The Toxicity of Ochratoxin to Ruminants

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ABSTRACT

Among the mold toxins the most toxic ochratoxin, ochratoxin A, commonly occurs in many grains, other feedstuffs, and in soil but in low concentrations. The amount required to produce acute toxicity in ruminants makes such occurrences unlikely. Toxic effects are more likely to occur in chronic low-level intoxication. The lethal single oral dose in cattle is high, probably being a few milligrams more than 13 mg/kg. The lethal level produced by repeated feeding to goats was 3 mg/kg. Ochratoxin A occurred in cows milk and urine but only when massive doses were ingested. Abortion or fetal death, though occurring in rodents, are unlikely to be induced in cattle.

RÉSUMÉ

Parmi les toxines des moisissures, l'ochratoxine la plus toxique, c'est-à-dire l'ochratoxine A, se retrouve souvent dans plusieurs grains, dans d'autres aliments et dans le sol, mais en faible concentration. La quantité requise pour provoquer une intoxication aiguë, chez les ruminants, rend cette dernière peu probable. Les effets toxiques sont plus susceptibles de se produire lors d'une intoxication chronique résultant de l'ingestion de plusieurs petites doses. Chez les ruminants, une seule dose orale létale est élevée et s'établit probablement à quelques milligrames au delà de 13 mg/kg. Chez la chèvre, la concentration létale d'ochratoxine A, résultant de l'ingestion répétée de cette toxine, se situait à 3 mg/kg. On ne décela de l'ochratoxine A que dans le lait et l'urine des vaches qui avaient ingéré des doses massives de cette toxine. Bien que de l'avortement ou de la mortalité foetale se produisent chez les rongeurs, il est peu probable qu'on puisse les provoquer chez la vache.

INTRODUCTION

Ochratoxins are a family of compounds with varying toxicity, with the general structure of β-phenylalanine linked by an amide bond to dihydroisocoumarin, the most toxic member being ochratoxin A. At least seven fungal species of the genus *Aspergillus* and six of the genus *Penicillium* are known to produce ochratoxins. The most common source in the United States of America is probably *Penicillium viridicatum* (14).

Despite extensive studies of ochratoxin A in various laboratory animal species and despite the compound's association with mold nephrosis of swine (8), we know of only one study in domestic ruminants. That project involved intravenous infusion of ochratoxin A into pregnant sheep (11). When one considers the time required to grow the fungus and purify even small quantities of the toxin or when one considers its high cost (U.S. $2,500/g) it is not surprising that amounts required for ruminant feeding are rarely available.

Most organisms which produce ochratoxin either produce other toxins or are found in association with organisms which do so. Toxins commonly found along with ochratoxin are citrinin, penicillic acid, hydroxyaspergillic acid and oxalic acid (7, 15, 17).

The toxin is considered as the cause of swine nephrosis in Denmark (8). In North America it has not been implicated in known clinical cases of mycotoxicosis nor is it widely suspected as a farm problem. Nevertheless, the frequency of its contamination of corn, oats, soybeans, buckwheat, barley, rye, rice and sorghum as well as in many nonanimal foodstuffs (4) causes concern. Of 127 moldy barley samples examined, ochratoxin was found in 18 (12).

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The highest concentration so far reported as occurring naturally is 27 ppm (15). The toxin is quite stable, 45% of a laboratory sample still being present after 12 weeks' storage (20). Similar persistence would be expected in nature. The causative fungi require greater than 16% moisture levels for growth but produce toxin over a wide range of environmental temperatures, apparently down to -2°C (10) or perhaps even lower. Even at optimum conditions, seven to 14 days' growth are required for production of sufficient toxin to cause intoxication.

Ochratoxin A causes nephropathy and hepatopathy in experimental animals (11, 19), thus it becomes suspect in cases wherein those syndromes occur and other causes have been eliminated from the differential diagnosis. Verification of such cause and effect has not been reported in domestic ruminants.

