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MYCOTOXINS OF POSSIBLE IMPORTANCE IN DISEASES OF CANADIAN FARM ANIMALS

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Introduction

Mycotoxicoses are diseases of man and animals caused by the consumption of foods and feeds containing toxic substances (mycotoxins) produced by molds. Not all molds produce toxic compounds but under certain conditions some molds form substances that are harmful to man and animals. Mold spores or other mold particles are commonly present in or on plant materials or in the surrounding atmosphere. Many species of molds can grow in plants in the field or in stored plant products but are inactive as long as certain conditions (plant resistance, temperature, moisture, level of oxygen, carbon dioxide, or other gases, acidity, type of product) limit their growth and spore production. In many stored products, the moisture content is the most important factor limiting mold growth. Once a certain moisture level is exceeded, the material will become moldy, unless other limiting factors are present. Moldy feed may have a bad odor, low palatability and lower nutritional value (160). Mycotoxicoses are, in general, characterized by the following features (45): (a) the disease is not transmissible, (b) treatment with antibiotics has little effect on the course of the disease, (c) the disease is often seasonal as certain climatic conditions favour toxin production by a given mold, (d) the disease is usually associated with consumption of a specific food or feedstuff, and (e) the suspected food or feed shows signs of moldiness. These may, however, become less apparent as a result of processing.

Mycotoxins produce disease and acute toxic effects when consumed in large amounts but more often the effects are subacute or chronic. Mycotoxicosis is usually suspected only when all other potential causes have been excluded or when the feed is obviously moldy. To make a diagnosis of mycotoxicosis caused by a specific toxin, the typical clinical and pathological features associated with the particular toxin should be observed in the affected animals and a significant level of the toxic compound must be demonstrated in the suspect feed and, if possible, in the urine and tissues (111). In practice, mycotoxicoses are difficult to diagnose because the etiological agent(s) may not be easily identified analytically and their toxic effects may be non-specific or in some cases unknown. A review on the principles involved in analytical methods for mycotoxins has been published (154).

The economic importance of mycotoxicoses in Canada and in other countries and their implications in animal and human health are largely unknown. It has been speculated that these effects may be considerable (46). This may be particularly true in years of unusually high rainfall and where drying of grain after harvesting and storage facilities are inadequate. High moisture grain to be used as feed can be preserved by treatment with acetic or propionic acid or a combination of these acids (110, 131).

Although many toxic compounds have been isolated from pure cultures of molds in the laboratory, the role of most of these in diseases of farm animals is obscure. Methods of analysis for their presence in feeds may be lacking or there are no data on their natural occurrence. The review on mycotoxins presented herein therefore is restricted largely to those toxins which, to date, have been shown to occur in foods or feeds and which have been linked to recognized diseases of animals. It should be clear, however, that many cases of suspected mycotoxicosis may be due to unidentified mycotoxins.

A number of relatively well-recognized mycotoxicoses, including ergotism of cattle, sheep,

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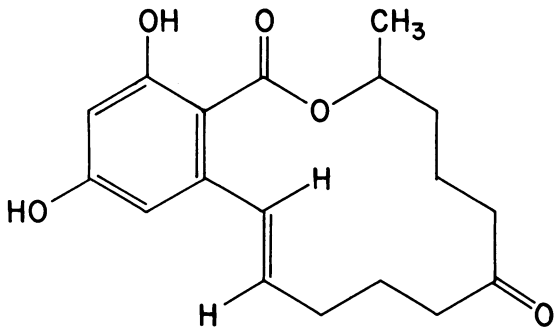

FIGURE 1. Zearalenone.

and swine (12), slobbering of cattle induced by red clover that is infected by *Rhizoctonia leguminicola* (32), and facial eczema of sheep and cattle in New Zealand and Australia (128) will not be discussed in this review.

ZEARALENONE

Zearalenone, also known as F-2 toxin, FES (fermentation estrogenic substance), or RAL (resorcylic acid lactone) (Figure 1) is produced by *Fusarium roseum, Fusarium tricinctum, Fusarium oxysporum* and *Fusarium moniliforme* (15, 91, 92, 103). The natural occurrence of this compound in high moisture corn stored in cribs in the fall and winter is attributed mainly to growth of *Fusarium roseum* (91, 103). Zearalenone is the major toxin responsible for vulvovaginitis or estrogenic syndrome, a disease of swine consuming some types of moldy corn. It has been recognized in Canadian swine (8). In young gilts suffering from the disease, swelling and edema of the vulva appears initially, followed in severe cases by prolapse of the vagina. The uterus is greatly enlarged with proliferation of the epithelium and myometrium and the ovaries are atrophied. Normal estrus may be interrupted. Young males may develop mammary gland hyperplasia and testicular atrophy. The estrogenic syndrome reverses when the diet is changed (91).

The syndrome can be induced experimentally by administration of the purified toxin to swine and other animals. Naturally occurring derivatives of zearalenone may act synergistically with the parent compound (91, 103).

One to five ppm zearalenone in the feed of swine produces physiological effects (95). In 60 lb gilts, 1 mg fed daily produces swollen vulvae within five days (93). At lower doses, zearalenone and some of its derivatives such as zearalanol have growth-promoting properties in young farm animals similar to that observed with diethylstilbestrol (91, 141).

Feeding pregnant sows naturally moldy corn in which *F. roseum* and *F. moniliforme* were the predominant fungi caused a reduction in litter size, number of live piglets born per litter, and increased fetal mummification. Corn cultures of strains of *F. roseum* also increased embryonic mortality in pregnant sows (140). It is unlikely that these latter effects were due to zearalenone since no zearalenone-like signs were noted in the treated animals.

Zearalenone has been detected in moldy hay, suspect feed, marketed corn, and naturally infected corn at harvest (Table I). Levels in some contaminated feeds far exceeded those required to induce estrogenism. The zearalenone-containing moldy hay was associated with decreased fertility in cattle (93). Zearalenone-containing feed given to laying hens caused reduced egg production (31).

Zearalenone may occur in sow's milk as signs of estrogenism occur in piglets before they are weaned (86, 117).

