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Mycotoxicological Analysis in Feed Used in Swine

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Abstract: Mycotoxins are a diverse group of chemicals that can affect many organ systems, mainly the liver, kidneys and nervous system. Mycotoxigenic fungi studied in this research were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp. and *Fusarium* sp. The objectives of this study understood the detection of mycotoxins and mycotoxigenic fungi in feeds sold in the city of Concordia (Santa Catarina). Analyses were performed according to the methodology of the Instituto Adolfo Lutz. The analysis included 18 samples in triplicate with a positive result for the presence of fungi in 13 of 18 samples (72.2%). The presence of *Fusarium* sp. was 9-13 samples (69.2%), *A. niger* (23.1%), *A. flavus* (7.7%), other unidentified species of fungi (46.2%). The presence of only one species of fungi occurred in 61.5% of samples while the presence of 2 species of fungi in 38.5% of samples. The presence of mycotoxins was detected in 11.1% of samples (deoxynivalenol). Thus, the method used to detect the presence of mycotoxins in feeds has proven reliable and moreover, characterized by being a quick detection test, serving as an important method of screening to test samples suspected of contamination by these substances.

Key words: Micotoxin, feed, fungi, deoxynivalenol, *Aspergillus niger*

INTRODUCTION

The term mycotoxins derives from the Greek word Mikes which means fungi and Latin word Toxicum meaning poison, i.e., mycotoxin is a toxin produced by fungi (Cover *et al*., 2010).

Mycotoxins are chemicals produced during secondary metabolism in filamentous fungi that contaminate food and animal feed, producing acute effects (mycotoxins) or chronic, usually, carcinogenic. Secondary metabolism comprises biochemical reactions whose products synthesis terminal does not play any role in the biology of self-producing fungus. Compared to other more developed organisms within the animal/vegetable scales, secondary metabolism is extremely well developed in micromicetos leading to a considerable range of molecules of similar chemical structure and functions, often toxic to other organisms (Teixeira *et al*., 2010).

The toxigenic fungi can grow both in the field during harvest storage, due to several factors that are denominated intrinsic (when inert substrate) and extrinsic factors (when inert conditions involving the substrate) (Teixeira and Fuentefria, 2009).

The climatic conditions of tropical countries (high temperature and humidity) allow the proliferation of fungi in agricultural products, especially grain, causing high levels of mycotoxins in foods from these regions (Oldoni *et al*., 2012). This can lead to difficulties in exporting these products with serious consequences for the economy of countries, such as Brazil, holds the balance of trade based on export of large amounts grain (Teixeira *et al*., 2010). The factors that favor the development of fungi and production of toxins are classified into 3 categories: Physical, chemical and biological factors: Relative humidity, temperature, light and mechanical damage among others (Cover *et al*., 2010).

Despite being 100 of known mycotoxins (over 300), the most frequent in food and feed are aflatoxins, zearalenone, ochratoxin A, citrine, trichothecenes, patulin, penicillic acid and ergot alkaloids (Oga *et al*., 2008).

This research aimed to the detection of mycotoxins and mycotoxigenic fungi in feed for swine, marketed in the city of Concordia (Santa Catarina), due to the importance that the suinoculture has on the local economy and the consequences caused by the mycotoxin when ingested by animals.

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MATERIALS AND METHODS

Sampling was conducted from May to October, 2013 and samples of feed (100 g) in storage silos from the city of Concordia (Santa Catarina) were collected in accordance with legal procedures prescribed by the laws within the monitoring program of mycotoxins in foods from the federal government.

In the laboratory, the samples were placed in sterile plastic bag, properly labeled with type of feed, silo number, place of origin of the sample, batch weight, date of collection, sample weight (kg), name and signature of the person has been collected.

Subsequently, the samples were homogenized and withdrawn four aliquots of 25 g of each sample for analysis by mycotoxicological and mycological by the quartering technique. The isolation and identification of the fungal flora was carried out at the Laboratory of Biochmistry and Toxicology, at the Instituto Federal Catarinense Campus Concordia, according to the techniques recommended by the Instituto Adolfo Lutz. For the detection of mycotoxins in solid samples, initially standardized extraction process was performed in the laboratory. The toxins were extracted with chloroform and identified in Thin Layer Chromatography (TLC) by comparing the fluorescence of the sample with known standards of mycotoxins (deoxynivalenol, ochratoxin A and aflatoxin B1). Data were analyzed using the statistical software Stats D+ (version 1.0) by descriptive statistical method.

RESULTS AND DISCUSSION

The results in mycological tests are shown in Table 1. They were analyzed 18 samples in triplicate with a positive result for the presence of fungi in 13 of 18 samples (72.2%). The presence of *Fusarium* sp., was 9-13 samples (69.2%), *Aspergillus niger* (23.1%), *Aspergillus flavus* (7.7%), unidentified species of fungi (46.2%) (Fig. 1). The fungi *Aspergillus fumigatus* and *Penicillium* sp., were not identified in the samples tested. The presence of only one specimen of fungi occurred in 61.5% of samples while the presence of 2 species of fungi in 38.5% of samples. The presence of mycotoxins was found in 11.1% of samples (samples 2-18) with the only detected mycotoxin deoxynivalenol.

