corn and soybean.

Table 1 summarizes the ability of producing fumonisins of the isolates. The majority of the *F. verticilliodes* (97.1%) and *F. proliferatum* (91.7%) isolates were capable of producing fumonisins. The levels of production of FB1 and FB2 by *F. verticilliodes* ranged from 5.6 to 25,846.4 mg/kg, and 3.4 to 7507.5 mg/kg for FB1 and FB2, respectively. Production of fumonisins by *F. proliferatum* isolates was much lower, with levels ranging from 6.9 to 3885.0 mg/kg for FB1 and 34.3 to 373.8 mg/kg for FB2. *F. subglutinans* isolates did not produce fumonisins under the conditions tested.

Discussion

Corn is one of the most prevalent grains colonized by *Fusarium* [8], and the majority of investigations that report *Fusarium* contamination on crops around the world, have been conducted mainly on this grain. Few reports were found that describe *Fusarium* isolation from other substrates than corn. Gamanya and Sibanda [9] evaluated the distribution of *Fusarium* in cereals and oilseeds from Zimbabwe [3]. They found that corn and sorghum were the most contaminated substrates, while sunflower samples were not contaminated. These results are in agreement with the results of the present study, where a high level of contamination was found in corn and in sorghum samples, and no contamination was found in sunflower seed meal.

In the present study, *Fusarium* fungi were present in 41 of the 57 samples analyzed (71.9%), being the predominant species those from section *Liseola* (*F. verticilliodes*, *F. proliferatum*, and *F. subglutinans*) and the most contaminated feedstuffs corn, sorghum and cottonseed meal. These results substantiate previous reports where *F. verticilliodes* has been the major *Fusarium* species isolated from corn and sorghum [10–12]. *F. proliferatum* and *F. subglutinans* have also been isolated in these substrates previously [13–15]. *F. verticilliodes* and *F. proliferatum*, the major species isolated in the present study, are the most common fumonisin producing fungi associated with corn [16]. In the present study, only these two species were capable of producing fumonisins, which is in agreement with the findings of Marasas et al. [16]. *F. verticilliodes* had a much higher toxigenic potential than *F. proliferatum*, and the predominant fumonisin species produced was fumonisin B1. These results also confirm results from previous studies [13, 14]. Furthermore, almost all strains of *F. verticilliodes* (97.1%) isolated were capable of producing fumonisins. This finding is in agreement with that from Almeida et al. [14] who found that all strains of *F. verticilliodes* isolated from Brazilian corn were able to produce fumonisins. The least common *Fusarium* species from section *Liseola* isolated in the present study, *F. subglutinans*, did not produce fumonisins. Several authors describe the scarce or null ability of *Fusarium subglutinans* to produce these toxins [17, 18]. However this fungus has been reported as a major producer of fusaproliferin and beauvericin, two mycotoxins with cytotoxic potential [19]. It would be worth to investigate the presence of these

Table 1. Production of fumonisins B1 (FB1) and B2 (FB2) by section *Liseola Fusarium* species isolated from different feedstuffs used in animal nutrition in Colombia

Fusarium species	Number of isolates	Fumonisin producers (number of isolates)	Frequency %	Fumonisin production (mg fumonisin/kg substrate)	
				FB1	FB2
F. verticilliodes	34	33	97.1	5.6–25,846.4	3.4–7507.5
F. proliferatum	12	11	91.7	6.9–3885.0	34.3–373.8
F. subglutinans	2	0	0	0	0

Detection limit: 50 μg/kg of each fumonisin.

toxins in the Colombian cereals found to be contaminated with *F. subaglutinans*.

An incidence of fumonisin contamination in Colombian corn and corn products of 65% was reported by Perilla and Diaz [6]. This high level of contamination suggests the existence of toxigenic *Fusarium* strains in the field, hypothesis that was confirmed in the present study. Fumonisins are capable of causing diseases in farm animals and humans [2]. In the present study, a *F. verticilliodes* strain with a very high production potential of FB1 (25,846.4 mg/kg) was isolated. The presence of highly toxigenic strains in the field obviously pose an important risk for animal health, and suggests that continuous monitoring of these mycotoxins in animal feeds and human foods based on corn and corn-derived products is necessary. The results of the present study indicate that there is a large *Fusarium* contamination in Colombian feedstuffs, particularly in corn, sorghum and cottonseed meal. The predominant *Fusarium* species isolated correspond to section *Liseola*, and include *F. verticilliodes*, *F. proliferatum*, and *F. subglutinans*. The majority of strains of *F. verticillioides* and *F. proliferatum* have the potential to produce fumonisins. The current findings, along with previous reports of fumonisin contamination in Colombian corn and corn products, indicate that fumonisins are important mycotoxins in Colombia. Continuous monitoring of these toxins in human foods and animal feeds is needed in order to prevent that batches contaminated with potentially toxic levels of fumonisins find their way into the food chain.