The toxin is relatively heat stable. Heating at 60°C in 50% ethanol for 60 min caused no destruction but at 111°C slow decomposition took place (94). As it has been detected in commercially prepared pelleted pig feed (23, 103), it can be concluded that it survives processing procedures.

Methods for the chemical detection and quantitation of zearalenone in foods and feeds have been described by Mirocha *et al* (94), Eppley (44) and Stoloff *et al* (155).

OCHRATOXIN A

Ochratoxin A is the major toxin of a group of related compounds produced by species belonging to the *Aspergillus ochraceus* group (60, 89). A number of *Penicillium* spp. also produce the toxin (27, 138, 139, 170). Available evidence indicates that ochratoxin A occurring in naturally moldy substances is associated with growth of *Penicillium viridicatum* and perhaps other *Penicillium* spp. (138, 139). Some strains of *P. viridicatum* produce both ochratoxin A and citrinin, another mycotoxin. In wheat with a moisture content of 22% and stored at 12 or 25°C, *P. viridicatum* competes effectively with the native mycoflora and produces large amounts of ochratoxin A and citrinin (57).

Ochratoxin A is a dihydroisocoumarin linked through its 7-carboxy group to L-β-phenylalanine (Figure 2). Ochratoxin B, which is considerably less toxic, is the dechloro derivative.

TABLE I

NATURAL OCCURRENCE OF ZEARALENONE

Sample	No. positive samples/no. tested	Levels (ppm)	Location	Reference
Moldy hay	1/1	14	England	93
Suspect corn (feed)	28/65	0.1–2,909	United States	95
Marketed corn	38/223	0.1–5	United States	43
Naturally infected corn at harvest	33/41, 2/10	0.1–10	United States	16, 17
Feed for laying hens	?	?	Canada	31

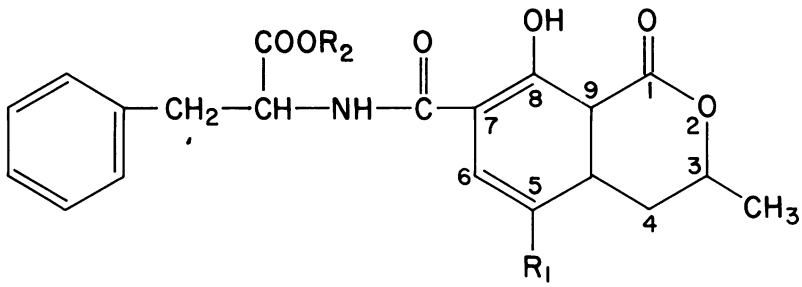

FIGURE 2. Ochratoxins; ochratoxin A: R_1 = Cl, R_2 = H; ochratoxin B: R_1 = H; R_2 = H; ochratoxin C: R_1 = Cl, R_2 = C_2H_5; methyl ester of ochratoxin A: R_1 = Cl, R_2 = CH_3; methyl or ethyl ester of ochratoxin B: R_1 = H; R_2 = CH_3 or C_2H_5. Hydrolysis of ochratoxin A yields L-β-phenylalanine and ochratoxin α (7-carboxy-5-chloro-3, 4-dihydro-8-hydroxy-3-methylisocoumarin).

The toxic methyl and ethyl esters of ochratoxin A and ochratoxin B have been isolated from cultures of *A. ochraceus* (88, 152).

A disease in Danish pigs known as swine nephropathy can be reproduced in rats and pigs by feeding barley infected by *P. viridicatum* (73). In Denmark, swine nephropathy recently has been associated with feed containing ochratoxin A and citrinin (72). Nephrotoxicity in swine associated with the consumption of naturally moldy barley also has been recognized in Ireland (11). Cultures of some strains of *P. viridicatum* isolated from moldy corn in the United States are known to be nephrotoxic to experimental animals but their nephrotoxicity cannot be attributed to known mycotoxins (20). There are no reports of swine nephropathy in Canada although ochratoxin A has been detected in Canadian feeds (Table II).

Ochratoxin A is a potent mycotoxin (Table III). The principal pathological changes accompanying administration of large doses are necrosis of the renal tubular epithelium and periportal liver cells, and enteritis (39, 119, 121, 169). At a dietary level of 0.2 ppm, the toxin induced kidney damage in rats consisting of hyaline changes and pyknosis in epithelial cells of the proximal convoluted tubules within three months (97, 98). This level of toxin in the feed of pigs caused nephropathy in the course of three to four months (72). Oral, daily doses of 1 mg/kg body weight caused death of pigs in six days with clinical features consisting of anorexia, depression, diarrhea, fever, polydipsia, polyuria, dehydration and prostration (157). Dogs are more sensitive to ochratoxin than pigs. Beagles receiving an oral daily administration of 0.3 mg/kg body weight survived 11–15 days. Clinical and pathological features reflected kidney damage (158) and histopathological alterations in the kidneys consisted of necrosis and desquamation of epithelial cells, mainly in the proximal convoluted tubules (159).

Ochratoxin A also has deleterious effects when fed to poultry. Sublethal doses in chicks produced dose-related growth depression, visceral gout, renal and hepatic lesions and enteritis (108). Diets of young laying hens mixed with cultures of *Aspergillus sulphureus* to provide dietary levels of 1, 2 or 4 ppm ochratoxin A resulted in high mortality and morbidity, depression of body weights, delayed sexual maturity and reduced egg production and feed efficiency (22). Growth depression and other toxic effects also have been observed in male broiler chicks fed diets containing ochratoxin A at 0.5 ppm and higher (164).

Ochratoxin A has been implicated in an outbreak of bovine abortion (153) but subsequent experimentation in cattle with the pure compound suggested it was not an abortifacient (127).