Pregnant ewes given ochratoxin A (1 mg/kg body weight) intravenously died in less than 24 hours without aborting (11). Death was attributed to either pulmonary congestion and edema or to massive liver necrosis. In this present paper we show that cattle fed up to 100 mg/kg of ochratoxin A convert almost all of the ochratoxin A to the much less toxic hydrolysis product, ochratoxin α. Thus the intravenous dose of 1 mg/kg of ochratoxin A is seen to be quite massive in comparison to that amount of ochratoxin A which might naturally enter the circulation.

Very little ochratoxin A apparently penetrates the ruminant placenta since no ochratoxin was found in the ewes amniotic fluid and fetal tissue levels were 1/400th to 1/1000th the levels in the maternal blood (11).

Other properties of ochratoxin A have been studied but their significance to ruminants is unknown. Ochratoxin A is nontumorigenic in rats and mice (2, 9, 13). However, when given with the noncarcinogen, sterulic acid to trout hepatomas resulted (3). The compound is teratogenic to mice (5). Lastly, orchratoxin A and penicillic acid often occur together and in such combination the toxicity of each is potentiated in chicken embryos, rats, mice and quail (9, A. Ciegler, personal communication, 1973).

This study was designed to determine how much ochratoxin A is toxic to cattle and goats and what are the lesions which are produced by the lethal dosage.

MATERIALS AND METHODS

Cattle

Two Holstein calves had been born and held in individual pens at the University's Charmany Farm. At five weeks of age they were given single doses of 11 mg/kg and 25 mg/kg of body weight ochratoxin A by stomach tube. The housing and diet remained unchanged.

Four Holstein cows which had been housed at the same farm since birth and were three to six months pregnant were used. Three were given ochratoxin A by stomach at daily doses of 0.2, 0.75 and 1.66 mg/kg for four to five days. The fourth cow was given 13.3 mg/kg as a single dose. As before, neither housing nor diet were changed.

Goats

Three American La Mancha female goats were purchased and held in individual pens. They were fed alfalfa hay and commercial dairy feed. After one week they were given daily oral doses of ochratoxin A at rates of 3, 2 and 1 mg/kg of body weight. The treatment period was to be 14 days but the goat treated at the rate of 3 mg/kg died on the sixth day. The latter goat was the only one that was pregnant.

Goats that did not die were stunned and exsanguinated. All calves and goats were necropsied and microscopic examination of the major organs was performed.

A number of hematological and urological parameters were examined in the goats. These are listed in Table I.

Milk and urine samples, collected once each morning and evening from each cow, were examined on thin layer chromatograms (TLC) for ochratoxins A and α (1). Milk was quick-frozen, freeze-dried, then stored at -20°C. It was extracted with methanol/water, centrifuged, filtered and the filtrate adjusted to pH 2.0. This was extracted with chloroform for TLC. Urine was merely adjusted to pH 2.0 and extracted with chloroform for TLC.

One and 10 μl of extract and of standards of ochratoxin A and α were spotted on 0.25 mm TLC plates of silica gel. The concentration of the standards was 0.006 mg/ml. After developing with benzene/ethyl acetate/formic acid (60:40:1), sam-

ples were examined under longwave ultraviolet light. Ochratoxin A had an Rf value of 0.5 and a blue-green fluorescence. Exposure to ammonia fumes caused the fluorescence to change to deep blue and deepened the blue of ochratoxin α. If the initial intensity of the extracts was not within the range of the two standards, the extracts were concentrated or diluted as necessary. Milk extracts required preliminary development of TLC plates with ether to move interfering oily substances to the top of the plate. Due to a natural yellow fluorescent substance in milk it was sometimes necessary to develop the plate a second time to detect ochratoxin α.

RESULTS

Both calves died within 24 hours. Epicardial hemorrhages were the only grossly detectable lesions. No changes were detected microscopically.

The cow given the single dose of ochratoxin A had difficulty in arising, diarrhea, anorexia and abrupt cessation of milk production, all commencing one day after dosing. Recovery was complete by day 4 but milk production never increased above one-third normal during that lactation period.