References

1. Hussein HS, Brasel JM. Review: Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 2001; 167: 101–134.
2. Diaz GJ, Boermans HJ. Fumonisin toxicosis in domestic animals: A review. Vet Hum Toxicol 1994; 36: 558–555.
3. Placinta CF, D'Mello JP, MacDonald AM. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim Feed Sci Technol 1999; 78: 21–37.
4. De Nijs MF, Notermans S. *Fusarium* moulds and their mycotoxins. J Food Safety 1996; 16: 15–58.
5. Diaz GJ, Céspedes AE. Natural occurrence of zearalenone in feeds and feedstuffs used in poultry and pig nutrition in Colombia. Mycotoxin Res 1997; 13: 81–87.
6. Perilla NS, Diaz GJ. Incidence and levels of fumonisin contamination in Colombian corn and corn products. Mycotoxin Res 1998; 14: 74–82.
7. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium* Species: An Illustrated Manual for Identification. University Park, PA: Pennsylvania State University Press, 1983.
8. Fandohan P, Hell K, Marasas WFO, Wingfield MJ. Infection of maize by Fusarium species and contamination with fumonisins in Africa. Afr J Biotechnol 2003; 2: 570–579.
9. Gamanya R, Sibanda L. Survey of *Fusarium verticilliodes* (*F. moniliforme*) and production of fumonisin B1 in cereal grains and oilseeds in Zimbabwe. Int J Food Microbiol 2001; 71: 145–149.
10. Julian AM, Wareing PW, Phillips SI, Medlock VF, MacDonald MV, Del Rio LE. Fungal contamination and selected mycotoxins in pre- and post-harvest maize in Honduras. Mycopathologia 1995; 129: 5–16.
11. Gonzalez HH, Martinez EJ, Resnik SL. Fungi associated with sorghum grain from Argentina. Mycopathologia 1997; 139: 35–41.
12. Rodríguez DB, Sabino M. Mycotoxin research in Brazil: The last decade in review. Braz J Microbiol 2002; 33: 1–11.
13. Magnoli CE, Saenz MA, Chiacchiera SM, Dalcero AM. Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains isolated from poultry feeds in Argentina. Mycopathologia 1999; 145: 35–41.
14. Almeida AP, Correa B, Mallozzi M, Sawaki E, Valente Soares L. Mycoflora and aflatoxin/fumonisin production by fungal isolates from freshly harvested corn hybrids. Braz J Microbiol 2000; 31: 321–326.
15. Labuda R, Tancinova D, Hudee K. Identification and enumeration of *Fusarium* species in poultry feed mixtures from Slovakia. Ann Agric Environ Med 2003; 10: 61–66.
16. Marasas W, Miller D, Riley R, Visconti A. Fumonisins – Occurrence, toxicology, metabolism and risk assessment. In: Summerell BA, Leslie JF, Backhouse D, Bryden W, Burgess L, eds. *Fusarium* Paul E. Nelson Memorial Symposium. St Paul Minessota APS Press, 2001; 122–137.
17. Nelson PE, Plattner RD, Shackelford DD, Desjardins AE. Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and by some related species. Appl Environ Microbiol 1992; 58: 984–989.
18. Reynoso MM, Torres AM, Chulze SN. Fusaproliferin, beauvericin and fumonisin production by different mating populations among the Giberella fujikuroi complex from maize. Mycol Res 2004; 108: 154–160.
19. Logrieco A, Moretti A, Fornelli F, Fogliano V, Ritieni A, Caiaffa MF, Randazzo G, Bottalico A, Macchia L. Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells, and IARC/LCL 171 human B lymphocytes. Appl Environ Microbiol 1996; 62: 3378–3384.

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