Research Article

Cooccurrence of Mycotoxins in Maize and Poultry Feeds from Brazil by Liquid Chromatography/Tandem Mass Spectrometry

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The objective of this study was to quantitatively evaluate mycotoxins in samples of maize and poultry feed produced in Brazil. A multimycotoxin method based on HPLC-MS/MS was applied to investigate the occurrence of toxical fungal metabolites in 119 samples collected from poultry feed factory integrated poultry farms: maize grain (74), poultry feed (36), and feed factory residue (9). Twenty of 101 fungal metabolites investigated were detected and quantified in the samples: aflatoxins B₁, B₂, G₁, and G₂, fumonisins B₁, B₂, and B₃, hydrolyzed fumonisin B₁, zearalenone, agroclavine, chanoclavine, deoxynivalenol, and nivalenol, and enniatin A, A₁, B, B₁, beaurevinic, kojic acid, and moniliformin. Most samples were contaminated with more than one mycotoxin. All samples were contaminated with fumonisins, with medians values of 1,840 μg/kg, 239 μg/kg, and 23,676 μg/kg for maize, feed, and factory residue samples, respectively. Surprisingly, beaurevinic was detected in more than 90% of samples. The median contaminations of aflatoxin and trichothecenes were low, near LOD values. The factory residue presented highest contamination levels for all mycotoxins. This is the first study dealing with agroclavine, chanoclavine, enniatin A, A₁, B, B₁, beaurevinic, and kojic acid contamination of maize and poultry feeds from Brazil.

1. Introduction

Brazil is the third major maize producer country of the world after United States and China. In particular, in 2012, it produced 71.5 million tons [1] which represents about 8.31% of the total world production [2]. Maize is produced all over the country; nevertheless, more than half of the national production is concentrated in three states. Considering the Brazilian production in 2012, Paraná is the major producer state with 23.4%, followed by Mato Grosso (21.9%) and Goias (11.5%). Brazilian maize production is destined mainly for animal feeding (82%) especially for poultry and pigs production [3].

The infection of cereal crops by phytopathogenic Fusarium fungi in the field as well as by fungi of the genera Aspergillus and Penicillium during processing and storage leads to the contamination of the food chain by toxic secondary fungal metabolites, the mycotoxins [4]. The most common mycotoxins in cereals are the Fusarium mycotoxins deoxynivalenol (DON), zearalenone (ZEA), and the fumonisins (FUM) and Penicillium and Aspergillus mycotoxins ochratoxin A (OTA) and aflatoxins (AFs) [5]. Toxicity,
metabolism and impact of these mycotoxins on human and animal health are already well-known and were subject for many reviews [6–9].

Research efforts to establish the magnitude of the mycotoxin occurrence in Latin America were initiated in the late 1960s after the outbreak of Turkey X disease. The bulk of mycotoxin research in Latin America has been conducted on maize and specifically on aflatoxins, although other toxins such as zearalenone, T-2 toxin, DON, penicillic acid, kojic acid, and ochratoxin have been detected in that cereal [10]. Recently, the Brazilian Regulation has changed including other mycotoxins beyond aflatoxins as well as decreasing the maximum tolerable levels for many commodities, especially for children's feed [11].

According to Salay and Zerlotti Mercadante [12], the incidence of aflatoxins, ochratoxin A, and zearalenone in maize cultivated in São Paulo State was much lower than the one from the northern and southern states. However, the incidence of fumonisins in maize seems to be widespread all over Brazil. Although the number of samples analyzed was small, the contamination of moniliformin, cyclopiazonic acid, sterigmatocystin, deoxynivalenol, and toxin T-2 seemed to not be relevant in Brazilian maize. The maize companies and feed industries consider expensive costs for the control of mycotoxins; therefore, few of them monitor other toxins than aflatoxins.

In a review dealing with mycotoxin research in Brazil between 1991 and 2000, Rodriguez-Amaya and Sabino [13] observed that thirty percent of the published articles surveyed mycotoxins in foods and feeds. AF occurrence in maize was low and occasional. As in other parts of the world, including other countries in Latin American, high contamination of maize and maize-based products with fumonisins (FBs) is widespread. Contamination with other mycotoxins, such as zearalenone (ZON), ochratoxin A (OTA), and trichotheccenes, is low.

