Mycotoxin Determinations on Animal Feedstuffs and Tissues in Western Canada

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ABSTRACT

Results of examination of specimens of plant or animal origin for various mycotoxins are presented. Analyses for aflatoxins and ochratoxins were most frequently requested, usually on the basis of visible mouldiness. Aflatoxin B_1 was found in one of 100 specimens at a level of 50 ppb in a sample of alfalfa brome hay. Ochratoxin A was detected in seven of 95 specimens comprising six samples of wheat at levels between 30 and 6000 ppb and one sample of hay at a level of 30 ppb. An overall detection rate of 4.2% involving significant levels of potent mycotoxins suggests that acute or chronic mycotoxicoses may occur in farm livestock or poultry more frequently than presently diagnosed.

RÉSUMÉ

L'auteur présente les résultats de la recherche de mycotoxines dans des échantillons d'origine végétale et animale. Il procéda le plus souvent à la recherche d'aflatoxines et d'ochratoxines, à cause de la présence de moisissures visibles dans les échantillons suspects. La recherche de l'aflatoxine B_1, dans 100 échantillons, se solda par la découverte de 50 ppb de cette substance dans un échantillon de foin de luzerne. Par ailleurs, la recherche de l'ochratoxine A, dans 95 échantillons lui permit d'en déceler entre 30 et 6000 ppb, dans six échantillons de blé, et 30 ppb dans un échantillon de foin. Le fait d'extraire de 4.2% des échantillons une quantité appréciable de mycotoxines puissantes laisse supposer que des mycotoxicoses aiguës ou chroniques affectent les animaux de la ferme et les volailles plus souvent qu'on le diagnostique actuellement.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by moulds. The diseases caused by these metabolites whether by contact or by inadvertent ingestion of the toxin when present in feeds or foods are called mycotoxicoses (9). This group of diseases may be divided into three classes according to the degree of knowledge of the clinical entity and mycotoxin as shown in Table I. Clinical diagnosis is often difficult due to the lack of adequate diagnostic criteria and mycotoxicoses may be suspected only when all other known causes have been eliminated. The degree of visible mould infestation is not necessarily an indication of the level of toxin production. Moreover mouldiness may not be apparent after milling or processing. The mycotoxins contain a variety of chemical types producing acute or chronic effects on liver, kidney, nervous system, skin, blood, gastrointestinal tract or reproductive organs

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TABLE I. A Summary of Known and Suspected Mycotoxicoses, after Pier (11)

Fungal Toxicoses		
Known	Suspected	
Disease Entity and Toxin Known	Disease Thought to be Caused by Toxin	Toxin with Poorly Defined Field Syndrome
Ergotism	Fescue foot (trichothecenes)	Ochratoxin A (hepato-, nephro-toxic)
Facial eczema	Mouldy corn toxicoses (trichothecenes)	T-2 toxin (skin, haemopoietic system)
Aflatoxicosis	Porcine nephropathy (ochratoxin A, citrinin)	Rubratoxins (hepato-, nephro-toxic)
Alimentary toxic aleukia		
Oestrogenic syndrome		
Slobber factor		
Stachybotrotoxicosis		
Tremortin A		

and may also be carcinogenic, mutagenic or teratogenic depending upon mycotoxin, level in feed, period of exposure and animal species.

Every year moulds infest stored grain in Western Canada. Grain that is mouldy must be downgraded and is hard to sell, thus causing financial loss (14). Prairie farmers harvested a large volume of damp grain in the fall of 1968. By the time winter set in some had been dried but millions of bushels remained in farm storage and piled in fields under less than optimal storage conditions (5). The situation was aggravated by the surplus of grains in subsequent years and many farmers moved into the raising of cattle in order to utilize the surplus. Concern at the effects of mycotoxins on livestock following the feeding of mouldy grains led to the establishment in 1971 of mycotoxin testing facilities in this laboratory as part of its overall interest in veterinary toxicology. The results of mycotoxin analyses from 1971 to date are now reported.

