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Fumonisin occurrence in naturally contaminated wheat grain harvested in Argentina

Eugenia Cendoya [a], Maria P. Monge [b], Sofia A. Palacios [a], Stella M. Chiacchiera [b], Adriana M. Torres [a], Maria C. Farnochi [a], Maria L. Ramirez [a, *]

[a] Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Fco-Qcas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina
[b] Departamento de Química, Facultad de Ciencias Exactas Fco-Qcas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, 5800 Río Cuarto, Córdoba, Argentina

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ABSTRACT

A survey was carried out to determine fumonisin contamination in 135 common and 40 durum wheat samples collected during 2011 harvest season (non-FHB epidemic year) in the main wheat production area of Argentina using LC–MS/MS. A 93% of total samples showed fumonisin contamination, with levels ranging from 0.16 to 680.44 ng/g in common and from 0.15 to 1304.39 ng/g in durum wheat samples, respectively. FB_1 was the fumonisin most frequently found during the evaluated year. Twenty five wheat samples (15 common and 10 durum) were selected for a deoxynivalenol (DON) analysis among all the samples analyzed for fumonisin content using different contamination levels as selection criteria. DON contamination was present in 24 out of 25 wheat samples, the levels ranging from 50.60 to 28650 ng/g. Nine out of 25 wheat samples reached values higher than 1000 ng/g. However there was no correlation between fumonisin and DON contamination. This is the first report of natural fumonisin presence in common wheat grains in Argentina, as well as of DON co-occurrence in both types of wheat.

1. Introduction

Fumonisins are toxic fungal metabolites produced mainly by *Fusarium* species. Fumonisin B_1 (FB_1) is the most significant in terms of occurrence and toxicity. FB_1 can cause severe disorders in animals such as leukoencephalomalacia in horses (Marasas et al., 1988), as well as pulmonary edema syndrome and hydrothorax in pigs (Haschek et al., 1992); this toxin has also shown nephrotoxic, hepatotoxic and hepatocarcinogenic activities in rats (Wan Norhasima, Abdulamir, Abu Bakar, Son, & Norhafniza, 2009). Further, consumption of fumonisin-contaminated maize has been epidemiologically associated with esophageal cancer (Marasas, 2001) and neural tube defects in some human populations (Missmer et al., 2006). The International Agency for Research on Cancer (IARC) designated FB_1 in Group 2B as "a possible carcinogenic to humans" (IARC, 2002).

Several *Fusarium* species are able to produce fumonisins, but the two most important ones are *Fusarium verticillioides* and *Fusarium proliferatum*, which are common fungi associated with maize, but can also be isolated from other substrates such as wheat. Fumonisins are geographically widely distributed and their natural occurrence has been reported mostly in maize, but also in other grains and grain-based products such as wheat, wheat based foods, semolina, farro, bread and others (Scott, 2012).

Wheat is the most important cereal consumed by the Argentine population. In this country human consumption of products manufactured with wheat, either semolina (*Triticum turgidum* L. var. *durum*) or bread (*Triticum aestivum*), is much greater than for products made from other cereals (Food Balance Sheet, 2007; Pacin, Ciancio Bovier, Canoa, Taglieri, & Hernandez Pezzani, 2012). Durum wheat in Argentina is mainly used for pasta elaboration, with its production reaching 604,651 tons in 2011. Pasta national production reached almost 183,000 tons in 2011, and the consumption per capita was estimated at 7.9 kg/year. On the other hand, common wheat is mostly used for bread elaboration, breakfast cereals, cookies and cupcakes, its production was of 15,271,000 tons in 2011. It is remarkable that wheat flour consumption per capita in Argentina was estimated at 7.4 kg/habitant/month. Sixty three percent of the total wheat cultivation area is concentrated in Buenos Aires province (MAGyP, 2013).

The main pathogen associated with *Fusarium* Head Blight (FHB) wheat disease in common and durum wheat in Argentina is

* Corresponding author. Tel.: +54 358 4676429; fax: +54 358 4676231.
E-mail address: mramirez@exa.unrc.edu.ar (M.L. Ramirez).

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Table 1
Geographic locations and climatic conditions of the sampled grain storage companies and commercial fields.