Epidemiological studies show that the most common foods to be contaminated by Deoxynivalenol (DON) are cereals, predominantly grains like wheat, barley, oats, rye and maize (De Oliveira *et al*., 2002; Cover *et al*., 2010). Since 1990, the DON came to be regarded as a high priority in mycotoxin monitoring programs, however only a few countries determined allowable limits of this toxin in food (FAO, 2004).

Table 1: Identification of samples taken from the feed storage silos according to the presence of mycotoxins and fungal agents. Samples were collected from May to October, 2013

Samples	Fungal agent	Mycotoxin
A1	*Aspergillus niger*	Not detected mycotoxin
A2	*Fusarium* sp.	Not detected mycotoxin
A3	There was no fungal growth	Not detected mycotoxin
A4	*Aspergillus flavus* PUSF	Not detected mycotoxin
A5	*Fusarium* sp. PUSF	Not detected mycotoxin
A6	*Fusarium* sp.	Deoxynivalenol detected
A7	*Fusarium* sp. PUSF	Not detected mycotoxin
A8	There was no fungal growth	Not detected mycotoxin
A9	*Aspergillus niger* *Fusarium* sp.	Not detected mycotoxin
A10	PUSF	Not detected mycotoxin
A11	*Fusarium* sp. PUSF	Not detected mycotoxin
A12	There was no fungal growth	Not detected mycotoxin
A13	There was no fungal growth	Not detected mycotoxin
A14	*Fusarium* sp.	Deoxynivalenol detected
A15	There was no fungal growth	Not detected mycotoxin
A16	*Aspergillus niger* PUSF	Not detected mycotoxin
A17	*Fusarium* sp.	Not detected mycotoxin
A18	*Fusarium* sp.	Not detected mycotoxin

PUSF = Presence of Unidentified Species of Fungi

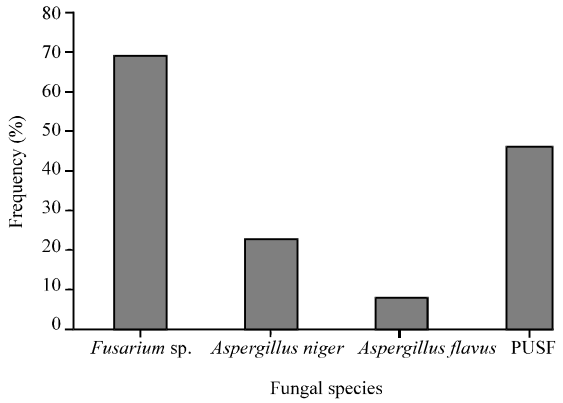

Fig. 1: Identification of samples taken from the feed storage silos according to the presence of fungal agents. Samples were collected from May to October, 2013; Presence of Unidentified Species of Fungi (PUSF)

In the literature, there are studies that prove the presence of mycotoxins in food and feed in amounts earlier those permitted by law. This is alarming, since the presence of mycotoxins can cause serious risks to human and animal health. De Oliveira *et al*. (2002) evaluated the incidence of DON between 1998 and 2000 on inputs used in baking, such as flour and bran in Minas Gerais (Brazil) and the result found was the presence of this mycotoxin in 32 of 47 samples analyzed. In a study done by Calori-Domingues *et al*. (2007), the presence of DON

in 100 wheat samples was detected with 50 national wheat (from the states of Sao Paulo, Parana and Rio Grande do Sul) and 50 imported (Argentina and Paraguay) wheat. Of the total samples tested, 94% of the national wheat and 88% of the imported wheat was contaminated with DON. In a similar study conducted in the years 2010-2011, there was 96.3% of the contamination of feed samples with fungi and in 7.4% of samples were positive for the presence of DON (Oldoni *et al*., 2012).

In addition to research DON, there are other studies in the literature regarding the detection of mycotoxins in feeds. In a study by Simas *et al*. (2007), the feeds analyzed from January, 2001 to February, 2004 were contaminated by Zearalenone (ZEA) in 15.93% of the samples. In another study, Sassahara *et al*. (2003) showed an index of 17% of samples of feeds contaminated by aflatoxins and ZEA.

The relevance of monitoring the detection of mycotoxins in feeds used in animal production is related to some aspects, such as feed intake and weight gain. Thus, the producer will have a lower feed conversion in live weight, resulting in an economic loss, besides the condemnation of the carcass due to concentration of mycotoxins detected in it because the international market is quite strict about the amount of mycotoxins in flesh. To reduce the presence of mycotoxins in the feed, the producer should be aware of some points, as the quality and origin of maize, as when maize is produced in regions of mild and wet weather the incidence of mycotoxin producing fungi found is greater. The storage is another important and determining factor in the occurrence of mycotoxins, the silo should be closed not allowing contact with the external environment and keep the humidity and temperature control, thus avoiding the proliferation of species of mycotoxigenic fungi. Furthermore, the presence of mycotoxigenic fungi not means the presence of mycotoxins, since mycotoxin production occurs in a slow manner, as are secondary metabolites (Cover *et al*., 2010).

CONCLUSION

These results indicated that the method is efficient for identification and detection of mycotoxins in feed samples. The presence of mycotoxin producing fungi does not mean the presence of the same, as in only one sample was positive for mycotoxins (DON). The quantification of mycotoxins requires a more advanced methodology, as for high performance liquid chromatography with an imperative for the risk assessment of the consumption of food or feed, based on the concentrations recommended by national and international laws quantification.

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