Cows treated at doses of 0.2, 0.75 and 1.66 mg/kg for four to five days remained clinically normal. All cows delivered normal calves.

The cow given the single 13.3 mg/kg dose had 650 mg of ochratoxin A and 4500 mg of ochratoxin α in the milk on the following day. There-after, only ochratoxin α could be detected. The cow given 1.66 mg/kg daily for four days had ochratoxin α in the milk on days 1 through 6 but traces of ochratoxin A were detected only on days 3, 4 and 5. Cows treated at lower than 1.66 mg/kg doses had no ochratoxin A in their milk. All cows had traces of ochratoxin α in milk and urine.

The goat treated with the dose of 3 mg/kg developed watery diarrhea, became dehydrated and died on day 5. There were no gross lesions. Microscopic lesions were confined to centrolobular cloudy swelling of the liver.

After 14 days of feeding, the goats treated at the doses of 2 mg/kg and 1 mg/kg had no clinical signs of illness or lesions when killed. Both apparently did have functional changes as shown in Table I.

DISCUSSION

The cow with signs of illness was treated with the dose of 13 mg/kg, which approaches the 20 mg/kg dose considered lethal for rats (13). Inasmuch as 13 mg/kg is about 23 times that which could be accomplished by feeding the most contaminated grain so far found in nature it is unlikely that acute poisoning will be a common farm problem. The lower of the two lethal single doses for calves in this study (11 mg/kg of body weight) if fed as contaminated grain at the rate of 2½ kg per day would require six times the highest level of contaminated grain so far detected in nature (15). Repeated feeding would likely require a somewhat lesser dose to be lethal.

Since the cow given 13.3 mg/kg had only transient illness, whereas calves given 11 and 25 mg/kg died, there appears to be a difference in age susceptibility. We consider the lesser toxicity in older animals to represent hydrolysis of ochratoxin A in the rumen for the following reasons: 1) prominent toxicosis developed in calves without a functional rumen, 2) as a preliminary to the present study we determined that ochratoxin A given intravenously to cattle resulted in only ochratoxin A in the urine, whereas a similar dose given orally resulted in urinary excretion of only ochratoxin α (19), 3) the rapidity (60 minutes) with which ochratoxin α could be detected in the urine of cows fed ochratoxin A and 4) there has been demonstrated hydrolysis of ochratoxin A to ochratoxin α and phenylalanine by normal rumenal contents extracted from the stomachs of cows (6). Contents of reticulum and omasum were also active but that of the abomasum was not.

The small size of goats makes them desirable substitutes for cattle in studies of material having limited availability such as ochratoxin A. The physiological and biochemical changes in goats fed ochra-