Locations		Elevation (m)	Annual precipitation (mm)	Average annual temperature (°C)
Common wheat				
Junin	34° 31′ S, 60° 52′ W	81	953.52[a]	16[a]
Baigorrita	34° 45′ S, 60° 59′ W	80		
Alberti	35° 1′ S, 60° 15′ W	38		
Bragado	35° 7′ S, 60° 30′ W	50		
9 de Julio	35° 27′ S, 60° 52′ W	78		
Casares	35° 37′ S, 61° 22′ W	72		
Durum wheat				
La Dulce	38° 20′ S, 59° 0′ W	72	685.4	14.1
Balcarce	37° 45′ S, 58° 18′ W	130	758.9	13.3
Miramar	38° 10′ S, 58° 0′ W	50	776.5	14,3
Barrow	38° 20′ S, 60° 13′ W	120	688.5	14.7
Bordenave	37° 50′ S, 63° 1′ W	212	711.4	15.1

[a] Conditions for all the whole area between latitude 34°–37°S and longitude 58°–62°W.

Fusarium graminearum Schw, perfect stage *Gibberella zeae* (Schw) Petch (Lori, Sisterna, Haidukowsky, & Rizzo, 2003; Ramirez, Reynoso, Farnochi, & Chulze, 2006; Ramirez et al., 2007). Also deoxynivalenol (DON) has been reported in both types of wheat (Dalcero, Torres, Etcheverry, Chulze, & Varsavsky, 1997; González, Pacin, Resnik, & Martinez, 1997; Lori et al., 2003). However Ramirez, Oviedo, Farnochi, and Chulze (2006) carried out a mycological survey during a non-FHB epidemic year in common wheat, and found that the predominant *Fusarium* species were *F. proliferatum*, *Fusarium subglutinans* and *F. verticillioides*. Also, Palacios et al. (2011) found that the predominant *Fusarium* species isolated from durum wheat during a non-FHB epidemic year were *F. proliferatum*, *F. subglutinans*, *F. verticillioides* and *Fusarium equiseti*. Moreover, fumonisin contamination has been reported in both kinds of wheat and sub-products in many countries (Busman, Desjardins, & Proctor, 2012; Chehri, Jahromi, Reddy, Abbasi, & Salleh, 2010; Cirillo, Ritieni, Galvano, & Amodio Cocchieri, 2003; Jakšić et al., 2012; Roscoe et al., 2008) but not in Argentina, where fumonisins were reported only in durum wheat (http://www.ncbi.nlm.nih.gov/pubmed?term=Palacios%20SA%5BAuthor%5D&cauthor=true&cauthor_uid=21999326 Palacios et al., 2011).

Due to the importance of wheat in Argentinean population diet, and because it has been proposed, in a study in the Netherlands, that fumonisin intake occurs mainly via the intake of wheat and wheat-products (Bakker, Speijers, & Paulsch, 2003), the aim of this work was to determine the natural occurrence of fumonisins in common and durum wheat during a non-FHB epidemic year, and its possible co-occurrence with DON.

2. Materials and methods

2.1. Sample collection

One hundred thirty-five common wheat samples (harvest 2011) were obtained upon arrival from 6 local grain storage companies located in Junin, Bragado, Casares, Baigorrita, 9 de Julio and Alberti (Table 1), in Buenos Aires Province, the main wheat production area in Argentina. Wheat samples were taken from trucks, with a load capacity between 25 and 30 tons. Wheat was taken in six different truck positions using a vacuum sampling device in order to obtain an aggregate sample of 10 kg of randomized seeds. This sample was homogenized and sub-samples of 1 kg were taken, finely milled using a Romer mill (Romer, Union, MO, USA), thoroughly mixed and stored in bags in the dark at 4 °C until analysis. Also, forty durum wheat samples (500 g) were randomly collected during the 2011 harvest season in 5 different commercial fields located in the major durum wheat production area in Argentina, south of Buenos Aires province (Table 1). Samples were collected from each field and pooled; from this pool, a subsample of 500 g was taken. These subsamples were immediately stored at 4 °C until mycotoxin analyses. All wheat samples (common and durum) were asymptomatic i.e. without evident kernel damage.

2.2. Analytical reagents

The standard of DON was purchased from Sigma–Aldrich (Buenos Aires, Argentina). FB_1 and FB_2 stock solutions in acetonitrile:water (1:1) were provided by Biopure (Tull, Austria). Analytical grade reagents, HPLC grade solvents and HPLC grade water were purchased from Panreac Quimica S.A.U. (Barcelona Spain). MycoSep®227 Trich+ cleanup columns were obtained from Romer (Romer Labs Inc., Union, MO). Bond-Elut strong anion-exchange (SAX) cartridges were obtained from Agilent Technologies Inc. (Agilent Technologies Inc., Santa Clara, CA).