The results from another study indicate a low occurrence of trichotheccenes mycotoxins in maize-based products commercialized in the city of São Paulo in spite of high levels of T-2 and HT-2 toxins found in one sample and show no immediate cause of concern. Nonetheless, more extensive surveys conducted for several years are advisable in order to furnish a more complete picture of the incidence of these toxins as well as other eventual (emergent) toxins in Brazilian products [14].

Maize is the major crop frequently exposed to the risk of contamination by all these mycotoxins. In particular, for maize, the European Commission has established maximum permitted levels for aflatoxins (AFB1, 2 µg/kg; total AFs, 4 µg/kg), OTA (5 µg/kg), ZON (100 µg/kg), and DON (1250 µg/kg); FBs (2000 µg/kg, FB1 + FB2) and limits for T-2 and HT-2 toxins are currently under discussion [15].

While most screening methods for mycotoxins addressed by legislation are based on immunoassays, unambiguous analytes confirmation can be easily achieved with mass spectrometric methods, such as gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). During the last few years, this technical and instrumental progress had also an increasing impact on the expanding field of mycotoxin analysis [16]. The development of multimycotoxin methods [17–19] enables analyzing a larger fraction of the 300–400 fungal metabolites which are currently recognized as mycotoxins.

The present work aimed to investigate mycotoxin contamination in a poultry maize-based feed chain in Brazil by using an HPLC-MS/MS multimycotoxin method.

2. Materials and Methods

2.1. Chemicals and Reagents. Methanol and acetonitrile (both LC gradient grade) were purchased from J.T. Baker (Deventer, The Netherlands) and ammonium acetate (MS grade) and glacial acetic acid (p.a.) from Sigma-Aldrich (Vienna, Austria). Water was purified successively by reverse osmosis and a Milli-Q plus system from Millipore (Molsheim, France). Details concerning standards of the investigated mycotoxins (which include trichothecenes, zearalenone derivatives, fumonisins, ergot alkaloids, aflatoxins, ochratoxins, and some other metabolites produced by Aspergillus and Penicillium species) are described by Sulyok et al. [19].

2.2. Collection of Samples. A total of 119 samples of maize grains, subproducts, and poultry feeds were collected from a poultry feed factory and integrated poultry farms in Paraná State, in Brazil, from 2005 to 2006. The samples obtained were as follows: (i) 74 samples of maize grains were randomly withdrawn from trucks (from each truck one sample of 10 kg) in the poultry feed factory reception and factory processing steps (3 kg); (ii) 36 samples of poultry feeds (3 kg) in the integrated poultry farms; and (iii) 9 samples of maize factory residues (10 kg each) collected in the discarding of first cleaning (after sieving).

All samples were ground in a TREU mill (7.5 CV, 1720 rpm) with a 20 mesh sieved at Embrapa Food Technology, homogenized during 15 min (Chopin MR10L), packed under vacuum, and frozen stored until analyzed.

2.3. Sample Preparation and LC-MS/MS Determination. To 5 g of milled sample, 20 mL of extraction solvent (acetonitrile/water/acetic acid 79 : 20 : 1, v/v/v) was added. Extraction, dilution, and analysis were performed as described by Sulyok et al. [19]. Detection and quantification were performed with a QTrap 4000 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with a Turbo Ion Spray electrospray. Ionization (ESI) source and an 1100 Series HPLC System were brought from Agilent, Waldbronn, Germany. Chromatographic separation was performed at 25°C on a Gemini C18 column, 150 × 4.6-mm.i.d., 5-µm particle size, equipped with a C18 4 × 3-mm.i.d. security guard cartridge (all from Phenomenex, Torrance, CA, US). Both eluents contained 5 mM ammonium acetate and were composed of methanol/water/acetic acid 10 : 89 : 1 (v/v/v; eluent A) or 97 : 2 : 1 (eluent B), respectively. After an initial time of 2 min at 100% A, the proportion of B was increased linearly to 100% within 12 min, followed by a hold-time of 3 min at 100% B and 4-min column reequilibration at 100% A. The flow rate of 1 mL/min ESI-MS/MS was performed in the multiple
reaction monitoring (MRM) modes both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte.

