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MYCOTOXINS IN ANIMAL FEEDSTUFFS OF THAILAND

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ABSTRACT

Contamination by mycotoxins in human food and animal feed is a worldwide problem because mycotoxins are naturally occurring toxic substances. Human ingestion of mycotoxins mainly occurs from the consumption of mycotoxins in residues and metabolites in animal-derived foods such as milk or meat products. Thailand is in the tropical area, thus it is hard to avoid mold-contaminated food and feed. So, the main purpose of this study was to investigate the possible incidence of mycotoxins in animal feeds. In this study a survey has been carried on the natural occurrence of mycotoxins in commercially available animal feeds. The study was divided into two parts, the first was to identify the genus of molds present in the feed samples, and the second was to determine the mycotoxins in these animal feeds. The results showed that all the samples were contaminated with molds. These molds are *Aspergillus* spp., *Penicilium spp*., *Fusarium* spp., and non-septate fungi. Determination of mycotoxins showed that aflatoxin B1 was detected in 23/25 samples (92%), and the average was 7.56 ppb. Ochratoxin A was detected in 3/10 samples (30%) in levels of 10.48, 11.14 and 12.35 ppb. Deoxynivalenol was detected in 13/15 samples (86%), and the average was 33.77 ppb. T-2 toxin was detected in all samples (10 samples), and the average was 6.91 ppb. Extent of mycotoxins contamination was determined from 10 samples. The results revealed that 3 out of 10 samples were contaminated with 4 mycotoxins (aflatoxin B1, ochratoxin A, deoxynivalenol and T-2 toxin), and 7 out of 10 samples were contaminated with 3 mycotoxins (aflatoxin B1, deoxynivalenol and T-2 toxin). The results obtained in this study suggest a high risk for human health because of the possibility of indirect exposure through meat and other products from animals consuming contaminated feedstuffs.

KEYWORDS: mycotoxins, animal feed

1. INTRODUCTION

Mycotoxins are low molecular weight secondary metabolites produced by certain strains of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium*, which invade crops in the field and may grow on foods during storage under favorable conditions of temperature and humidity. They are regularly implicated in toxic syndromes in animals and humans [1, 2]. Such mycotoxins are usually prepared from cereals or oilseeds and products derived from them. Mycotoxins have been detected in various food commodities from many parts of the world and are presently considered as one of the most dangerous contaminants of food and feed [3]. Adverse effects on animal health and production have been recognized in intensively

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farmed animals such as poultry, swine and cattle as a consequence of the consumption of high levels of cereals and oilseeds in the diet [4, 5]. Animals may have varying susceptibilities to mycotoxins depending on physiological, genetic and environmental factors. There is increasing evidence that intensively farmed animals are being exposed to multiple mycotoxins both in the field and through compounded feedstuffs. The capacity of some mycotoxins to alter normal immune function when present in foods at levels below observable toxicity is of particular interest. This may predispose food animals to infectious disease and could result in decreased productivity as well as increased animal-to-human transmission of pathogens.

Most mycotoxins such as aflatoxin B_1, T-2 toxin and ochratoxin A inhibit protein synthesis. This inhibition may not be the primary mechanism involved in their immunotoxic effects. They may have selective effects on various target organs, affect membranes or interfere with macromolecular synthesis and function. They can directly or indirectly influence immunological functions. Some of the mycotoxins are neurotoxic or cause other organ pathology, and these compounds may activate endocrine mechanisms. For example, the stress-induced release of corticosteroids inhibits immune function [6, 7]. Aflatoxins are potent liver toxins, and their effects in animals vary with dose, length of exposure, species, breed, and diet or nutritional status. Acute aflatoxicosis in cattle has been thoroughly described. Clinical signs consist of reduced feed consumption, dramatic drops in milk production, weight loss, and liver damage [8]. Chronic exposure of a dairy herd to aflatoxin-contaminated corn (120 ppb) resulted in severe herd health problems, including the birth of small, unhealthy calves, diarrhea, acute mastitis, respiratory disorders, prolapsed rectum, hair loss, and reduced feed consumption [9]. Ochratoxin A is a nephrotoxin. However, it can cause damage to the liver of animals, particularly at higher doses. Smith and Moss [10] reported that liver damage in broiler chicks was present in concert with nephrotoxicity. Deoxynivalenol has been found to induce vomiting, headache, fever and nausea in humans [2]. T-2 toxin, deoxynivalenol and ochratoxin A have been found to inhibit the proliferation of bovine peripheral blood mononuclear cells and induced apoptosis [11]. Owing to these adverse health effects of mycotoxins a survey was conducted in animal feedstuffs of Thailand.

2. MATERIALS AND METHODS

2.1 Sampling and mold determination

Animal feeds samples from commercial sources were collected from various places. A minimum of 1 kg sample was collected. Mold contamination was determined by the agar plating method on malt extract salt agar. For detection of mycotoxins, the samples were stored under cool conditions until analysis.