DON stock I solution of 500 μg/mL was prepared in methanol, a second stock was obtained diluting the stock I in methanol to achieve a final concentration of 100 μg/mL. They were stored in the darkness in glass-stoppered bottles under secure conditions at −20 °C. DON working standard solutions for HPLC calibration curve were prepared by dissolving diluting adequate amounts previously evaporated to dryness under nitrogen stream of the stock II solution in water:methanol (95:5), previously evaporated to dryness under nitrogen stream. Stock solution of FB_1 and FB_2 (50 μg/g) was diluted with acetonitrile:water (1:1) in order to obtain the appropriate working solutions, and were stored in darkness at −20 °C until LC–MS/MS analysis.

2.3. Wheat fumonisin extraction

The fumonisin analysis performed was based mainly on the method of Shephard, Sydenham, Thiel, and Gelderblom (1990) as described by Doko, Rapior, Visconti, and Schjoth (1995). Subsamples of about 100 g were finely ground in a Buehler laboratory mill and thoroughly mixed. Aliquots of the ground subsamples (25 g) were shaken with 50 mL of methanol:water (3:1) for 30 min and filtered through Whatman N° 4 filter paper. While the flow rate was maintained below 2 mL/min, 10 mL of the filtered extract was applied to a Bond-Elut strong anion-exchange (SAX) cartridge (Agilent Tecnologies Inc., Santa Clara, CA) fitted to a Supelco solid-phase extraction (SPE) manifold (Supelco, Bellefonte, PA), previously conditioned by the successive passage of methanol (5 mL) and methanol:water (3:1, 5 mL). The cartridge was then washed with methanol:water (3:1, 8 mL) followed by methanol (3 mL), and fumonisins were eluted with 0.5% acetic acid in methanol (14 mL). The elute was evaporated to dryness at 40 °C, under a moderate stream of nitrogen, and stored dry at 4 °C until HPLC LC–MS/MS analysis.

2.4. Fumonisin LC–MS/MS analysis

Fumonisin detection was performed using a Waters 2695 Alliance HPLC (Waters Corporation, Milford, MA, USA) equipped with a Waters Alliance 2685 pump, a Waters Alliance 2695 autosampler, a diode array detector Waters 2996 PDA interfaced to a Quattro Ultima Platinum tandem quadrupole mass spectrometer with electrospray ionization (ESI) source. An XBridge™ C18 column (3.5 μm, 2.1 × 150 mm) with a guard column of the same material (Waters, Milford, MA) was used. An isocratic chromatographic procedure was performed with 5 mM ammonium acetate in

Table 2
MS parameters used to investigate fumonisins by LC–MS/MS.

Compound	$[M + H]^+$	t_R/min	Product ions (m/z)	CV (V)	CE (V)
FB_1	722	5.4	334		57
			352	91	55
FB_2	706.3	17.6	318.5		51
			336.3	96	59
FB_3	706.3	9.3	318.5		51
			336.3	96	59

$[M + H]^+$, precursor ion; t_R, retention time; Product ions (m/z), daughter ions; CV (V), cone voltage; CE (V), collision energy.

methanol:water:acetic acid (39.5:59.5:1 v/v) as mobile phase. The flow rate was 0.18 mL/min. The column temperature was maintained at 35 °C. The nitrogen flow was adjusted to 109 and 726 L/h for cone and desolvation gases, respectively. Blank matrix extracts were investigated to confirm that no spectrometric interferences came from the matrix. Data acquisition and processing were performed using Mass Lynx V.4.1, Waters INC software. The interface was operated in a positive ion mode. Nebulization and desolvation temperatures were 150 and 200 °C, respectively. The capillary voltage was 3.00 kV. Multiple-reaction monitoring (MRM) was used for toxin determination. The precursor peak $[M + H]$ and two product peaks, monitored to accomplish both quantification and qualification criteria, as well as the retention times and the detector settings, are collected in Table 2. Trace m/z 722 > 352 was used for the quantification of FB_1, while 706 > 336 was used for both FB_2 and FB_3, respectively. Aliquots of 45 μL of sample extracts were injected into the HPLC unit. Four points of identification were used to identify FB_1, i.e. retention time, the precursor ion $[M + H]^+$ and two product ions (m/z 334 and 352). A calibration curve was obtained injecting 10 μL of a different mixed standard solution ($FB_1 + FB_2$) (0.25, 0.5, 1.0, and 2.0 μg/mL). Good linearity with a correlation coeffcient higher than 0.996 was obtained for the calibration range. The calculated instrumental LOD ($S/N = 3$) for FB_1 and FB_2 was 0.01 ng/g and LOQ ($S/N = 5$) was 0.05 ng/g, and the relative within-day and between-day standard deviations (% RSD) were 6.5.