2.4. Recovery of Mycotoxins and Limits of Detection from Spiked Samples. The recovery was determined in duplicate by spiking in three different maize and feed sample. It spiked 0.5 g of sample in an open vial with appropriate amounts of a multianalyte working solution. The samples were subsequently stored for one day at room temperature to allow solvent evaporation. After this period, 2 mL of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) was added, and the same analytical procedure used as for the investigated samples was followed. Because all the investigated samples were naturally contaminated by fumonisins, the samples with the lowest levels were used for spiked experiments.

Limits of detection were calculated from the signal to noise ratios (LOD = 3 × S/N) of the respective multiple reaction monitoring (MRM) chromatograms deriving from the analysis of spiked samples.

3. Results and Discussion

The significance of mycotoxin contamination in food gained much attention over the past four decades. The coocurrence of mycotoxins had been already described in maize and others foods [20–24]. It can affect both the level of mycotoxin production and the toxicity of the contaminated grains resulting in additive and synergistic effects. The surveillance of mycotoxins in maize is important for further toxicological studies especially for poultry industry that could indicate which toxins are relevant for further investigations.

Quantitative analysis of raw extracts by LC-MS/MS can be disturbed by signal suppression due to matrix effects. As these were investigated in maize only for a smaller set of 39 analytes [17], recovery tests were performed by spiking three individual samples of both matrices (maize and poultry feed). As we have previously observed that matrix effects may also vary between individual samples of a given matrix [19], three different samples per matrix were spiked. Table 1 lists the spiking levels, the limit of detection (LOD), and average recoveries of the investigated mycotoxins. In general, the values obtained for the apparent recoveries were in good agreement with the results obtained earlier [17, 18]. In that aspect, apparent recoveries significantly lower than 100% occurred for fumonisins (due to incomplete extraction), aflatoxins (due to matrix effects), ergot alkaloids (due to incomplete extraction and epimerization in case of ergopeptides—note the difference between -ines and -inines), and some other polar analytes. However, the apparent recoveries of some additional analytes (such as gliotoxin, chaetoglobosin A, and chaetomin) were unexpectedly low, which indicates that any findings concerning a specific matrix or analyte should not be overgeneralized. For example, the apparent recoveries in feed were slightly lower in comparison to those in maize for just a few analytes (e.g. fumonisins, aflatoxins B1, and G2), although the former matrix is considered to be far more complex.

Nevertheless, it must be emphasized that matrix effects have to be carefully reevaluated for every analyte if the method is transferred to a new matrix. For most analytes, differences between the recoveries of individual samples of a given matrix were within the precision of the method.

Figure 1 shows total ions chromatogram (TIC) in positive and negative mode of 101 analytes analyzed by HPLC-MS/MS (ESI).

Table 2 gives the contamination range, median, and percentage of contaminated samples for each mycotoxin found in maize, poultry feed, and factory maize residue samples collected from the poultry feed factory reception and integrated poultry farms. All samples were contaminated with FB1, FB2, and FB3. The average contamination levels in poultry feed samples were lower than in maize samples, probably due to the processing or the adding of other ingredients beside maize. As reported by Soriano and Dragacci [25], Silva et al. [26], and Rodriguez-Amaya and Sabino [13] in their reviews on mycotoxins, the distribution of fumonisins is widespread. Compared with other grains, fumonisin contamination of maize is not only more frequent but also accompanied by larger toxin concentrations. The FB1 concentrations always exceeded FB2 and FB3 concentrations; this follows the general pattern of fumonisin contamination in maize and maize-based foods [26, 27]. In the present study, FB1 concentrations ranged from 32 to 6,000 µg/kg with a median of 1,300 µg/kg in maize, while this median is 185 µg/kg in poultry feed samples. For FB2 and FB3 the concentration ranges varied, respectively, from 9 to 2,450 µg/kg and from 7 to 820 µg/kg.

The median of total fumonisins (FB1 + FB2) in maize samples found in the present study (1,840 µg/kg) was below the maximum limit of fumonisins (FB1 + FB2) recently established by Brazilian regulation [11] for unprocessed maize (5000 µg/kg); only one maize samples analyzed exceed this limit reaching 8760 µg/kg. On the other hand, the poultry feed samples did not exceed the recommended value by the FDA (100,000 µg/kg) [28].