MATERIALS AND METHODS

This study is based upon the analysis of 190 specimens submitted to this laboratory from Western Canada for mycotoxin examination due to concern about mould infested feedstuffs or suspected mycotoxicoses. With the exception of the procedure for trichothecenes, examination was by qualitative and quantitative analytical chemical methods using authentic mycotoxin standards for reference[1,2]. Methylated or ethylated derivatives were formed to confirm identity. A qualitative bioassay was used for the trichothecenes. The mycotoxins examined for and the procedures used are given below.

i) Aflatoxins were extracted with chloroform-water-diatomaceous earth, followed by cleanup on a silica gel column eluted with methanol-chloroform and characterized by silica gel thin layer chromatography (TLC) (1). Ten specimens were also examined by a rapid qualitative method involving extraction with methanol-water, cleanup with benzene and characterization by fluorescence spectroscopy (8).

ii) Citrinin extraction was by methanol-water-hexane acidified to pH 2, with cleanup by liquid-liquid partitioning into chloroform. Characterization was by silica gel TLC (12).

iii) Ergot alkaloids were obtained by acid extraction with partition by liquid-liquid chromatography and characterized employing silica gel TLC (2).

iv) Ochratoxin acids and esters were extracted with chloroform-aqueous phosphoric acid. Acids were entrapped on an aqueous sodium bicarbonate-Celite 545[3] column, esters and fats removed with hexane chloroform and acids eluted with formic acid chloroform. Esters were entrapped on a methanol-aqueous sodium bicarbonate-Celite 545 column, fats removed by hexane-benzene and esters eluted with formic acid-hexane-benzene. Ochratoxins were charac-

[1]Calbiochem, La Jolla, California, USA.

[2]Makor Chemicals Ltd., Jerusalem, Israel.

[3]Fisher Scientific Co., Ltd., Edmonton, Alberta.

TABLE II. Summary of Results of Mycotoxin Analyses on 190 Specimens

Type of Specimen	Number Received for Analysis	Number Positive for Mycotoxin	Percent
Corn	6	0	0
Feeds	36	0	0
Barley, wheat, oats	68	6	8.8
Legumes, hay, forage	70	2	2.9
Liver, kidney	10	0	0
Total	190	8	4.2

TABLE III. Summary of Results of 213 Mycotoxin Analyses

Mycotoxin	Detection Limits (ppb)	Number of Analyses in which Mycotoxin		
		Detected	Range (ppb)	Not Detected
Aflatoxin B_1	10 — 150	1	50[a]	99
Citrinin	400	0		2
Ergot alkaloids	1,000	0		1
Ochratoxin A	30 — 200	7	30-6,000[b]	88
Trichothecenes	1,000	0		6
Zearalenone	500	0		9
Total		8		205

[a] one sample of alfalfa brome hay
[b] six samples of wheat, one sample of hay

terized on silica gel TLC using a spectrophoto-fluorometer[4] (10).

v) Trichothecenes were extracted in ethyl acetate with cleanup by washing with hexane, followed by liquid-liquid partitioning into methanol-water (18, 19). Characterization was by bioassay using the guinea pig skin test (20).

vi) Zearalenone was extracted with acetonitrile-water, defatted with isooctane and transferred by liquid-liquid partitioning into chloroform. Characterization was by silica gel TLC (15).

The detection limits for the six mycotoxins varied with the type and amount of specimen submitted and the specific procedure (Table III). In general, samples containing plant tissue, e.g. legumes and mixed feeds, gave the least sensitivity.

RESULTS

The types of specimen received for mycotoxin analysis are summarized in Table II. The results of 213 analyses are summarized in Table III. Aflatoxin B_1 was found in one sample of alfalfa brome hay at a level of 50 ppb. Ochratoxin A was detected in six samples of wheat at levels ranging from 30 to 6,000 ppb and in one sample of hay at a level of 30 ppb.

DISCUSSION

Summarizing previous reports Feuell (6) has noted certain useful diagnostic features that characterize outbreaks of mycotoxicoses: (a) the diseases are not transmissible from one animal to another, (b) the disease is often seasonal as particular climatic sequences may favour toxin production by the mould, (c) treatment with drugs or antibiotics usually has little effect on the course of the disease, (d) the disease is usually associated with a specific food or feedstuff and (e) the suspected food or feedstuff usually shows signs of mouldiness. However mouldiness may not be apparent in ground feed.

