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Detection of multiple mycotoxin occurrences in soy animal feed by traditional mycological identification combined with molecular species identification

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ABSTRACT

Soy products are a main component of animal feed. Because mycotoxins may harm farm animals, undermining productivity and health, a mycological and toxigenic screening was carried out on 36 batches used in animal feed, collected in 2008, 2009 and 2010 in Italy. The investigated mycoflora of a subset of soy seed ($n = 6$) suggested that *Aspergillus* spp. and *Fusarium* spp. frequently colonize soy seeds. Aflatoxins, fumonisins and deoxynivalenol were detected in 88.9%, 72.2% and 30.6% of samples, respectively. Co-occurrence of at least two toxins was observed in 72% of cases. The molecular analysis of the *Fusarium* spp. population identified *Fusarium verticillioides* as potential producers of fumonisins, but no known deoxynivalenol producers were detected. It is suggested that the widespread presence of toxins can be due to non-optimal storing conditions of the feed. Moreover, our results suggest that mycotoxin thresholds should be adapted to consider the frequent case of toxin co-occurrence. This approach would better reflect the real toxigenic risk of feedstuffs.

1. Introduction

Mycotoxins are secondary metabolites produced by several fungi mainly belonging to the genera *Fusarium*, *Aspergillus* and *Penicillium*. Their global occurrence is considered to be a major risk factor, affecting human and animal health. It is estimated that up to 25% of the world's crop production is contaminated to some extent by mycotoxins [11,12,19,28,35,39]. Mycotoxin contamination may occur in the field before harvest, during harvesting, or during storage and processing. Environmental factors such as substrate composition, humidity and temperature govern the mycotoxin production and thus the degree of contamination of feed and food commodities. According to their various chemical structure, mycotoxins have a wide spectrum of toxicological effects. The nature and intensity of these effects is related to the dose and duration of exposure [18]. A major concern is chronic low-dose contamination that may even remain undetected, but may result in reduced weight gain, reduced reproduction and increased susceptibility to infections [27].

A large number of predominant mycotoxins are produced by the *Fusarium* fungi, probably constituting the most prevalent toxin-producing fungi found on cereals in the northern temperate regions of Europe, America and Asia [10]. There is compelling evidence for the implication of fusariotoxins in livestock disorders in different parts of the world. Outbreaks of fusariotoxicoses have been

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reported for Europe, Asia, New Zealand and South America. Moreover, chronic intake of these mycotoxins is reported on a regular and more widespread basis due to continuing global contamination of cereal grains and animal feed [12].

The most important fusariotoxins with respect to animal health and productivity are deoxynivalenol (DON) and fumonisins (FBs) [35]. Co-occurrence of *Fusarium* mycotoxins [9] has also become an important issue, with complex and indeterminate implications on animal health and welfare [35].

Exposure to these mycotoxins has been positively linked with a number of specific syndromes in farm livestock [6]. In spite of enhanced awareness of the debilitating effects of these mycotoxins and chronic exposure of farm animals to DON, the risk of exposure to fusariotoxins has not diminished in the past years, presenting a continuous hazard in continental Europe, Canada and the USA [12].

DON, also known as vomitoxin due to its emetic effects in pigs, is produced principally by *Fusarium graminearum* and *Fusarium culmorum* and is considered to be a major cause of economic losses due to reduced growth performance. The mode of action of DON is explained by its ability to bind to the 60S ribosomal subunit and to inhibit protein synthesis. Moreover, DON activates mitogen-activated protein kinases (MAPKs) and cause apoptosis through a process known as "ribotoxic stress response" [34]. DON exposure is generally associated with feed refusal, depressed feed intake, and possibly impaired immune function in many animal species [30]. The European Commission (EC) has published guidance levels for DON in products intended for animal feed. These guidance values for DON are 8 mg kg^{-1} in cereals and cereal products, 12 mg kg^{-1} in maize by-products and 5 mg kg^{-1} in complementary and complete feeding stuffs with the exception of feeding stuffs for pigs (0.9 mg kg^{-1}), calves (<4 months) and lambs (2 mg kg^{-1}) [16].