TABLE II

NATURAL OCCURRENCE OF OCHRATOXIN A

Sample	No. positive samples/no. tested	Levels	Location	Reference
Commercial corn	1/283	110–150 ppb	United States	143
Export cargo corn	3/293	83–166 ppb	United States	144
Commercial barley	18/127	trace–38 ppb	United States	104
Moldy wheat from the same pile	4/4	20–100 ppb	Canada	138
Heated grain	18/29	0.03–27 ppm	Canada	139
Mixed feed	1/3	0.53 ppm	Canada	139
Dried white beans	3/?	0.02–2.1 ppm	Canada	139
Moldy peanuts	1/1	4.9 ppm	Canada	139
Barley and oats associated with swine nephropathy	19/33	0.03–27.5 ppm	Denmark	72
Tissues of swine consuming above grain				
kidneys	18/19			
livers	7/8	up to 67 ppb	Denmark	53
adipose tissue	8/8			
High quality barley	3/50	9–189 ppb	Denmark	72

TABLE III

LD_{50} VALUES OF OCHRATOXIN A

Route	Animal	LD_{50}	Reference
Oral	Duckling	150 μg/kg	119
Oral	Rat	20–22 mg/kg	121
Intraperitoneal	Trout	4.64 mg/kg	39
Oral	Chick	166 μg/kg	25
Oral	Chick	116–135 μg/kg	108

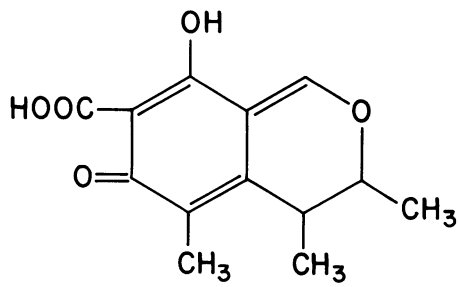

FIGURE 3. Citrinin.

There is some evidence for embryotoxic effects of ochratoxin A. Fetal deaths and resorptions have been noted in rats given a single oral dose on the tenth day of gestation. Administered intraperitoneally, on one of gestation days 7–12 to pregnant mice at 5 mg/kg, it caused increased prenatal mortality, decreased fetal weight and fetal malformations (59). It remains to be determined if oral ingestion of the toxin produces malformations in mice.

When ochratoxin A is fed to cows in low doses, the hydrolysis product, ochratoxin α (Figure 3) appears in the urine; with large doses, however, both ochratoxin A and α are present in the milk and urine (24). Ochratoxin α is also present in the urine and feces of rats given ochratoxin A (102, 169). Demonstration of these compounds in such excretions may help in the diagnosis of ochratoxin A poisoning in farm animals but high levels of the parent compound may have to be present in the feed before they are detectable analytically.

Low levels of ochratoxin A have been detected in marketed corn and barley in the United States (Table II); in poor quality grain, much higher levels may be present, as indicated by the data from Canada. Feed (mainly barley) from Danish pig farms where swine nephropathy had occurred contained levels comparable to those found in Canada; 19 of 33 samples of feed of pigs suffering nephropathy contained ochratoxin A and nine of the positive samples contained more than 0.2 ppm. Three of 50 samples of good quality barley contained ochratoxin A at levels lower than 0.2 ppm.

Small amounts of ochratoxin A may be transmitted to humans through the tissues of swine consuming ochratoxin A-containing feed (Table II).

Ochratoxin A is a heat stable toxin. Oatmeal and rice cereal autoclaved for three hours still contained 30% of added toxin (163). Heat

processing of ochratoxin A-containing canned beans at 121°C for one hour did not significantly decrease levels of the toxin (55).

Methods for the detection and quantitation of ochratoxin A recently have been reviewed (24, 56). Analytically useful spectroscopy data are available (101).

Citrinin

Citrinin is a cyclic, low molecular weight compound with a free carboxylic acid group (Figure 3). It is produced by a number of *Aspergillus* and *Penicillium* spp. including *P. viridicatum* (69, 74, 138, 139). Citrinin is nephrotoxic in laboratory animals (2) and induces a disease syndrome comparable to swine nephropathy when fed at levels of 200–400 ppm for one to two months (49, 72, 74). The nephrotoxicity of cultures of *Penicillium citrinum* may be partly or wholly due to citrinin (18, 34, 130).

The toxin has been detected in naturally moldy grain that also contained ochratoxin A. In Canada, 13 of 18 of such poor quality samples of wheat, oats, barley and rye concontained citrinin at levels of 0.08–80 ppm (139).

In Denmark, three of 19 ochratoxin A-containing samples of a total of 33 samples from farms with swine nephropathy contained citrinin at levels of 0.16–2 ppm. Considering its low toxicity compared to that of ochratoxin A, citrinin is likely to play only a minor role in swine nephropathy in Denmark (72).

Citrinin may readily react with certain compounds. It loses its antibacterial activity in the presence of cysteine and may bind to the albumin fraction of human blood serum (33, 69). It cannot be readily recovered when added to apple juice and levels in grain extracts decrease with time (58, 139). The phytotoxic activity of citrinin disappears in the soil (146).

Citrinin dissolved in hexane or 95% ethanol decomposes slowly on refluxing but it is thermolabile in acid and alkaline solution (67, 100). It undergoes some photodecomposition both in solution and in the solid state (100).

Two relatively sensitive methods are available for the analysis of citrinin in naturally moldy grain. Both methods involve thin layer chromatography and make use of the fluorescence properties of citrinin under ultraviolet light for detection and quantitation (52, 139).

Aflatoxins

The aflatoxins are potent hepatotoxins and are among the most carcinogenic agents known. They are a group of toxins primarily produced by *Aspergillus flavus* and *A. parasiticus*. Chemically, they are difuranocoumarin derivatives (Figure 4). Under long wave UV light, aflatoxins B_1 and B_2 fluoresce blue while aflatoxin G_1 and G_2 fluoresce green (10).

Aflatoxin-producing strains of *A. flavus* and *A. parasiticus* occur commonly in soil, air, seed and forage throughout the world (38). Aflatoxins are sometimes present in crops at harvest, e.g. in cotton seeds (85), peanuts (64) and corn (176). Conditions of high humidity and temperature during harvesting, drying, transportation, and storage favour growth of *A. flavus* and lead to subsequent toxin production (5, 38). Accordingly, aflatoxin-contaminated products present a problem particularly in the hot and humid regions of the world but may be introduced into countries of temperate climate that depend on these products for food or feed (168).