2.2 Reagents

All reagents were of analytical grade. Solvents used for chromatography were of HPLC grade. Mycotoxin standards, aflatoxin B1, B2, G1, G2, ochratoxin A, T-2 toxin and deoxynivalenol were purchased from Supelco, USA. The immunoaffinity columns for aflatoxin and ochratoxin A were purchased from Vicam L.P., USA. The quantitative ELISA test kits for T-2 toxin and deoyxnivalenol were purchased from Veratox, Neogen, USA.

2.3 Extraction and clean up of the samples for HPLC/ ELISA determination

All the feed samples were finely ground and 50g aliquots weighed before following the procedures shown in Table 1.

Table1 Sample extraction and cleanup procedure for mycotoxins from animal feed

Mycotoxins	Aflatoxin	Ochratoxin A	T-2 toxin	Deoxynivalenol
Sample weight and extraction solvent	25g sample + 5g NaCl + 100ml methanol (80%)	25g sample + 5g NaCl +100ml methanol (80%)	5g sample + 25ml water:methanol shake vigorously 3 min	10g sample + 50 ml deionized water, shake vigorously 3 min
Dilution of filtered extract	10 ml extract + 40 ml deionized water	10 ml extract + 40 ml deionized water	5ml extract through Whatman#1	5ml extract through Whatman#1
Column wash	20 ml deionized water	10 ml PBS and 10 ml deionized water		
Eluting solvent	2 ml methanol	2 ml methanol		

2.4 HPLC determination
Aflatoxin and ochratoxin were determined following the procedures described in Table 2.

Table2 HPLC conditions for the determination of mycotoxins in animal feed

HPLC conditions	Aflatoxin	Ochratoxin
Column	ODS-3 150x4.6 mm	ODS-3 150x4.6 mm
Mobile phase	Methanol:water:acetonitrile(45:55:5)	Water:acetonitrile:acetic acid (99:99:2)
Flow rate(ml/min)	1 ml/min	1 ml/min
Detector	Fluorescence detector Ex.360nm, Em.440 nm	Fluorescence detector Ex.333nm, Em.477nm
Injection volume	10 μl	10ul

2.5. ELISA Determination
Determination of T-2 toxin and deoxynivalenol, after extraction and filtering as in Table1, the sample is ready for testing by following the test kit direction and reading the result at 650 nm.

3. RESULTS AND DISCUSSION

Examination for molds in the feed samples showed that all the samples were contaminated with molds. These molds are *Aspergillus* spp., *Penicilium* spp., *Fusarium* spp., and non-septate fungi. The mold incidence ranged from $5x10^2$-$2x10^6$ CFU/g. According to these results it is very clear that some of the feed contaminants may be toxigenic, mainly *Aspergillus* spp., *Penicilium* spp., and *Fusarium* spp. Determination of mycotoxins showed that aflatoxin B1 was detected in 23/25 samples (92%), and the average concentration was 7.56 ppb. Ochratoxin A was detected in 3/10 samples (30%) at levels of 10.48, 11.14 and 12.35 ppb. Deoxynivalenol was detected in 13/15 samples (86%), and the average was 33.77 ppb. T-2 toxin was detected in all samples (10 samples), and the average was 6.91 ppb. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species [10]. Often more than one mycotoxin is found on a contaminated substrate [10]. So, multi-mycotoxins contamination was determined from 10 samples. The results revealed that 3/10 samples were contaminated with 4 mycotoxins (aflatoxin B1,

ochratoxin A, deoxynivalenol and T-2 toxin),and 7/10 samples were contaminated with 3 mycotoxins (aflatoxin B1, deoxynivalenol and T-2 toxin). The concentration of mycotoxins did not correlate with demonstrated mold contamination; this may be due to the toxin-producing fungi being killed or removed during processing, but the mycotoxins remaining in the final product. Mycotoxins may be produced when fungal growth occurs, however the presence of these molds on food or feed do not automatically mean the presence of mycotoxins because they require many suitable factors such as substrate, pH, moisture and temperature [10]. On the other hand, the absence of these molds does not guarantee that the food or feed is free of mycotoxins.

The results obtained in this study suggest a high risk for human health because of the possibility of indirect exposure through meat and other animal products. It has been estimated that 25% of the world's crops may be contaminated with mycotoxins and the worldwide contamination of foods and feeds with mycotoxins is a significant problem [12]. Since 1994, many countries have developed regulations for aflatoxin, ochratoxin A and deoxynivalenol in animal feeds and human food but the regulation has varied by country. For example, the European Union set the maximum levels of aflatoxins in agricultural commodities at 4 ppb with aflatoxin B1 at 2 ppb, but in the US, the Federal Food Drug and Cosmetic Act has regulated aflatoxin in foods and feeds at 20 ppb maximum [12]. Efforts have continued internationally to establish guidelines to control mycotoxins. So, the further studies are required to determine mycotoxins residues in meat and animal products.

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