Mycotoxins in Animal Feedstuffs and Tissues in Western Canada 1975 to 1979

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ABSTRACT

Results of analyses of specimens of plant or animal origin for various mycotoxins are presented. Analyses for aflatoxins, ochratoxins and zearalenone were most frequently requested. Aflatoxin B_1 was found in one of 474 specimens at a level of 60 ppb in a sample of hay. Ochratoxin A was detected in four of 148 specimens of grains and two of 19 specimens of corn at levels up to 500 ppb. Trichothecenes were qualitatively found in two of 108 specimens of forage, three of 182 specimens of feeds and one of 148 specimens of grains. Ergot was detected qualitatively in three specimens of rye and one of forage. An overall detection rate of 3.8% of potent mycotoxins suggests that acute or chronic mycotoxicoses may occasionally occur in farm livestock or poultry.

RÉSUMÉ

Cet article présente les résultats de la recherche de diverses mycotoxines, dans des échantillons d'origine végétale ou animale. Les demandes les plus fréquentes d'analyses concernaient les aflatoxines, les ochratoxines et la zéaralénone. Des 474 échantillons soumis pour la recherche d'aflatoxines, un échantillon de foin recelait 60 ppb d'aflatoxine B_1. Quatre des 148 échantillons de grain et un des 108 échantillons de fourrage contenaient de 30 à 400 ppb d'ochratoxine A. Un des 148 échantillons de grain et deux des 19 échantillons de maïs recelaient jusqu'à 500 ppb de zéaralénone. La recherche qualitative de trichothécènes en révéla la présence dans deux des 108 échantillons de fourrage, trois des 182 échantillons de moulée et un des 148 échantillons de grain. La recherche qualitative d'ergot en révéla aussi la présence dans trois échantillons de seigle et dans un échantillon de fourrage. La détection de mycotoxines virulentes, dans 3,8% des échantillons impliqués dans cette étude, permet de penser que des mycotoxicoses aiguës ou chroniques puissent occasionnellement affecter les animaux domestiques ou les volailles.

INTRODUCTION

In order to assess the significance of mycotoxins in animal disease it is necessary to determine both hazard and risks. There is an increasing amount of information on the effects of various mycotoxins on livestock and poultry (6, 18, 20, 32), but little on the incidence of mycotoxins in Canada (5, 14, 17, 22). This report is concerned with the incidence of mycotoxins in animal feedstuffs and tissues in Western Canada for the period 1975 through 1979.

MATERIALS AND METHODS

This report is based on the analyses of 499 specimens submitted to this laboratory for mycotoxin examination due to concern about mould-infested feedstuffs or possible presence of mycotoxins associated with animal disease. With the exception of the procedure for trichothecenes, examination was by qualitative and quantitative analytical chemical methods using authentic mycotoxin standards for reference.[1] Methylated or ethylated derivatives were formed to confirm identify. 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 thin layer chromatography (TLC) (2).
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 (22).
iii) Ergot alkaloids were obtained by acid extraction with partition by liquid-liquid chromatography and characterized by silica gel TLC (3).
iv) Ochratoxin acids and esters were extracted with chloroform-aqueous phosphoric acid. Acids were entrapped on an aqueous sodium bicarbonate-

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

Celite 545[2] column, esters and fats removed with hexane-chloroform and acids eluted with formic acid-chloroform (11). Ochratoxins were characterized on silica gel TLC using a spectrophotofluorometer[3] (4). Thin layer chromatography plates were exposed to ammonia fumes before quantitation (24). Animal tissues were homogenized with phosphoric acid, extracted with chloroform and centrifuged at 6000 rpm (9). Aliquots of the bottom layer were then processed by the method of Nesheim *et al* (11). In order to maintain a pH of 2-3 during homogenization, the molarity of phosphoric acid was modified according to the tissue being analyzed (12).

v) Zearalenone was extracted with acetonitrile-water, defatted with iso-octane and transferred by liquid-liquid partitioning into chloroform. Characterization was by silica gel TLC (23).

vi) Trichothecenes were extracted in ethyl acetate with cleanup by washing with hexane followed by liquid-liquid partitioning into methanol-water (25, 26). Characterization was by bioassay (28).

Detection limits varied with types of specimens and are given in Table II.