Outbreaks of a disease involving liver damage have been reported in many countries, including Canada, and have been attributed to aflatoxins. The disease has occurred in turkey poults, ducklings, young pheasants, chickens, pigs, calves, dogs and rabbits. All were associated with consumption of rations containing peanut meal (1, 41, 87). Hepatoma and other chronic toxic effects in trout have been attributed to aflatoxin-containing cotton seed or peanut meals (54).

In general, young animals are more susceptible to aflatoxicosis than older ones and males more susceptible than females. Factors such as species, breed, and diet composition also influence susceptibility. The order of susceptibility in poultry, as estimated by feeding of aflatoxin-containing peanut meal, is as follows: duckling > turkey poult and pheasant chick > chickens and quail; in mammals, it is: pigs (three to 12 weeks old) and pregnant sows > calves > fattening pigs and mature cattle > sheep (1). Clinical signs and gross and microscopic lesions associated with acute aflatoxicosis in chickens, ducks, calves, turkeys, dogs, pigs and sheep have been reviewed by Edds (41), Carlton and Tuite (19) and Newberne (105). Edds (41) also tabulated aflatoxin levels causing poisoning in farm animals. Maximum permissible levels of aflatoxin B_1 in feed have been established in Rhodesia for cattle, sheep, poultry, horses, chinchillas, rabbits, dogs and trout (40).

In general, the clinical signs of acute intoxication in most species studied include lack of appetite, weight loss, unthriftiness, neurological abnormalities, icterus of mucous membranes, convulsions and death. Damage to the

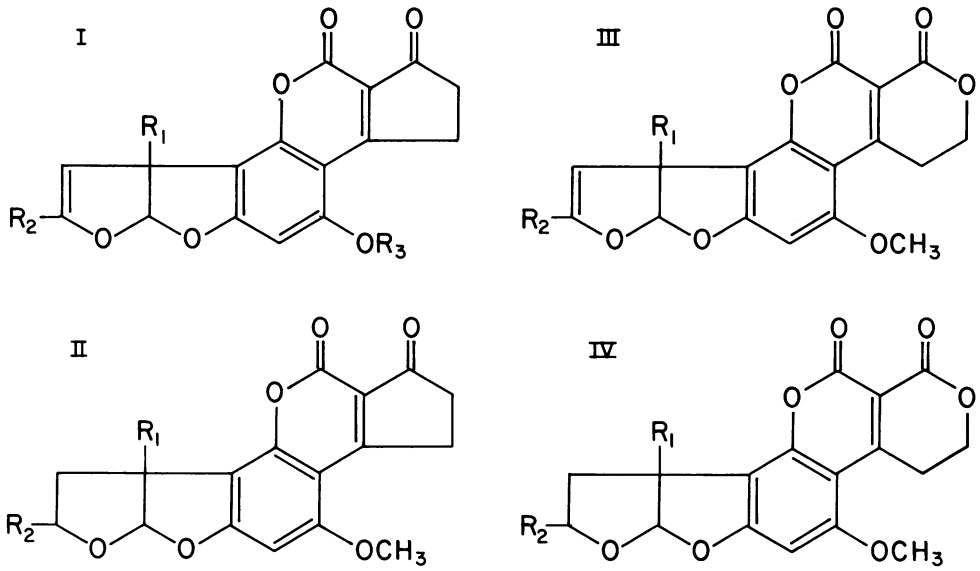

FIGURE 4. Aflatoxins and their metabolites. I. Aflatoxin B_1: R_1 = H, R_2 = H, R_3 = CH_3; aflatoxin M_1: R_1 = OH, R_2 = H, R_3 = CH_3; aflatoxin P_1: R_1 = H, R_2 = H, R_3 = H. II. Aflatoxin B_2: R_1 = H, R_2 = H; aflatoxin B_{2a}: R_1 = H, R_2 = OH; aflatoxin M_2: R_1 = OH, R_2 = H; III. Aflatoxin G_1: R_1 = H, R_2 = H; aflatoxin GM_1: R_1 = OH, R_2 = H; IV. Aflatoxin G_2: R_1 = H, R_2 = H; aflatoxin GM_2: R_1 = OH, R_2 = H; aflatoxin G_{2a}: R_1 = H, R_2 = OH. Cyclopentenone reduction of aflatoxins B yields the secondary alcohols aflatoxicol and dihydro-aflatoxicol (107).

liver is the most outstanding pathological change. Grossly, the livers are pale or discolored while microscopically, they show diffuse and centrilobular necrosis and fat accumulation. Fluid in the body cavities and, frequently, hemorrhage of the kidney and gastrointestinal tract also may be noted. Chronic aflatoxin poisoning results in icterus of the carcass and cirrhosis of the liver. Liver cell necrosis is less pronounced than in acute poisoning but bile duct proliferation and periportal fibrosis occur (105). Economically important consequences of chronic aflatoxicosis in pigs are decreased feed conversion and weight gain. In dairy cattle, milk production is decreased and in beef cattle weight gain is lowered (105).

Ducklings may develop cholangiocarcinoma, hepatocellular carcinoma, or both on prolonged feeding of low dietary concentrations (105). Trout develop hepatic tumors at dietary aflatoxin levels of a few parts per billion (54).

There is some evidence that prolonged exposure to low levels of aflatoxins may result in lowered resistance to some animal species to infection by certain pathogens (112).

Another area of concern to public health organizations is the transfer of aflatoxins to tissues and milk of animals consuming aflatoxin-containing feed (176). Experimentally, it has been shown that aflatoxins and their metabolites or both may be transmitted to the blood, organs, and tissues of pigs and poultry and to chicken eggs (4, 71, 81, 132, 187). Livers, kidneys, and carcasses of such pigs may be in reasonably good condition and pass meat inspection (71). Aflatoxin M_1, a hydroxylated derivative of aflatoxin B_1 (Figure 4), has been found in some commercial milk samples in the United States, South Africa and Germany (51, 68, 118). Aflatoxin M_1 has comparable carcinogenicity to aflatoxin B_1 in rainbow trout and produces local tumors on subcutaneous injection into rats (125, 145).