FBs are a group of mycotoxins produced primarily by *Fusarium verticillioides* and *Fusarium poliferatum*. The known forms are FB_1, FB_2 and FB_3, of which in particular FB_1 is considered the most common and harmful [7].

Due to their structural similarity to the sphingoid bases, FBs interfere with the *de novo* biosynthesis of ceramide and sphingolipid metabolism by specifically inhibiting sphingosine N-acyltransferase (ceramide synthase). Ceramide synthase inhibition leads to accumulation of the sphingoid bases (sphinganine and sphingosine) in tissues that exert proapoptotic, cytotoxic, and growth inhibitory effects [40].

FBs are likely involved in the incidence of many diseases such as leukoencephalomalacia in horses and lung edema in pigs, and they are also suspected to be a cause of esophageal tumors in certain human populations [36,6]. Regulatory authorities have established guidance levels for FBs (total including FB_1, FB_2 and FB_3) in animal feed. These guidance values, concerning complementary and complete feeding stuffs, are 5 mg kg^{-1} for horses, rabbit, pigs and pet animals, 10 mg kg^{-1} for fish, 20 mg kg^{-1} for poultry, calves (<4 months) and lambs and 50 mg kg^{-1} for adult ruminants (>4 months) and mink [16].

Animal exposure to a mixture of several mycotoxins from commercial feed, derived not only from *Fusarium* but also from *Aspergillus*, has been reported [3]. However, the occurrence of single-mycotoxin contamination seems to be rare [4]. Generally, data on possible interactions between mycotoxins upon ingestion are poor and often outdated. The effects on some intestinal parameters, including morphology, histology, expression of cytokines and junction proteins, induced by a combined exposure to DON and FBs, were investigated in piglets [4]. In the gastrointestinal tract of piglets for example, four different interactions at different levels of the intestine were reported for the combined effects of DON and FB_1: synergistic (number of goblet cells and eosinophils in the ileum), additive (expression of IL-10, TNF-α and adherent proteins), less-than-additive (histological lesions and expression of IFN-γ) and antagonistic effects (some cell populations such as goblet cells, plasma cells, eosinophils and lymphocytes in the jejunum and some cytokine expression such as IL-1β and IL-6) [4]. Synergistic and additive effects are potentially mediated by both DON and FBs [4] through the activation of MAPKs that are known to be involved in several physiological processes such as cell growth, apoptosis and immune response [13]. No explanations were found for the antagonistic effects [4].

An experimental interaction between aflatoxins (AFs) and DON was reported in broiler chickens, and additive toxicity was demonstrated on broiler performance and health [25].

AFs, a group of mycotoxins able to infect a wide range of crops, are produced by several different species of *Aspergillus*, including *A. flavus*, *A. parasiticus*, *A. nomius*, *A. pseudotamarii*, *A. flavus* being the most common. Four different forms of AFs have been identified, including AFB_1, AFB_2, AFG_1, AFG_2 [7]. AFs cause liver injury in a wide variety of animal species, and may have effects on production aspects (eggs, milk and weight gains) and on the immune system. AFs are also carcinogenic, teratogenic and mutagenic, with AFB_1 being the most toxic [36]. AFB_1 is responsible for hepatic cancer by inducing DNA adducts in the target cells that consequently undergo genetic changes [23]. The limits of AFB_1 established by the European Community concerning complete feeding stuffs, are 20 μg kg^{-1} for cattle, sheep and goats, 5 μg kg^{-1} for dairy animals, 10 μg kg^{-1} for calves and lambs and 20 μg kg^{-1} for pigs and poultry [17].

The study of mycological composition of feed may help guiding the detection of toxins [33] despite the impossibility to predict the amount of toxins produced, given the fact that mycotoxin production is linked to different environmental factors such as climate [38]. Few studies have so far focused on the potential contamination of soy by multiple types of mycotoxins [26]. The aim of this work was to assess the mycotoxigenic risk of soy samples used for animal feed by a combined study of the mycological composition of soy samples and their toxin content with special attention to potential co-occurrence of fusariotoxins.

2. Materials and methods

2.1. Sampling and mycological analysis

Soy samples were collected randomly from a feed manufacturing company located in the Lombardy region