The clinician is hampered by the lack of adequate diagnostic criteria. Laboratory examination for mycotoxins can be time-consuming and analytical methods are not always transferable from one tissue to another without modification. The recov-

[4]Model SPF, American Instrument Co., Silver Spring, Maryland, USA.

ery rate, precision, accuracy, detection limits and effective range need to be ascertained for each type of tissue. Mixed feeds pose a severe problem for the analyst. The numerous excipients of green plant origin must be removed by such techniques as acidic copper or lead precipitation or by two dimensional TLC. These procedures are time-consuming, reduce sensitivity and may be contraindicated, e.g. acidic copper precipitation will remove zearalenone presumably due to its exposed phenolic group. The skin bioassay utilizes the dermatitis inducing property of the trichothecenes and butenolides, the sensitivity varying with the particular scirpene derivative (18).

The results of this study indicate that the incidence of aflatoxin production by members of the genus *Aspergillus* is low, despite the widespread occurrence of members of this genus. The one positive finding would indicate that conditions favourable for aflatoxin production do occasionally occur, i.e. toxigenic strain, suitable substrate, moisture greater than 14-15%, temperature range of 20-32°C for optimum toxin production and adequate aeration.

Aflatoxicosis in Canadian farm animals would appear more likely to occur as a result of eating aflatoxin-containing imported feeds than from ingestion of home grown feeds. Generally, young animals are more susceptible than older animals, males more susceptible than females and cattle more suspectible than horses and sheep to aflatoxicosis. The aflatoxins are stable at room temperature, readily absorbed by the gastrointestinal tract and excreted in the urine and milk. Susceptibility is increased by dietary protein deficiency. In farm livestock the target organ is the liver. The aflatoxins have been reviewed recently by Detroy *et al* (4).

Ochratoxins have been reported previously, being found in Canadian wheat that had heated during storage and subsequently fed to cattle (12). Later 18 of 29 grains and feeds were found to contain ochratoxin A at levels up to 27 ppm. The mould responsible for toxin production was *Penicillium viridicatum* (13). Ochratoxin producing moulds include the *A. ochraceus* group and *P. viridicatum,* with a substrate moisture content exceeding 16% at 20-26°C being favourable conditions for toxin production. It is significant that five of the six wheat samples containing ochratoxin A had heated during storage. Ochratoxicosis has been incompletely defined as either an experimental or a field syndrome. The target organ is the kidney and depression, reduced appetite, loss of body weight and diarrhea in pigs (16), lowered milk yield in cattle (Personal communication, PhD Thesis, P. E. Still, University of Wisconsin, 1973) or egg production in poultry (17) have been reported. The ochratoxins have been recently reviewed (3).

Few requests were received for analysis for the scirpene derivatives. The prime toxin producers of trichothecenes and butenolides are *Fusarium* species, with an optimum temperature range of 0-8°C for toxin production. In view of the characteristic temperatures of the Prairie winter it is very likely that the trichothecenes could occur in the prairies. The present qualitative method of bioassay would be replaced by a fully quantitative gas chromatographic procedure (7) if there is further concern about the incidence of this group of mycotoxins.

No mycoytoxin residues were found in the ten liver and kidney specimens submitted from cases where a mycotoxicosis was included in the differential diagnosis. In some cases a diagnosis was established that excluded mycotoxicosis, in others autolysis or insufficient tissue reduced sensitivity. Only analyses for aflatoxins and ochratoxins were conducted on these tissues, thus other mycotoxins would not have been detected.

Specimens were submitted because of concern at the possible adverse effects of visible mould infestation. Infrequently was there an association with a clinical syndrome. The overall incidence of 4.2% of mycotoxin contaminated samples indicates that caution should be exercised in the feeding of mouldy feeds to animals. The finding of significant levels of potent mycotoxins suggests that acute or chronic mycotoxicoses may occur in farm livestock or poultry more frequently than presently diagnosed.

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