The metabolic fate of aflatoxins upon entering the liver cell has been recently reviewed by Patterson (107). Aflatoxin B_1 may bind to nuclear DNA, inhibit RNA synthesis, and interact with sex-related binding sites of the endoplasmic reticulum. Such events are thought to be related to carcinogenesis. This may involve transformation of aflatoxins to epoxides by liver enzymes. Recognized transformations of aflatoxins by liver enzymes are (Figure 4): (a) 4-hydroxylation to form aflatoxins M_1, M_2, GM_1 and GM_2, (b) *O*-demethylation to a phenolic derivative; the derivative of aflatoxin B_1 is known as aflatoxin P_1, (c) hydration of the vinyl ether double bond; the 2-hydroxy derivatives of aflatoxins B_1 and G_1 are the hemiacetals aflatoxin B_{2a} and G_{2a} and (d) cyclopentenone reduction of aflatoxins B to yield the secondary alcohols known as aflatoxicol or

aflatoxin F_1 from aflatoxin B_1 and dihydroaflatoxicol or aflatoxin F_2 from aflatoxin B_2. Another transformation observed in fungal cultures involves hydrolytic fission and decarboxylation of the δ-lactone ring of aflatoxin G_1 yielding aflatoxin B_3 or parasiticol. Reactions (a) and (c) are mediated by microsomal enzymes (mixed function hydroxylases of the endoplasmic reticulum) in the presence of reduced nicotine amide diphosphate ($NADPH_2$). Microsomal enzymes also mediate reaction (b) while transformation (d) is performed by cytoplasmic, $NADPH_2$-dependent liver enzymes.

In rat liver cells, aflatoxin B_1 *per se* may survive long enough to cause primary tissue damage but in ducklings, the toxin is rapidly metabolized (107). The toxic action in ducklings therefore may be due to a metabolite. Phenobarbitone pretreatment enhances microsomal activity and decreases the effects of acute aflatoxin poisoning in rats, thereby stimulating the conversion of aflatoxin B_1 to aflatoxin M_1 and P_1. These may be more readily eliminated by the rat's biliary excretion as taurocholic acid conjugates or as glucuronides than aflatoxin B_1. In contrast, phenobarbitone pretreatment does not stimulate aflatoxin metabolism in the duck liver. In this species, and in the chicken, turkey and rabbit, aflatoxins B_1 and B_2 are reduced to aflatoxicol and dihydroaflatoxicol by $NADPH_2$-dependent enzymes present in the cytoplasmic fraction of the liver. A soluble $NADPH_2$-linked 17-hydroxysteroid dehydrogenase may be involved in this reaction. This pathway is reversible so that it may either act as a "reservoir" for aflatoxin which may then be bound to intracellular structures and cause chronic liver injury, or it may be subsequently converted to hemiacetals. The acute toxic action of aflatoxins has been suggested to hinge upon formation of these hemiacetals. They are non-toxic when given to intact animals but once formed within the hepatocyte may be toxic by binding to and inhibiting enzymes. Many of the species of animals studied are capable of transforming aflatoxin B_1 to aflatoxin B_{2a} (107).

Plant products considered in the United States to have demonstrated potential for aflatoxin contamination include peanuts, cotton seed, corn, copra, Brazil nuts, pistachio nuts, almonds, pecans and walnuts; some of these are used as components of animal feed (4, 176). Products such as rye, wheat, oats, buckwheat, barley, millet and rice have not been implicated in detectable aflatoxicosis (4) but low levels of aflatoxins have been found in some of these commodities (4, 135). Low levels may also be present in soybeans (142).

It is of economic importance to note that in 1969 alone Canada rejected 7.5 million pounds of imported peanuts due to aflatoxin contamination (168).

Aflatoxins are relatively heat stable. For example, roasting of peanuts leads to a loss of only 40–50% of aflatoxins B_1 and G_1 and 20–40% of aflatoxins B_2 and G_2 (172). Ensiling aflatoxin-containing, high moisture corn fails to remove aflatoxins (77). Inactivation of aflatoxins in peanut and cotton seed meals by ammoniation appears to be a promising possibility (3, 46, 50, 82).

Numerous methods of analysis for aflatoxins are now available. Some of these have been tested by collaborative studies, each for specific use in products such as corn, peanuts, peanut products, coffee, cocoa, copra, cotton seed and cotton seed meal (4). Reviews on aflatoxin analysis include those by Pons and Goldblatt (116), Armbrecht (4) and Jones (65). A recent publication describes a method for its determination in mixed feeds (129).

T-2 Toxin and Other 12, 13-epoxytrichothecenes

T-2 toxin and related compounds are produced by a number of *Fusarium* spp. including *F. tricinctum*. They belong to a group of fungal toxins that are derivatives of a ring system named trichothecane and are characterized as 12, 13-epoxytrichothecenes. Their toxicity can be attributed to the epoxy group at carbons 12 and 13 and the olefinic bond at carbons 9 and 10 (6). Related toxins produced by *Fusarium* spp. are diacetoxyscirpenol, neosolaniol, HT-2 toxin, nivalenol, fusarenon-X, diacetylnivalenol and deoxynivalenol (Figure 5) (14, 165, 186).

In the Midwestern states of the United States *F. tricinctum* is particularly common in corn associated with problems of toxicity in livestock, generally referred to as moldy corn toxicosis. Because of similar climatic conditions, this disease may occur on Canadian farms although it has not been reported to date. Among the molds isolated from such corn, *F. tricinctum* is consistently the most toxic (147). In culture, strains of this mold can produce T-2 toxin, HT-2 toxin, neosolaniol, diacetoxyscirpenol, a toxic butenolide (4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone) and zearalenone (15, 165, 185). Moldy corn toxicosis therefore may be the result of a number of toxins. The disease is usually associated with corn that is late to mature or high in moisture at the time of the first killing frost; such corn becomes moldy during fall and winter when stored in cribs without artificial drying (148). Under laboratory conditions, temperatures of 8 and 15°C

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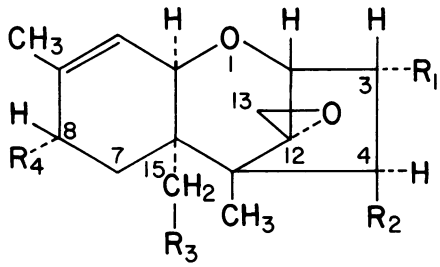

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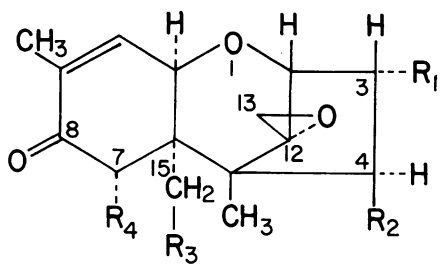

FIGURE 5. 12,13-Epoxytrichothecenes produced by *Fusarium* spp.