RESULTS

During the period covered by the survey 499 specimens were analyzed for mycotoxins. Of these 19 (3.8%) contained detectable levels of mycotoxins. One specimen of hay contained 60 ppb aflatoxin B_1. Ergot alkaloids were detected qualitatively in three specimens of rye and one farm-mixed feed. Ochratoxin A was found in four specimens of grains and one of forage at levels ranging from 30-4000 ppb.

TABLE I. Summary of Results of Mycotoxin Analyses on 499 Specimens of Animal Feedstuffs and Tissues from Western Canada

Type of Specimen	No. Received for Analysis	No. Positive for Mycotoxin	Percent
Corn	19	2	10.5
Feeds	182	4	2.2
Barley, wheat, oats, rye	148	9	6.1
Legumes, hay, forage	108	4	3.7
Silage	18	0	0
Animal tissues	24	0	0
Total	499	19	3.8

Zearalenone was detected at a level of 500 ppb in one specimen of grain and two specimens of corn. Trichothecenes were found in two forage specimens, three feed specimens and one grain specimen. Table I summarizes the types of specimens received for mycotoxin analysis. The results of 1595 analyses are summarized in Table II.

DISCUSSION

The majority of requests for mycotoxin analyses were for aflatoxins, ochratoxins and zearalenone (Table I). Ochratoxins were detected most frequently and aflatoxins least frequently. This in contrast to the findings of Funnell (5) who reported that zearalenone was most frequently detected, with a considerably lower incidence of aflatoxins and ochratoxins. This is perhaps a reflection of the importance of the amount of corn grown for animal feed in Ontario. The lower incidence of ochratoxin contamination in the present study may be due to increased grain sales during the period of the survey, with less grain being held in storage. There was a marked increase in the number of requests for analyses for the trichothecenes and, in contrast to a previous report (14), there were several indications that this group of mycotoxins merit further investigation to determine frequency of occurrence. It is difficult to evaluate the incidence of ergot, for this contamination is frequently diagnosed in the field by the presence of ergot bodies.

The aflatoxins are hepatotoxins. Dietary aflatoxin levels of 0.03 to 0.8 ppm were toxic to ducklings, broilers and chickens (1,28); levels of 0.1 to 0.5 ppm reduced weight gain and feed efficiency in pigs and anorexia and some fetal deaths in pregnant sows (1). Dietary aflatoxin levels toxic to cattle range from 0.2 to 0.7 ppm (1). The aflatoxin-contaminated hay identified in the present survey contained insufficient aflatoxin B_1 to induce other than mild aflatoxicosis, provided that sufficient hay was ingested for sufficient time. The ochratoxins are nephrotoxins. Dietary levels of 0.5 to 4 ppm lowered egg production and reduced body weight of hens (15), levels of 0.5 to 2 ppm reduced the

TABLE II. Summary of Results of 1595 Mycotoxin Analyses Conducted on 499 Specimens of Animal Feedstuffs and Tissues from Western Canada

Mycotoxin	Detection Limits (ppb)	No. of Analyses in Which Mycotoxins Detected	Not Detected
Aflatoxins	10 - 150	1	473
Ochratoxins	30 - 100	5	469
Zearalenone	50 - 200	3	490
Citrinin	400	0	34
Trichothecenes	1,000	6	94
Ergot alkaloids	1,000	4	16
Totals		19	1576

[2]Fisher Scientific Co. Ltd., Edmonton, Alberta.
[3]Model SPF, American Instrument Co., Silver Spring, Maryland.