A recovery experiment was performed in triplicate by spiking 25 g of ground fumonisin-free wheat samples with FB_1 and FB_2 toxins at levels of 10, 100, and 200 ng/g. Spiked samples were left overnight at room temperature to allow solvent evaporation prior to proceed with the extraction step. Mean recoveries for FB_1 and FB_2 were 98.98% and 94.9%, respectively.

2.5. Deoxynivalenol analysis

Twenty five wheat samples (15 common and 10 durum) were selected among all the samples analyzed for fumonisin content, using different contamination levels as the selection criteria. DON analysis was performed based mainly on the method proposed by AOAC (1995). Subsamples of about 100 g were finely ground in a Buehler laboratory mill and thoroughly mixed. Aliquots of the ground subsamples (25 g) were shaken with 100 mL of acetonitrile:water (84:16) for 30 min and filtered through Whatman N° 4 filter paper. While the flow rate was maintained below 2 mL/min, 8 mL of the filtered extract were transferred to a glass tube and then pushed all through MycoSep®227 Trich+. After that, 4 mL were removed to evaporate to dryness at 40 °C, under a moderate stream of nitrogen, and stored dry at 4 °C until HPLC analysis.

The HPLC system consisted of a Hewlett–Packard 1100 pump (Palo Alto, CA, USA) connected to a Hewlett–Packard 1046A programmable fluorescence detector and a data module Hewlett–Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a Luna™ C18 reversed phase column (100 × 4.6 mm, 5 μm particle size) connected to a guard column SecurityGuard™ (4 × 3.0 mm) filled with the same phase. The mobile phase consisted of methanol:water (12:88), at a flow rate of 1.5 mL/min. The detector was set at 220 nm with an attenuation of 0.01 AUFS. Injection volume was 50 μL and the retention time of DON was 800 s. Quantification was relative to external standards of 0.5–4 μg/mL in methanol:water (5:95). The LOQ was 50 ng/g.

A recovery experiment was performed in triplicate by spiking 25 g of ground DON-free wheat samples with DON toxin at levels of 500, 1000, and 1500 ng/g. Spiked samples were left overnight at room temperature to allow solvent evaporation before proceeding with the extraction step. Mean recoveries for DON was 88.5%.

Table 3
Incidence rate and concentration of fumonisin in common and durum wheat grain from Argentina.

	Common wheat		Durum wheat	
	FB_1	FB_2	FB_1	FB_2
Sample size[a]	131/135	69/135	31/40	17/40
Incidence (%)	97	51	77.5	42.5
Range (ng/g)	0.16–680.44	0.25–23.67	0.15–1304.39	0.43–46.94
Mean[b] (ng/g)	30.07	1.47	65.69	4.45

[a] Number of positive samples/Number of total samples.
[b] Mean concentration in total samples.

2.6. Statistical analysis

To determine differences among fumonisins content in relation to the wheat type and location, the non-parametric test Kruskal–Wallis one-way analysis of variance was used. Pearson correlation coefficient between fumonisins and DON content was calculated. All the statistical analyses were performed using SigmaStat for Windows version 2.03 (SPSS Inc.). Statistical significance was determined at the level $p < 0.05$.

3. Results and discussion

In this study, natural fumonisin contamination was present in 93% of all the wheat samples (162/175) analyzed. Total fumonisin amounts ($FB_1 + FB_2 + FB_3$) ranged from 0.15 to 1304.39 ng/g, with a mean level of 42.11 ng/g. A total of 131 out of 135 common and 31 out of 40 durum wheat samples were contaminated with fumonisins. Fumonisin B_1 was present in all positives samples, being the most abundant fumonisin found (high ratio FB_1/FB_2). Most common and durum wheat samples had FB_1 and FB_2 presence, while 36% of common wheat samples only had FB_1, and 13% of common wheat samples had FB_1, FB_2 and FB_3. On the other hand 29% of durum wheat samples contained FB_1, FB_2 and FB_3, whereas 16% contained only FB_1.

Table 3 shows the FB_1 and FB_2 contamination differences between common and durum wheat samples. In all samples of both types of wheat FB_1 incidence percentage and amounts were higher than FB_2. FB_1 mean level was 10 and 8 folds higher than FB_2 mean level in common and durum wheat samples, respectively.

Considering that FB_1 is the most important fumonisin, from toxicity point of view, Table 4 was constructed in order to classify FB_1 amounts in wheat samples into three categories: FB_1 < LOQ; LOQ < FB_1 < 100 ng/g; and FB_1 > 100 ng/g. Fumonisin B_1 levels mostly ranged from LOQ to 100 ng/g in 88.5% of common and 70% durum wheat samples, respectively. Only four common and 9 durum wheat samples showed no detectable fumonisin levels. Nine percent of common wheat samples showed levels higher than 100 ng/g, reaching 680 ng/g; while 7.5% durum wheat samples reached levels higher than 100 ng/g, reaching 1304 ng/g, the highest level found in this study.