Group I toxins:	R_1	R_2	R_3	R_4
T-2 toxin	OH	OAc	OAc	$OCOCH_2CH(CH_3)_2$
Diacetoxyscirpenol	OH	OAc	OAc	H
Neosolaniol	OH	OAc	OAc	OH
HT-2 toxin	OH	OH	OAc	$OCOCH_2CH(CH_3)_2$
Group II toxins:				
Nivalenol	OH	OH	OH	OH
Deoxynivalenol	OH	H	OH	OH
Fusarenon-X	OH	OAc	OH	OH
Diacetylnivalenol	OH	OAc	OAc	OH

are more favourable for T-2 toxin production by *F. tricinctum* than temperatures of 25 and 32°C (6, 13).

Signs of moldy corn toxicosis may include refusal to eat, digestive disorders accompanied by diarrhea, lack of weight gain and death. Hemorrhagic lesions in the stomach, heart, intestines, lungs, bladder and kidneys may be present (148).

T-2 toxin has a high oral toxicity. The LD_{50} values for rats, trout and calves are, respectively, 3.8 mg/kg, 6.1 mg/kg and 0.6 mg/kg (70, 84, 127). Animals dying from toxic doses of T-2 show severe edema in the body cavities and hemorrhage of the large intestine. Neurotoxic effects have been noted in chickens (179). Similar symptoms have been observed in animals and humans that ingested naturally toxic feed and grain. For this reason, T-2 and related toxins are thought to play a causal role in moldy corn toxicosis of pigs, poultry and cattle, and in such diseases as alimentary toxic aleukia of humans, fescue foot disease of cattle, and stachybotryotoxicosis of horses, sheep, swine, calves and humans (6, 177, 179, 182, 184).

T-2 toxin is known to cause emesis in pigeons when administered orally at nonlethal doses (42) and may be responsible for a disease syndrome observed in commercial broiler flocks. This disease is characterized by yellowish-white lesions in the oral cavity which can be produced experimentally by feeding chickens small amounts of T-2 toxin (180, 181). The oral lesions may prevent chickens from eating.

Available data indicate that T-2 toxin is not carcinogenic (83).

When applied to the skin of rats, rabbits, and guinea pigs, T-2 causes inflammation and tissue necrosis. This property is used in bioassays for T-2 toxin. These methods are quick, convenient and sensitive (detection limit about 50 ng) (26, 175) but are non-specific when applied to extracts of natural products. Skin tests may be useful for rapid screening of suspect toxic samples but positive samples need to be analyzed chemically.

T-2 toxin has been detected by gas-liquid chromatographic methods in moldy corn associated with the death of cattle (62). The dead animals showed extensive hemorrhaging in the intestines. Another report on the natural occurrence of a 12, 13-epoxytrichothecene involves corn contaminated in the field with *Fusarium* spp. Pigs either refused to eat the corn or vomited after its ingestion. The corn contained an emetic factor that was tentatively identified as the trichothecene 3, 7, 15-trihydroxy-12, 13-epoxytrichothe-9-en-8-one (167). In Japan, nivalenol and deoxynivalenol have been detected in field-infected barley (96, 186). The rabbit skin test applied to extracts of corn from terminal elevators and food establishments in the United States showed the presence of a skin-irritating factor in 93 of 173 samples (43).

T-2 toxin and related compounds are chemically stable under laboratory conditions; they are not destroyed by normal cooking procedures but prolonged boiling results in structural rearrangement of some compounds (6).

It is evident that practical analytical methods are urgently needed for the detection of 12, 13-epoxytrichothecenes to assess their significance in animal and human health.

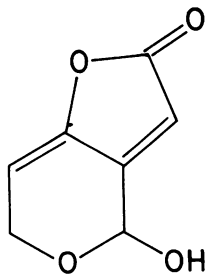

FIGURE 6. Patulin.

MISCELLANEOUS NATURALLY OCCURRING MYCOTOXINS

Patulin, penicillic acid and sterigmatocystin occur in naturally contaminated products but their role in causing animal or human disease is unknown.

Patulin (Figure 6) is now referred to as a mycotoxin but was previously known as an antibiotic. It was isolated from a variety of fungi in different laboratories (47, 69). It is an unsaturated lactone and possesses a structure that is similar to that of certain carcinogens. Synonyms of patulin are clavatin, clavacin, claviformin, expansin, penicidin, mycoin, leucopin and tercinin (115).

Molds producing patulin in culture are *Penicillium* and *Aspergillus* spp. and *Byssochlamys nivea* (47). *P. expansum* is known to produce large quantities of patulin in apples (7, 58, 171, 178.) *P. urticae* (*P. patulum*) produces the compound in wheat straw residues in the soil where it may have phytotoxic activity (106). There are no reports on the occurrence of patulin in feed. However, in Japan, the toxin has been implicated in the deaths of cattle consuming malted barley (183). *P. urticae* was isolated from this feed and was shown to produce patulin in culture (166). When grown on barley, the mold was lethal to mice and a mature bull (183). Toxic signs observed included paralysis of the motor nerves, convulsions and reflex excitement. The signs were similar to those observed in the cattle suffering from the naturally occurring disease. Patulin was produced in inoculated barley (183). There are, however, no reports on the effects of pure patulin on cattle.