In addition, fully hydrolyzed fumonisin B1 (HFB1), also named aminopentol (AP1), was found in 9% of maize samples. Although numerous fumonisins have been characterized, FB1 is usually the most abundant in contaminated foods, except when maize has been treated with base to produce maize flour for tortillas, which hydrolyzes FB1 to AP1. AP1 also appears to have the same liver cancer promoting activity as FB1. Heretofore, these in vivo effects of AP1 have been somewhat puzzling because AP1 is less potent than FB1 as an inhibitor of ceramide synthase in vitro; AP1 is converted to an even more potent metabolite [29]. Our results on the frequency and range of FB1 and FB2 contaminations in maize are comparable with the data reported by studies conducted in other countries. Sydenham et al. [30] reported a similar incidence of fumonisins in maize meal from USA with slightly lower levels of contamination when compared with our results. It was noted that mean positive values of FB1 reaches 1048 µg/kg in USA maize meal and 138 µg/kg in South Africa maize meal, while FB1 contaminations in maize reaches 1655 µg/kg in Ghana and 6600 µg/kg in Argentina and Honduras. The same happened in Brazil; when it was observed that all the samples of maize flour from São Paulo
Table 1: Spiking levels (SL), limit of detection (LOD), and average apparent recoveries of spiked maize and feed.

| Toxins            | SL  | LOD  | Recovery (%) | Maize | Feed |
|-------------------|-----|------|--------------|-------|------|
|                   | µg/kg | µg/kg |              |       |      |
| **Fumonisins**    |      |      |              |       |      |
| Fumonisin B₁ (FB₁)| 504 | 8    | 78 ± 5       | 51 ± 3|      |
| Fumonisin B₂ (FB₂)| 505 | 7    | 76 ± 2       | 53 ± 2|      |
| Fumonisin B₃ (FB₃)| 50.0| 4    | 98 ± 5       | 74 ± 7|      |
| Hydrolysed fumonisin B₁ (HFB₁) | 54.9 | 17 | 74 ± 5 | 75 ± 4 |
| **Aflatoxins**    |      |      |              |       |      |
| Aflatoxin B₁ (AFL B₁) | 25 | 0.8 | 71 ± 5 | 77 ± 3 |
| Aflatoxin G₁ (AFL G₁) | 25 | 0.3 | 80 ± 3 | 75 ± 6 |
| Aflatoxin B₂ (AFL B₂) | 25 | 0.7 | 83 ± 2 | 65 ± 7 |
| Aflatoxin G₂ (AFL G₂) | 25 | 1   | 87 ± 2 | 69 ± 9 |
| **Ochratoxins**   |      |      |              |       |      |
| Ochratoxin A (OTA) | 20 | 1   | 82 ± 5 | 94 ± 3 |
| Ochratoxin B (OTB) | 20 | 1   | 85 ± 5 | 93 ± 5 |
| Ochratoxin α (OTα) | 11 | 3   | 77    | 84    |
| **Zearalenone**   |      |      |              |       |      |
| Zearalenone (ZON) | 100 | 0.4 | 86 ± 3 | 81 ± 3 |
| Zearalenone-4-sulfate | 0.4 | 3 | 86 | 95 |
| α-Zearalenol (α-ZOL) | 20 | 3 | 100 ± 9 | 90 ± 6 |
| β-Zearalenol (β-ZOL) | 20 | 4 | 75 ± 7 | 69 ± 7 |
| α-Zearalenol-glucoside | 120 | 0.8 | 94 ± 11 | 99 ± 8 |
| β-Zearalenol-glucoside | 120 | 1 | 110 | 99 |
| Zearalenone-4-glucoside | 20 | 5 | 94 ± 19 | 112 ± 5 |
| **Hexadepsipeptides** |      |      |              |       |      |
| Beauvericin (BEA) | 10  | 2    | 66       | 86    |
| Enniatin A (EA)   | 0.8 | 0.1  | 100 ± 8 | 87 ± 6 |
| Enniatin A₁ (EA₁) | 0.56| 0.15 | 100 ± 6 | 88 ± 9 |
| Enniatin B (EB)   | 0.53| 0.3  | 66 ± 6  | 67 ± 13|
| Enniatin B₁ (EB₁) | 1.51| 0.2  | 100 ± 3 | 73 ± 2 |
| Enniatin B₃ (EB₃) | 0.63| 0.04 | 93    | 87    |
| **Ergot alkaloids** |      |      |              |       |      |
| Agroclavine       | 3.4 | 0.2  | 49 ± 7   | 60 ± 16|
| Chanooclavine     | 50  | 0.4  | 79 ± 4   | 80 ± 7 |
| Festuclavine      | 50  | 0.15 | 83 ± 2   | 70 ± 6 |
| Elymoclavine      | 50  | 1    | 47 ± 4   | 47 ± 11|
| Elymoclavine fructoside | 50 | 4 | 29 ± 4 | 32 ± 8 |
| Oxidized eymoclavine | 50 | 3 | 46 ± 3 | 51 ± 9 |
| Ergine            | 1.08| 0.1  | 57 ± 4   | 54 ± 9 |
| Ergotamine        | 1.08| 0.7  | 24       | 37    |
| Ergocornine       | 1.08| 1    | 36       | 30    |
| Ergocorninine     | 0.692| 0.15 | 52       | 62    |
| Ergocristine      | 1.08| 0.3  | 23       | 33    |
| Ergocristinine    | 0.692| 0.2 | 59       | 61    |
| α-Ergocryptine    | 1.08| 0.2  | 30       | 36    |