Table 4
Distribution of fumonisin B_1 concentration in common and durum wheat grain from Argentina.

Ranges	Common wheat	Durum wheat
$FB_1 < LOQ$[a]	3%	22.5%
$LOQ < FB_1 < 100$ ng/g	88%	70%
$FB_1 > 100$ ng/g	9%	7.5%

[a] LOQ = 0.05 ng/g.

There are several reports in the literature about natural fumonisin contamination in common and durum wheat all over the world. Busman et al. (2012) studied 43 wheat samples with kernel black point disease from different geographic regions in the United States and analyzed fumonisin presence by LC–MS. In most of the samples (34) the levels of the three fumonisins (FB_1, FB_2 and FB_3) were below the LOQ (1 ng/g), Four samples had low levels (<10 ng/g) of FB_1, two samples had moderate levels, while three samples had high levels (4500 ng/g). Khosrow, Saeed Tamadoni, Kasa, Saeed, and Baharuddin (2010) have analyzed fumonisin contamination in 82 wheat grain samples collected from different supermarkets in Iran, where the overall results demonstrate that 68.2%, 42.6% and 31.7% of wheat samples showed contamination with FB_1, FB_2 and FB_3, ranging from 15 to 155 ng/g, 12–86 ng/g and 13–64 ng/g, respectively. In Argentina, Palacios et al. (2011) studied 55 freshly harvested durum wheat samples during 2007 and 2008 harvest seasons and found that during 2007 harvest season, 29 out of 30 samples (97%) were positive for fumonisins (B_1 and B_2) by HPLC and LC–MS/MS with levels ranging from 10.5 to 1245.7 ng/g. During the second year (2008) none of the 25 samples analyzed showed fumonisin contamination by HPLC analysis, but further LC–MS/MS analysis demonstrated that 6 samples showed traces of fumonisin contamination. In another survey Castella, Bragulat, and Cabanes (1999) analyzed natural fumonisin occurrence in 17 wheat samples by HPLC analysis and confirmed these results by LC–MS, finding that 8 out of 17 wheat samples showed FB_1 contamination ranging from 200 to 8800 ng/g.

It is remarkable that in the present survey almost all wheat samples were contaminated with fumonisins at levels comparable to those found in the studies described above. The analytical method used in this study, LC–MS/MS, is also important because others techniques have been demonstrated that frequently produce false positive results in wheat (Shephard, van der Westhuizen, Gatyeni, Katerere, & Marasas, 2005).

There are also, other researches about fumonisin contamination in wheat and subproducts, but their results are slightly different from those mentioned in the present study, because the natural incidence percentage and/or the amounts of fumonisin found there are lower. Castoria, Lima, Ferracane, and Ritieni (2005) confirmed, by LC–MS, the presence of natural FB_1 and FB_2 contamination, in 5 out of 8 Farro samples (*Triticum monococcum* L., *Triticum dicoccon* Schrank and *Triticum spelta* L.) in low concentrations, up to 70 ng/g for FB_1, and below the LOQ (12 ng/g) for FB_2. Recently Kushiro, Zheng, Nagata, Nakagawa, and Nagashima (2009) have found natural FB_1 contamination in 1 out of 47 wheat samples collected in various areas of Japan, by LC–MS/MS, at trace level. Also in China Wang et al. (2013) have found that among 20 wheat samples analyzed, only 3 showed FB_1 contamination in levels ranging from 94.4 to 379.93 ng/g where the determination was done by IC-ELISA and the confirmation by LC–MS/MS. There is a report on wheat grains from Tunisia where the authors have found only FB_2 contamination in 2 samples out of 20 analyzed by LC–MS/MS (Serrano, Font, Ruiz, & Ferrer, 2012).