When administered at lethal levels by various routes of administration to rats, mice and rabbits, the toxin caused pulmonary edema (9, 66). For an evaluation of patulin as a health hazard, oral toxicity studies are most relevant. The oral LD_{50} in mice was 35 mg/kg (9) and 170 mg/kg in chicks (79); 0.5 mg administered daily by the oral route for two weeks caused lung edema and deaths in rats (9) while chicks given 0.2 mg daily for six weeks developed liver damage (90).

Current public health interest in patulin is based on its occurrence in commercial apple juice (136, 173, 178) and on its potential carcinogenic activity; 0.2 mg injected subcutaneously, twice weekly into rats for 61–64 weeks produced transplantable sarcomas at the site of injection (37).

Following oral administration, patulin cannot be detected in body fluids of rats and rabbits (48). *In vitro*, it is inactivated in kidneys, blood, liver and other tissues (9, 48). Radioactivity appeared in the eggs of laying hens fed ^{14}C-patulin (78). The metabolic products are not known.

Patulin is unstable under alkaline conditions (21) but is relatively stable under acidic conditions (21, 63, 80). The kinetics of destruction of patulin in aqueous solution by heat was studied by Lovett and Peeler (80).

Patulin reacts with sulfhydryl compounds such as cysteine and glutathione (36, 61). The reaction rate with gluthathione increases with increasing pH and the reaction products are non-toxic in tests involving chick embryo, rabbit skin and mice (61). This reaction may account for the disappearance of added patulin from apple juice and wheat flour (137), and from moist corn, wheat and sorghum (113). Its rate of disappearance from apple and grape juice, and dry corn is relatively slow. Patulin also reacts with SO_2 (113) and its antibacterial activity disappears in the presence of vitamin B_1 (162). Prompt analysis for patulin or appropriate storage of suspect feed is probably needed for the detection of patulin.

Methods of analysis of patulin in grain or grain products involving TLC or GLC have been described by Scott and Somers (137), Suzuki *et al* (156), Pohland *et al* (114) and Pero *et al* (109). An extensive review on patulin was published by Scott (134).

Like patulin, penicillic acid is an unsaturated lactone and was originally considered an antibiotic. In solution, it exists in two tautomeric forms, an open or keto-form and a lactone form (Figure 7).

Some of the *Penicillium* and *Aspergillus* spp. known to produce penicillic acid (29, 30) are common in grains and may become predominant under certain conditions of storage. In moist corn stored at temperatures of 1–10°C, *P. martensii*, *P. palitans*, *P. cyclopium* and *P. puberulum* produce large amounts of the toxin (29, 75).

The oral toxicity of penicillic acid is low; the oral LD_{50} in mice is 600 mg/kg (99). At

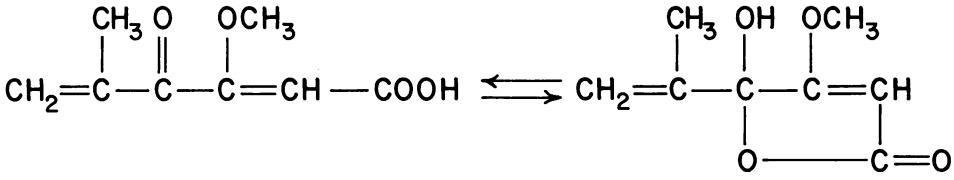

FIGURE 7. Penicillic acid.

lethal doses, penicillic acid administration induces fatty liver degeneration in quail and liver cell necrosis in mice (30). In pharmacological studies, penicillic acid was shown to dilate blood vessels and to have antidiuretic effects (99). Because of its high toxicity when administered at levels that are pharmacologically effective, penicillic acid has not come into use as an antibiotic (69).

Current interest in penicillic acid as a mycotoxin is partially based on its potential carcinogenicity. It produces transplantable sarcomas in rats that are injected subcutaneously, twice weekly, for 64 weeks at 1 mg/dose (37).

The toxicity of penicillic acid may be expected to be affected by other food components. Recovery of penicillic acid from sausage meat, flour and orange juice decreases with time after addition of the compound (30, 137). The toxin reacts with amines and amino acids. Reaction products of the sulfhydryl-containing amino acids glutathione and cysteine are non-toxic when administered intraperitoneally or orally to mice or quail, or when applied to rabbit skin. With the chick embryo test, they have reduced toxicity (30).

Penicillic acid has been detected in seven of 20 commercial corn samples at levels of 5–230 μg/kg and in five of 20 samples of commercial dried beans at levels of 11–179 μg/kg (161). Samples had been collected from areas in the United States with suspected mold problems. It has also been detected in moldy tobacco (149).

TLC methods for the toxin have been described by Ciegler and Kurtzman (28) and Scott and Somers (137). A GLC method also has been developed (109).

Sterigmatocystin consists of a xanthone nucleus attached to a bifuran structure and is a precursor of aflatoxin B_1 (Figure 8). In culture, *Aspergillus versicolor*, *A. nidulans*, *A. flavus*, *A. rugulosus*, *Penicillium luteum* (35) and *Bipolaris sorokiniana* (126) produce sterigmatocystin. Large quantities are produced by some strains of *A. versicolor* in moistened and autoclaved shredded wheat or rice. Most, if not all, isolates of *A. versicolor* produce sterigmatocystin (133).

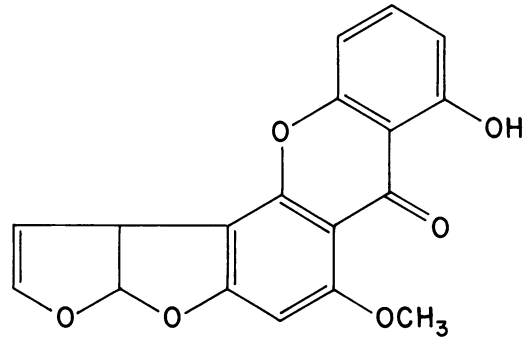

FIGURE 8. Sterigmatocystin.

It is difficult to arrive at a reliable estimate of the toxicity of sterigmatocystin because of its poor solubility in non-toxic solvents. Available data indicate it has low toxicity. The oral LD_{50} in mice is 800 mg/kg and the toxin is 125 times less effective in inducing liver hyperplasia in ducklings than aflatoxin B_1 (76). The oral LD_{50} in rats is 120–166 mg/kg but part of this toxicity may be attributable to the dimethylformamide used as solvent (124). Dissolved in dimethylsulfoxide, the LD_{50} (intraperitoneal) in vervet monkeys is 32 mg/kg (174). At high doses, the toxin causes necrosis of the liver and kidneys and bile duct proliferation.