weight gain of broilers (16). Ochratoxicosis occurs in swine fed dietary levels of 1 ppm (9). Ochratoxin A fed at 2 mg/kg reduced the weight gain of calves (13) and a single dose of 13 mg/kg caused inappetence, diarrhea and lowered milk yield in a cow (19). The four grain samples in the present survey that were contaminated with ochratoxin A at levels from 1 to 4 ppm might have induced toxicity in poultry, pigs and calves. Zearalenone is oestrogenic and toxicity has been reported in prepubertal gilts following daily dosing with 0.02 mg/kg and in sexually mature gilts fed 0.6 mg/kg (10). The zearalenone contaminated grain and corn samples might have induced oestrogenic toxicity in swine if fed for a sufficient period. Trichothecene mycotoxins, including T-2 toxin, have been implicated in several toxicoses of livestock. Since the detection method used in the present study did not distinguish between the necrotizing trichothecenes any positive was reported at T-2 toxin. Dietary levels of 1-16 ppm T-2 caused neural disorders, oral lesions and decreased egg production (29, 30); low levels of T-2 adversely affected production parameters in swine (26). It is possible that the levels of T-2 toxin detected in six specimens of the present survey could have caused feed refusal, oral lesions or systemic toxicity to poultry. The total concentration and proportions of alkaloids of ergot vary with species and environmental conditions. Sows fed 0.5 to 1.0% barley ergot delivered weak pigs and failed to lactate properly (7); in cattle, 0.02% of body weight as ergot produced gangrenous ergotism (8). No attempt was made in the survey to quantify the ergot or its alkaloids.

Mycotoxin incidence reported by the laboratory can be used only as a guide because of the variation in sampling techniques used by the submittors. The overall incidence (3.8%) of mycotoxin-contaminated animal feedstuffs would suggest that acute outbreaks of mycotoxicoses might occur in farm livestock or poultry. It would seem more likely that chronic or subclinical mycotoxicoses might be associated with the overall low incidence of mycotoxins in animal feedstuffs.

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LETTER TO THE EDITOR

Incomplete and Debatable Information?

DEAR SIR:

I would like to comment on some aspects of two papers published in the January 1981 issue of the Canadian Journal of Comparative Medicine.

I refer to the article "Porcine Haemophilus Pleuropneumonia Epizootic in Southwestern Ontario: Clinical, Microbioligical, Pathological and Some Epidemiological Findings", by S.E. Sanford and G.K.A. Josephson in Can.J-.comp.Med. 45: 2-7. 1981.

On page 5 of the article the authors say "the *H. pleuropneumoniae* organism, despite differing nomenclature, had probably been in Ontario for nearly 20 years having been implicated in meninitis, septicemia and abortion in pigs". The authors refer to two articles on Hemophilus infections in pigs: one which was associated with meningitis, reference no. 26, and the other reference no. 29, which was associated with septicemia. In both of these outthe organisms isolated were probably *Haemophilus parasuis* and not *Heamophilus haemolyticus (pleuropneumoniae).*

The summary on page 2 indicates that "Broad spectrum antibiotics were usually effective in stopping deaths". On page 3, under results, the statement is made that "there was usually a dramatic clinical response to mass medication with broad spectrum antibiotics administered via the drinking water combined with intramuscular injections of the same antibiotics to animals which were obviously ill". The paragraph goes on to say that deaths ceased after antibiotic therapy and sometimes recurred following withdrawal of the antibiotics.

However, there is no indication of which antibiotics were used, the dosages used or the duration of treatment. I would suggest that the article is incomplete without such important clinical information.

In the same issue of the Journal, there may be some debatable information in the article "Some Pathophysiological Changes Associated with Infection of *Eimeria zuernii* in Calves" by P.H.G. Stockdale *et al* (Can. J.comp. Med. 45: 34-37. 1981). On page 36, the authors cite a reference which apparently concluded that "depression of Na^+ and $C1^-$ levels occur in calves infected with *E. zuernii* and that the lowering of these ions may contribute to the nervous signs sometimes associated with bovine coccidiosis. This is debatable and based on very slim evidence.

The Large Animal Clinic of the Western College of Veterinary Medicine has studied the clinical and laboratory findings of approximately 100 cases of bovine coccidiosis accompanied by nervous signs, over the last ten years, and there is no indication that the nervous signs are associated with any electrolyte imbalances. Furthermore, severe serum electrolyte deficiencies occur in cattle and horses affected with enteritis (of the small and large intestine) and nervous signs are extremely rare, if they ever occur. I hope that your readers do not conclude that there is necessarily a cause and effect relationship between hyponatremia and hypochloremia in calves with coccidiosis and the observed nervous signs.

At the present time, I am unaware of any explanation for the pathogenesis of the nervous signs associated with bovine coccidiosis. It is indeed interesting that protozoologists who have experimentally reproduced fatal cocidiosis in cattle have not reported nervous signs of the kind associated with some of the naturally occurring cases.

Sincerely,

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