Table 1: Continued.

| Toxins        | SL  | LOD  | Recovery (%) | Maize | Feed |
|---------------|-----|------|--------------|-------|------|
| α-Ergocryptine| 0.692| 0.1 | 67       | 69    |
| Ergometrine   | 2.17| 0.1  | 90       | 80    |
| Ergometrine   | 0.432| 0.07 | 58 ± 5    | 43 ± 4|
| Ergosine      | 1.08| 0.13 | 37       | 31    |
| Ergosine      | 0.692| 0.02 | 82       | 48    |
| Dihydroergotamine | 1.08 | 0.5 | 49       | 43    |
| Oxidized luol | 50  | 0.3  | 72       | 75    |
| Dihydrolys ergol | 50 | 0.2  | 79 ± 2   | 66 ± 4|
| Lysergol      | 50  | 1    | 76 ± 2   | 65 ± 5|
| **Trichothecenes** |      |      |              |       |      |
| Deoxynivalenol (DON) | 100 | 20 | 107 ± 5 | 99 ± 2 |
| 15-Acetyl-deoxynivalenol | 50.4 | 50 | 104 ± 7 | 116 ± 18 |
| 3-Acetyl-deoxynivalenol | 100 | 20 | 91 ± 5 | 96 ± 3 |
| Deoxynivalenol-3-glucoside | 20 | 15 | 120 ± 11 | 75 ± 7 |
| Deepoxydeoxynivalenol | 25.5 | 15 | 127 | 114 |
| Nivalenol (NIV) | 100 | 50 | 110 ± 16 | 90 ± 3 |
| Fusarenon X (F-X) | 101 | 50 | 100 ± 8 | 101 ± 5|
| Toxin HT-2 (HT2) | 100 | 20 | 99 ± 6 | 104 ± 4|
| Toxin T-2 (T2) | 100 | 20 | 101 ± 2 | 98 ± 2 |
| Neosolaniol (NEO) | 27 | 3 | 92 ± 6 | 95 ± 4 |
| Monoaacetoxyscirpenol | 10 | 2 | 111 ± 16 | 110 ± 13 |
| Diacetoxyscirpenol | 100 | 1 | 91 ± 4 | 98 ± 1 |
| Verrucarol     | 200 | 180 | 80 ± 17 | 95 ± 8 |
| Verrucarin A   | 10.7| 5   | 95       | 91    |
| Roridin A      | 13.7| 1   | 89       | 87    |
| T2-Tetraol     | 42.7| 20  | 76       | 89    |
| T2-Triol       | 42.7| 20  | 79       | 77    |
| **Others**     |      |      |              |       |      |
| Moniliformin (MON) | 204 | 81 | 87       | 112   |
| Kojic acid.    | 300 | 160 | 83 ± 3    | 64 ± 9 |
| Emodin         | 8.5 | 4   | 89       | 65    |
| Penicillic acid | 62.5| 20 | 50 ± 11  | 39 ± 7 |
| Brefeldin A    | 62.5| 60  | 95 ± 12  | 93 ± 7 |
| Roquefortin C  | 62.5| 4   | 65 ± 5   | 61 ± 9 |
| Gibberellic acid | 85.4 | 20 | 102     | 101   |
| Patulin (PAT)  | 64.2| 100 | 16       | 22    |
| Gliotoxin      | 42.7| 12  | 58       | 12    |
| Fumitremorgin C | 6.4 | 4  | 90       | 79    |
| Altenuene      | 8.5 | 6   | 89       | 102   |
| Alternariol    | 17.1| 2   | 91       | 82    |
| Alternariol monomethyl ether | 8.5 | 0.1 | 99 | 81 |
| Sterigmatocystin | 8.5 | 0.4 | 78 | 84 |
| Citrinin (CTN) | 25.6 | 30 | 90 | 122 |
| Cytochalasin A | 62.5 | 30 | 18 ± 7 | 25 ± 8 |
and found an average of 140 and 0.4 µg/kg and maximum levels of 1950 and 8.4 µg/kg, respectively. Concerning other *Fusarium* toxins, zearalenone (ZON) concentration reached 9.80 µg/kg; thus, it did not exceed the norm setting the maximum amount for the mycotoxin at 400 µg/kg in 2012 and 150 µg/kg in 2016. The maximum concentration of moniliformin was 170 µg/kg.