It could be possible a high health risks result from maize simultaneously contaminated with FB_1 and combinations with other fusariotoxins, as reported along with DON, NIV and AFB_1 in Vietnam (Wang et al., 1995), with DON, NIV, ZEN and AFB_1 in Indonesia (Ali, Sardjono, Yamashita, & Yoshizawa, 1998), and with DON, NIV, other trichothecenes and ZEN in Korea (Sohn, Seo, & Lee, 1999). Little work has been done to study the co-occurrence of fumonisins and other mycotoxins in wheat grains all over the world (Mashinini & Dutton, 2006; Stankovic et al., 2012). Our co-occurrence results are summarized in Table 5, it shows that natural DON contamination was present in 24 out of 25 wheat samples and the levels ranged from 50.60 to 28650 ng/g. The average of positive samples was 3211 ng/g. Nine out of 25 wheat samples reached values higher than 1000 ng/g. However there was no correlation between fumonisin and DON contamination ($p > 0.05$). Samples containing high or even no natural fumonisin contamination had important DON levels. These results are comparable to others reported in the literature about natural co-occurrence of fumonisin and DON in bread and other varieties of wheat and wheat-based products. Stankovic et al. (2012) reported natural occurrence of FB_1 and its co-occurrence with DON and other mycotoxins on winter wheat. One hundred and three winter wheat samples were collected after four to six-month storage in family barns from different locations in Serbia and observed that all samples were mycotoxin positive, FB_1 was detected in 82.1% and 92.0% of all samples with ranges of 750–5400 ng/g and 750–4900 ng/g in 2005 and 2007, respectively. Moderate positive correlations were found between FB_1 and DON concentrations (r^2 0.56 in 2005 and r^2 0.54 in 2007). Recently Rodrigues and Naehrer (2012) studied the occurrence of DON, fumonisins and other toxins between January 2009 and December 2011 analyzing a total of 7049 corn, soybean/soybean meal, wheat, dried distillers grains with solubles (DDGS) and finished feed samples from the Americas, Europe and Asia. As a result no fumonisin was found in wheat and wheat bread samples in north America (7 samples) but DON average of positives samples was of 1029 ng/g (19 out of 25 samples). Something similar was found in northern Europe where no fumonisin was found, but they did find similar DON values. Two out of 40 samples had fumonisin in South America (positive average:

Table 5
Co-occurrence of fumonisin and deoxynivalenol in 15 common wheat and 10 durum wheat samples.

Type of wheat	Sample	Total fumonisin (ng/g)	DON (ng/g)
Common	101	nd[a]	1113.1
	82	2.7	627.2
	41	4.7	249.2
	62	5.7	286.5
	22	9.2	387.1
	50	23.0	214.3
	23	24.5	216.1
	113	100.1	757.4
	40	105.7	209.4
	5	209.4	188.3
	100	236.7	150.6
	61	266.3	12468.3
	1	328.6	221.8
	81	661.5	913.9
	49	680.4	865.7
Durum	106-7	nd	194.4
	101-7	nd	15141.3
	104-4	0.41	1731.5
	102-5	0.71	4117.9
	103-4	2.83	1261.9
	101-6	11.8	2871.0
	102-7	96.0	2727.7
	106-8	508.4	nd
	103-3	531.2	1458.1
	104-3	1304.4	336.3

[a] not detected, < LOQ.

1407 ng/g), and 9 out of 17 were contaminated with DON (positive average: 947 ng/g), and similar results were found in south and central Europe, north and south-east Asia, and Oceania.

Although there is no legislation for fumonisin levels content in wheat and wheat products, this legislation do exist in maize, where the maximum limits established for maize and sub-products for human consumption is 1000 ng/g in the European Union and from 2000 to 4000 ng/g in USA. It is remarkable that some reports about wheat fumonisin contamination exceeds (even one of the samples analyzed in this study) this limits. More surveys of fumonisin presence in wheat are necessary to establish, in the near future, regulation limits for this cereal devoted mainly to human consumption. Regarding DON legislation, the European Union sets limits of 1250 ng/g for unprocessed cereals, other than durum wheat, oats and maize, and 1750 ng/g for unprocessed durum wheat and oats, while USA and Canada legislation sets 2000 and 1000 ng/g respectively, in wheat and wheat subproducts for human consumption. Analyzing other reports it is noticeable that some wheat samples exceed these limits, although 9 out of 25 wheat samples analyzed in this study exceed 1000 ng/g.

During a non-FHB epidemic year, common and durum wheat can be contaminated not only with DON but also with fumonisins. Although in the present study fungi isolation was not done, the fact that the toxin was found in naturally contaminated samples demonstrates that fumonisins can be synthesized in common and durum wheat.

As a final conclusion wheat fumonisin contamination was found during non-FHB epidemic years and when conditions were appropriate. This is the first report of natural fumonisin occurrence in common wheat grains in Argentina, and its co-occurrence with DON during a non-FHB epidemic year. The co-occurrence is important because it is well known the toxic effect of each toxin separately, but is scarce the information related with the synergic effects of these toxins (Bracarense et al., 2012). Based on our results we consider that it is necessary to conduct further studies in order to determine the levels of fumonisin contamination on wheat subproducts such as flour, semolina and also on wheat manufactured food, such as pizza, noodles, pasta, cookies in order to determine the fumonisin intake in Argentina.