The most outstanding biological activity of sterigmatocystin is its carcinogenicity. Incorporation in the diet of rats to provide a dose of 0.15–2.25 mg/day results in hepatocellular carcinoma (123). It is also carcinogenic when applied to the skin of rats (122).

Sterigmatocystin has been detected at low levels in one of two samples of green coffee beans unfit for further use, and in a sample of moldy wheat (120, 139).

Methods of analysis for sterigmatocystin in grains are available (150, 151).

SUMMARY

Diseases in animals associated with the consumption of moldy feed have been recognized for many years. Environmental conditions that favour mold growth on certain agricultural

products are known to occur in Canada but the extent to which mycotoxins are present in feed is not known. The number of reported outbreaks in Canada of acute disease in farm animals allegedly due to moldy feed is limited but it is likely that subclinical mycotoxicoses exist at certain times of the year in some farm livestock. The extent to which this influences animal productivity is largely unknown.

It is important to recognize that many mycotoxins have been identified in molds grown under laboratory conditions but that only a few have been detected in natural products. Some of these, i.e. zearalenone, ochratoxin A, citrinin and the aflatoxins have been linked to recognized animal diseases. It is to be expected that analytical procedures for toxins of the 12, 13-epoxytrichothecene group will be improved and that these toxins will prove to be important agents of disease associated with feed.

In order to determine the importance of mycotoxicosis in Canada, data will be required on the natural occurrence of known and as yet unidentified mycotoxins in suspect feed, the levels of toxin that induce disease in various farm animals, the nature of the toxic signs and the synergistic action of multiple mycotoxins.

Résumé

On connaît, depuis plusieurs années, la relation entre certaines maladies animales et l'ingestion d'aliments moisis. Au Canada, on sait qu'il existe des conditions environnantes favorisant la croissance de moisissures sur certains produits agricoles; on ignore cependant jusqu'à quel point les mycotoxines sont présentes dans les aliments. Le nombre de rapports concernant l'éruption de maladies aiguës chez les animaux de la ferme, vraisemblablement attribuables à l'ingestion d'aliments moisis, est limité, au Canada; il semble cependant que des mycotoxicoses sub-cliniques affectent des animaux domestiques, à certaines périodes de l'année. On ignore jusqu'à quel point cet état de choses influence la productivité animale.

Il est important de réaliser qu'on a identifié plusieurs mycotoxines dans les moisissures cultivées au laboratoire, mais qu'on en a décelé seulement quelques-unes dans les produits naturels. Certaines d'entre elles, v.g. la zéaralénone, l'ochratoxine A, la citrinine et les aflatoxines ont été associées à des maladies animales reconnues. Il faut s'attendre à ce que les procédés analytiques pour les toxines du groupe 12, 13-époxytrichothécène s'améliorent; ces toxines pourraient dès lors s'avérer des agents importants de maladies alimentaires.

Afin de préciser l'importance des mycotoxicoses au Canada, il nous faudra obtenir des données sur la présence de mycotoxines connues, mais pas encore identifiées, dans les aliments suspects, les concentrations de toxine qui provoquent la maladie chez les différents animaux de la ferme, la nature des signes toxiques et l'action synergique de plusieurs mycotoxines.

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BOOK REVIEW

Atlas of Veterinary Opthalmoscopy. L. F. Rubin. Published by Macmillan of Canada, Toronto, Ontario. 1974. 470 pages. Price $79.75.

This book deals with the ocular fundus, that portion of the globe seen through the pupil with an ophthalmoscopic device, in health and disease. Primarily the dog and cat but also horses, cattle, swine, sheep, rat, mouse, rabbit, guinea pig and monkey are dealt with. The use of ophthalmoscopy has increased recently and this book is an excellent source of information for those interested in ophthalmic diseases of animals. The price is high but not unreasonably so since almost every second page is a colour plate of fundus photographs with some histological sections (182 pages). Twenty-five illustrations are also included. There are ten chapters, the first and last being primarily narrative. The rest of the book is designed as an atlas with brief descriptions of the many colour plates and a few references for each condition included. Some normal findings are also given.

Chapter one is an introduction to techniques used in ophthalmoscopy ranging from the common direct and indirect ophthalmoscope to more research oriented techniques of electroretinography and fluorescein angiography. Chapter 10 is concerned with treatment but is extremely short since some general methods and comments were given but few details. The author in his preface states "The brevity of the section on treatment requires explanation. Insofar as I can determine, there are few controlled data concerning the specific treatment of the fundus. For these conditions in which the fundus lesion is a manifestation of the systemic disease, I suggest the reader consult standard texts describing the general condition".

Chapter 2 entitled Ophthalmoscopic interpretation is a general overview and provides the reader with a view of the types of lesions seen that are more specifically dealt with under specific diseases in later sections. Chapter 3 deals with the dog on 177 pages, Chapter 4 is concerned with the cat on 43 pages, Chapter 5 with the horses on 36 pages and Chapter 6 with the ruminants and pig but primarily the ox on 39 pages. The chapter on the rat and rabbit fundus (30 pages) also includes a bit on the mouse, and guinea pig. Chapter 8 (25 pages) deals with the monkey but also includes a plate covering a variety of primates. The second last chapter (25 pages) deals with the fundus lesions of various drug and chemical toxicities. Much of this information is not readily available elsewhere.

The conditions covered for each species include those caused by malformations, degenerations, infectious agents and neoplasias. Some syndromes not described elsewhere were presented. Because of the practical way the various entities are presented, this atlas can be used as a diagnostic aid and a means for self education by practicing veterinarians, dealing with both large and small animals, and veterinary students. It will also be of use to research workers and toxicologists using laboratory animals. Because of the very close relationship between clinical ophthalmology and ophthalmic pathology the book could also be useful for pathologists. *T. W. Dukes.*