TABLE I. Physiological and Biochemical Parameters in Goats fed Ochratoxin A

	3 mg/kg		2 mg/kg		1 mg/kg	
	Pre-expos.	5th day	Pre-expos.	14th day	Pre-expos.	14th day
Respiration	32	24	48	40	24	28
Pulse	84	136	68	76	94	124
Temperature	102.8	100.2	102.7	103.4	102.3	102.6
Clotting time, minutes	6	6	7	4	5.5	7.5
Blood glucose (mg/dL)	72	202	61	40	35	97
Erythrocytes (10^6/mm^3)	5.6	25.8	9.6	10.5	7.9	14.8
Leucocytes (10^3/mm^3)	8.6	50.8	8.3	19.4	10.1	14.2
Hematocrit (%)	22	72	27	35	27	36
Hemoglobin (gm/dL)	6	17.7	9	9.8	8.5	10
Lymphocytes (% leucocytes)	61	19	70	32	61	49
Neutrophiles (% leucocytes)	38	71	27	64	39	40
Blood urea nitrogen (mg/dL)	13	80	19	26	19	28
Serum proteins (gm/dL)	7.0	9.8	6.4	7.4	7.5	7.3
Bilirubin (mg/dL)	—	—	0	1.1	—	—
Sodium (mEq/L)	—	—	177	150	—	—
Potassium (mEq/L)	—	—	4.8	4.1	—	—
SGOT (Karmen units)	48	110	39	200	25	60
SGPT (Wrobleski units)	6	2	5	12	6	2
LDH (Wacker units)	280	270	185	250	240	170
Alkaline phosphatase[a]	53	16	49	16	22	37
Isocitric dehydrogenase[b]	458	432	—	—	297	548
Leucine aminopeptidase[c]	1.375	2.225	—	—	2.075	2.425
Urine pH	8.5	5.0	7.5	6.6	8.5	8.4
Specific gravity	—	—	1.017	1.011	1.032	1.009
Urine protein (gm/dL)	trace	>100	0	0	trace	0
Urine glucose (Labstix)	0	0	0	0	0	0
Urine ketones (Labstix)	0	0	0	0	0	0
Urine blood (Labstix)	0	0	0	0	0	0
Urine bilirubin (mg/dL)	—	—	0	0.7	—	—
Urine creatinine (mg/dL)	—	—	—	—	100	20
Urine SGOT (Karmen units)	—	—	0	10	1	0
Urine SGPT (Wrobleski units)	—	—	0	7.9	0	1
Urine LDH (Wacker units)	—	—	0	2.5	—	—
Urine alkaline phosphatase[a]	—	—	29.5	1.7	—	—
Urine leucine aminopeptidase[c]	0	0	—	—	0	0
Urine isocitric dehyrogenase[b]	0	0	—	—	0	0

[a]King-Armstrong units
[b]Sigma units/ml
[c]μg B napthylamine/min/ml from leucine napthylamide

toxin A in part probably represented hemoconcentration due to fluid loss from diarrhea and polyuria. These changes were accompanied by a rapid, bounding pulse. The decline in lymphocytes and increase in neutrophils has also been described in swine fed ochratoxin A (16).

The goat's blood urea nitrogen content increased in all animals, however, microscopic kidney changes were minimal. Serum glutamic-oxalacetic transaminase activity increased in all cases and correlated with the hepatocellular degenerative changes which were the only degenerative changes encountered. At the doses of 3 and 2 mg/kg, serum alkaline phosphatase activity declined from 53 and 49 King-Armstrong units, respectively, to 16 units, whereas at the dosage of 1 mg/kg it increased slightly from the original 22 rising to 37 units. This latter alkaline phosphatase change is probably merely coincidental. However the decrease in animals fed higher levels may be treatment related but are without obvious explanation. Urine pH decreased markedly in the goat treated at the rate of 3 mg/kg presumably due to loss of bicarbonate by diarrhea with resultant acidosis. Urine enzyme determinations, except for leucine aminopeptidase and isocitric dehydrogenase, could not be made on the goat treated at the rate of 3 mg/kg because of insufficient sample size. These latter two enzymes were undetectable in all other animal urine samples. Other enzymes changed insignificantly in the 2 mg/kg animals. The specific gravity could be determined only in the goats treated at the rate of 2 mg/kg and 1 mg/kg. In both, it declined markedly.

It is our conclusion that ochratoxicosis, if it occurs naturally in adult ruminants, is most likely to result from prolonged ingestion and would likely result in loss of appetite and decreased milk production. If young calves were fed a sufficient amount of contaminated grain, more severe illness might occur.

As a consequence of our studies with ochratoxin and our experience with other mycotoxins in ruminants the following recommendation is made regarding feeding moldy feedstuffs to cattle. If it is necessary to feed moldy feed, dilute it as much as possible with good feed and feed this mixture for one week to a cow or steer old enough to have a functional rumen (i.e. four months). If problems do not occur, expand the feeding to include other cattle but avoid feeding such a mixture to lactating cattle.

Although ochratoxin A is known to accumulate in the kidney and muscle tissue of swine (8), we do not consider this a likely problem in ruminants because of ochratoxin hydrolysis within the rumen. Inasmuch as all farm grains contain fungi it is economically impossible to treat or discard all moldy feed and likewise unrealistic to reject as harmful the meat from such animals unless harmful residues have been demonstrated.

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