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References

Ali, N., Sardjono, T., Yamashita, A., & Yoshizawa, T. (1998). Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia. *Food Additives and Contaminants, 15*, 377–384.

Association on Official Analytical Chemist (AOAC). (1995). *Official method of analysis* (16th ed.). Arlington, VA, USA.

Bakker, M. I., Speijers, G. J. A., & Paulsch, W. E. (2003). *Risk assessment of fumonisin B_1 in the Netherlands*. Laboratory for Food and Residue Analysis, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, the Netherlands RIVM report 310301001/2003.

Bracarense, A. P., Lucioli, J., Grenier, B., Drociunas Pacheco, G., Moll, W. D., Schatzmayr, G., et al. (2012). Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. *Brazilian Journal of Nutrition, 107*, 1776–1786.

Busman, M., Desjardins, A. E., & Proctor, R. H. (2012). Analysis of fumonisin contamination and the presence of *Fusarium* in wheat with kernel black point disease in the United States. *Food Additives and Contaminants, 29*, 1092–1100.

Castella, G., Bragulat, M. R., & Cabanes, F. J. (1999). Surveillance of fumonisins in maize-based feeds and cereals from Spain. *Journal Agricultural and Food Chemistry, 47*, 4707–4710.

Castoria, R., Lima, G., Ferracane, R., & Ritieni, A. (2005). Occurrence of mycotoxin in farro samples from southern Italy. *Journal of Food Protection, 68*, 416–420.

Chehri, K., Jahromi, S. T., Reddy, K. R., Abbasi, S., & Salleh, B. (2010). Occurrence of *Fusarium spp.* and fumonisins in stored wheat grains marketed in Iran. *Toxins, 2*, 2816–2823.

Cirillo, T., Ritieni, A., Galvano, F., & Amodio Cocchieri, R. (2003). Natural co-occurrence of deoxynivalenol and fumonisins B_1 and B_2 in Italian marketed foodstuffs. *Food Additives and Contaminants, 20*, 566–571.

Dalcero, A., Torres, A., Etcheverry, M., Chulze, S., & Varsavsky, E. (1997). Occurrence of deoxynivalenol and *Fusarium graminearum* in Argentinian wheat. *Food Additive and Contaminants, 14*, 11–14.

Doko, M. B., Rapior, S., Visconti, A., & Schjoth, J. E. (1995). Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *Journal Agricultural and Food Chemistry, 43*, 429–434.

Food Balance Sheet. (2007). Available at http://www.faostat.fao.org/DesktopDefault.aspx?PageID=346&SelTab=4 Accessed 04.04.13.

González, H. H. L., Pacin, A., Resnik, S. L., & Martinez, E. (1997). Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinian wheat in 1993. *Mycopathologia, 135*, 129–134.

Haschek, W. M., Motelin, G., Ness, D. K., Harlin, K. S., Hall, W. F., Vesonder, R. F., et al. (1992). Characterization of fumonisin toxicity in orally and intravenously dosed swine. *Mycopathologia, 117*, 83–96.

IARC. (2002). Fumonisin B_1. In IARC (Ed.), *IARC (international agency for research on cancer) monograph on the evaluation of carcinogenic risk to human, some traditional herbal medicines, some mycotoxins, naphthalene and styrene* (Vol. 82; pp. 301–366). Geneve.

Jakšić, S., Abramović, B., Jajić, I., Baloš, M.Ž., Mihaljev, Ž., Despotović, V., et al. (2012). Co-occurrence of fumonisins and deoxynivalenol in wheat and maize harvested in Serbia. *Bulletin of Environmental Contamination and Toxicology, 89*, 615–619.

Khosrow, C., Saeed Tamadoni, J., Kasa, R. N. R., Saeed, A., & Baharuddin, S. (2010). Occurrence of *Fusarium spp.* and fumonisins in stored wheat grains marketed in Iran. *Toxins, 2*, 2816–2823.

Kushiro, M., Zheng, Y., Nagata, R., Nakagawa, H., & Nagashima, H. (2009). Limited surveillance of fumonisins in brown rice and wheat harvested in Japan. *Journal of Food Protection, 72*, 1327–1331.

Lori, G., Sisterna, M., Haidukowsky, M., & Rizzo, I. (2003). *Fusarium graminearum* and deoxynivalenol contamination in the durum wheat area of Argentina. *Microbiological Research, 158*, 1–7.