Among the mycotoxins most frequently found in the samples, there was also beauvericin (BEA) which was detected in 96% of maize samples with a media of 12 µg/kg and a maximum of 160 µg/kg and in 92% of feed samples in much lower levels (median of 3.6 µg/kg and maximum of 16.7 µg/kg). Enniatin concentrations in maize samples reached 0.1 µg/kg, 0.3 µg/kg, 5.0 µg/kg, and 1.3 µg/kg for enniatins A, A_1_, B, and B_1_, respectively. This is the first study detecting hexadepsipeptides in Brazilian maize. Uhlig et al. [34] identified this compound group as one of the two with the highest cytotoxicity of the *F. avenaceum* rice culture extracts in PK-15 cells. The cyclic hexadepsipeptides beauvericin (BEA) and enniatins are *Fusarium* secondary metabolites, which are less frequently investigated by routine methods. Beauvericin is toxic to several vertebrate and invertebrate cell cultures, inducing apoptosis, and is known to be a very potent channel-forming molecule inducing pores in biological membranes [35]. Enniatins are known for their phytotoxic and antimicrobial activity. Recently, BEA was found to exhibit phytotoxicity in tomato protoplasts—leading to protoplasts death and decrease in the ascorbate level [36].

Despite a relatively low amount of agroclavine (7.20 µg/kg) found in the samples, this is the first report in which a maize sample presented contamination by this ergot alkaloid. Agroclavine specifically modifies spatial memory in mice by impairing reproduction of conditioned navigation reflex in the Morris water test. This alkaloid modulates activity of the serotonin- and noradrenergic systems of the brain acting as antagonist and partially agonist of 2A-type serotonin receptors (5-HT2A receptors) and α1-adrenoceptor antagonist [37].

Unfortunately, there is only a limited number of surveys concerning *Fusarium* mycotoxins other than fumonisins in poultry feed mixtures, but they clearly show that this kind of feed should be of bigger concern from the mycotoxicological point of view.

Last column of Table 2 shows the factory residue samples contamination where the highest contamination levels were found for almost all toxins. In fact, the cleaning step was important to remove and discard broken maize grains, which were the most contaminated ones, although the mass fraction of this discard was obviously too low to cause any significant decrease of mycotoxins in the remaining grains. Contamination by fumonisins B_1_, B_2_, and B_3_ and BEA occurred in 100% of the samples with a prevalence of FB_1_.

Kojic acid was also detected in 100% of sample reaching concentrations of 344 µg/kg and median 28 µg/kg, respectively. In spite of no adverse effect of kojic acid (KA), its level has been established in chickens at 146 mg/kg in a 21-day feeding study, and the NOAEL (no observed adverse effect level) for thyroid tumor promoting effects of kojic...
Table 2: Mycotoxins and metabolites detected in maize, poultry feed, and factory residue by liquid chromatography-tandem mass spectrometry (LC-MS/MS). It shows the contamination range, median, and percentage of contaminated samples.