Marasas, W. F. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspective, 2*, 239–243.

Marasas, W. F., Kellerman, T. S., Gelderblom, W. C., Coetzer, J. A., Thiel, P. G., & Van der Lugt, J. J. (1988). Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort Journal of Veterinary Research, 55*, 197–203.

Mashinini, K., & Dutton, M. F. (2006). The incidence of fungi and mycotoxins in South Africa wheat and wheat-based products. *Journal of Environmental Science and Health, Part B, 41*, 285–296.

Ministerio de Agricultura, Ganaderia y Pesca de la Republica Argentina. (2013). Available at http//www.minagri.gob.ar Accessed 04.04.13.

Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Rothman, K. J., et al. (2006). Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. *Environmental Health Perspective, 114*, 237–241.

Pacin, A., Ciancio Bovier, E., Canoa, G., Taglieri, D., & Hernandez Pezzani, C. (2012). Effect of the bread making process on wheat flour contaminated by deoxynivalenol and exposure estimate. *Food Control, 21*, 492–495.

Palacios, S. A., Ramirez, M. L., Cabrera Zalazar, M., Farnochi, M. C., Zappacosta, D., Chiacchiera, S. M., et al. (2011). Occurrence of *Fusarium spp* and fumonisin in durum wheat grains. *Journal Agricultural and Food Chemistry, 59*, 12264–12269.

Ramirez, M. L., Oviedo, M. S., Farnochi, M. C., & Chulze, S. N. (2006). Mycological and mycotoxin survey on wheat during a non epidemic FHB year in Argentina. In *Proceeding of 9th European Fusarium Seminar.* September 12–22, (pp. 34). Wageningen, The Netherlands.

Ramirez, M. L., Reynoso, M. M., Farnochi, M. C., & Chulze, S. (2006). Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. *European Journal of Plant Pathology, 115*, 139–148.

Ramirez, M. L., Reynoso, M. M., Farnochi, M. C., Torres, A., Leslie, J., & Chulze, S. (2007). Population genetic structure of *Gibberella zeae* isolated from wheat in Argentina. *Food Additive and Contaminants, 24*, 1115–1120.

Rodrigues, I., & Naehrer, K. (2012). A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. *Toxins, 4*, 663–675.

Roscoe, V., Lombaert, G. A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., et al. (2008). Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey. *Food Additives and Contaminants, 25*, 347–355.

Scott, P. M. (2012). Recent research on fumonisins: a review. *Food Additives and Contaminants, 29*, 242–248.

Serrano, A. B., Font, G., Ruiz, M. J., & Ferrer, E. (2012). Co-occurrence and risk assessment of mycotoxins in food and diet from Mediterranean area. *Food Chemistry, 135*, 423–429.

Shephard, G. S., Sydenham, E. W., Thiel, P. G., & Gelderblom, W. C. A. (1990). Quantitative determination of fumonisins B_1 and B_2 by high performance liquid chromatography with fluorescence detection. *Journal of Liquid Chromatography, 13*, 2077–2080.

Shephard, G. S., van der Westhuizen, L., Gatyeni, P. M., Katerere, D. R., & Marasas, W. F. (2005). Do fumonisin mycotoxins occur in wheat? *Journal Agricultural and Food Chemistry, 53*, 9293–9296.

Sohn, H. B., Seo, J. A., & Lee, Y. W. (1999). Co-occurrence of *Fusarium* mycotoxins in mouldy and healthy corn from Korea. *Food Additives and Contaminants, 16*, 153–158.

Stankovic, S., Levic, J., Ivanovic, D., Krnjaja, V., Stankovic, G., & Tanci, S. (2012). Fumonisin B_1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. *Food Control, 23*, 384–388.

Wan Norhasima, W. M., Abdulamir, A. S., Abu Bakar, F., Son, R., & Norhafniza, A. (2009). The health and toxic adverse effects of *Fusarium* fungal mycotoxin, fumonisins, on human population. *American Journal of Infectious Diseases, 4*, 273–281.

Wang, D. S., Liang, Y. X., Chau, N. T., Dien, L. D., Tanaka, T., & Ueno, Y. (1995). Natural co-occurrence of *Fusarium* toxins and aflatoxin B_1 in corn for feed in North Vietnam. *Natural Toxins, 3*, 445–449.

Wang, Y.-K., Shi, Y.-B., Zou, Q., Sun, J.-H., Chen, Z.-F., Wang, H., et al. (2013). Development of a rapid and simultaneous immunochromatographic assay for the determination of zearalenona and fumonisin B_1 in corn, wheat and feedstuff samples. *Food Control, 31*, 180–188.