| Toxin                | Maize                  | Poultry feed             | Factory residue          |
|----------------------|------------------------|--------------------------|--------------------------|
|                      | Min. (μg/kg) | Median (μg/kg) | Max. (μg/kg) | % Min. (μg/kg) | Median (μg/kg) | Max. (μg/kg) | % Min. (μg/kg) | Median (μg/kg) | Max. (μg/kg) | % |
| Fumonisins           |                        |                          |                          |
| Fumonisin B₁ (FB₁)   | 32 1300 6000 100 50    | 185 1118 100 14085 17153 27145 100 |
| Fumonisin B₂ (FB₂)   | 9 540 2760 99 8        | 54 474 100 5927 7412 10867 100 |
| Fumonisin B₃ (FB₃)   | 7 190 820 nd           | 27 142 9 1422 1853 3090 100 |
| Fumonisin total (FB₁ + FB₂) | 41 1840 8760 100 58 | 239 1592 100 20012 23676 36040 100 |
| Hydrolysed fumonisin B₁ (HFB₁) | nd* 6.0 170 9 | nd nd nd 0 168 366 909 100 |
| Aflatoxins           |                        |                          |                          |
| Aflatoxin B₁ (AFLB₁) | nd nd 3.0 16 nd nd nd 0 nd nd 5.96 44 |
| Aflatoxin B₂ (AFLB₂) | nd nd nd 0 nd nd 0 nd nd 1.10 22 |
| Aflatoxin G₁ (AFLG₁) | nd nd 0.6 1 nd nd nd 0 nd nd 0.52 11 |
| Aflatoxin G₂ (AFLG₂) | nd nd 1.8 4 nd nd 1.43 14 1.0 1.73 2.51 100 |
| Trichothecenes       |                        |                          |                          |
| Deoxynivalenol (DON) | nd nd 30.0 4 nd 20 3 nd nd 0 |
| Nivalenol (NIV)      | nd nd 120.0 5 nd 67 17 nd nd 0 |
| Zearalenone (ZON)    | nd nd 9.8 12 nd 6.5 39 nd nd 0 |
| Hexadepsipeptides    |                        |                          |                          |
| Beauvericin (BEA)    | nd 12 160 96 nd 3.6 16.7 92 59 116 211 100 |
| Enniatin A (EA)      | nd nd 0.1 1 nd nd nd 0 nd nd 0 |
| Enniatin A₁ (EA₁)    | nd nd 0.3 12 nd nd 0.72 17 nd nd 0.27 22 |
| Enniatin B (EB)      | nd nd 5.0 34 nd nd 4.6 78 nd nd 3.21 44 |
| Enniatin B₁ (EB₁)    | nd 0.1 1.3 12 nd 12 67 nd nd 1.12 33 |
| Others               |                        |                          |                          |
| Kojic acid           | nd 12 230 65 nd 12 84 67 2.9 28 344 100 |
| Agroclavine          | nd nd 7.2 1 nd nd nd 0 nd nd 0 |
| Chanoclavine         | nd nd nd 0 nd nd nd 0 nd nd 3.95 33 |
| Moniliformin (MON)   | nd 170 8 nd 120 3 nd 220 336 89 |

*nd (not detected), nd < LOD.

Acid has been established at 15.5 mg/kg·day⁻¹ in mice and rats [38]. Takizawa et al. [39] provide strong evidence for a tumor-promoting behavior of a 2% KA in a rat diet. At this concentration, KA can be considered as a weak hepatocarcinogenic agent.

4. Conclusion

The HPLC-MS/MS method used in this study constituted an alternative to conventional techniques for mycotoxin analysis showing an ultralarge mycotoxin spectra, good sensitivity, rapidness, and applicability to complex matrices such as maize and maize-based feed. It could therefore be applied as routine method for different types of food as well as food production testing. The recovery was between 70 and 120% for 73 mycotoxins in maize while 65 mycotoxins in feed.

Concerning fumonisins, all samples were contaminated, and in some samples, contamination levels exceeded the maximum levels established by the EC. This would lead to increased risk to the consumer health from mycotoxins and emphasizes the urgency for establishing regular monitoring programs for mycotoxins in staple grains in developing countries. The results claim for an urgent regulation for fumonisins in Brazil.

This is the first study dealing with agroclavine, chanoclavine, enniatin A, A₁, B, beauvericin, and kojic acid contamination of maize and poultry feeds from Brazil. Although some mycotoxin content in maize was low, most samples were contaminated with more than one mycotoxin analyzed. This study suggests that more investigations are needed in this commodity since this survey only covers 2005/2006, and the occurrence may change from year to year implying that further monitoring of mycotoxin in Brazil is justified.

This result reinforces the need to know other mycotoxins in food products to verify the real extension of the mycotoxins in food and feed to protect public health.
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