Ochratoxins

Ochratoxins comprise a series of related metabolites (at least 7 in all) isolated from several species of *Aspergillus* and *Penicillium* molds. Ochratoxins A, B, C, D, and their methyl and ethyl esters have been isolated. Ochratoxin A is thus far believed to be the most toxic and most common of the ochratoxins.

Sources

Ochratoxin is primarily a contaminant of feed grains and cereals. The toxin occurs in barley, sorghum and especially wheat. It has also been found in corn, dried beans, rye, oats, mixed feeds, and peanuts.

Occurrence

- Ochratoxin A is presumed to be the cause of porcine nephropathy seen in Denmark, Sweden, and the USA.
- Production by *Penicillium viridicatum* occurs at temperatures as low as 4 °C and with a moisture content of the affected grain of 18.5 to 40.4%.
- Concentrations reported in Denmark were as high as 27.5 ppm in barley.
- During the porcine nephropathy epidemic in Denmark, over 50% of the barley had 0.2 ppm; and 0.2 ppm can cause experimental nephropathy in pigs.
- Has often been detected in Swedish barley and oats as well as in Japanese rice.
- Was found in one hay sample and six wheat samples (at up to 6 ppm) from Western Canada.
- Detected in concentrations (1.0 to 2.7 ppm) believed to be toxic to swine and cattle in Iowa.
- In 1976, ochratoxin was believed to be the biggest problem occurring in poultry in North Carolina.
- Occurrence generally tends to be sporadic.
Susceptible Species

Potentially hazardous to livestock. Toxicity has been demonstrated in swine, ducklings, chickens, turkeys, and dogs.

Toxicity

- Synergistic or additive effects with aflatoxin, citrinin or penicillic acid (other mycotoxins) (see below).
- The toxicity of ochratoxin A is approximately equal to that of ochratoxin C, although ochratoxin A is much more commonly encountered. Ochratoxin B is several times less toxic.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ochratoxin A Oral LD50 mg/kg</th>
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<tbody>
<tr>
<td>Chicken</td>
<td>2 to 4</td>
</tr>
<tr>
<td>Turkey poult</td>
<td>6</td>
</tr>
<tr>
<td>Japanese quail</td>
<td>16.5</td>
</tr>
<tr>
<td>Rat</td>
<td>22 to 30</td>
</tr>
<tr>
<td>Mouse</td>
<td>22 to 58</td>
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</tbody>
</table>

- **Swine are very sensitive.** Doses of 1 to 3 mg/kg in swine caused considerable gastrointestinal and lymphoid damage.
- In swine, ochratoxin A at 1 or 2 mg/kg BW caused toxicosis, and death occurred within 5 to 6 days.
- Also in swine, dietary concentrations of 1 ppm for 3 months caused polydipsia and reduced growth rate and feed conversion in addition to impaired renal function.
- In swine microscopically detectable renal lesions were induced by as little as 0.2 ppm of ochratoxin in the diet.
- **Cattle are reportedly extremely resistant to ochratoxin A, but calves are sensitive** until the rumen is developed. Thirty-day-old calves given 0.1 to 0.5 mg/kg/day for 30 days displayed polyuria, CNS depression, decreased weight gain, urine of low specific gravity and dehydration.
- In calves, an approximate lethal dose is between 11 and 25 mg/kg BW while doses of 0.1 to 0.5 mg/kg produced only polyuria.
- A dose of 3 mg/kg BW **in dogs** produced acute toxicosis.
- In dogs, doses of 0.2 to 0.4 mg/kg caused clinical signs to develop over 10 to 14 days, while no clinical signs developed during 21 days of dosing at 0.1 mg/kg.
- In rats, average doses of 210 and 70 µg/kg (5 days/week) for 2 years caused decreased weight gains on the order of 12% less than controls and decreased urine concentrating ability, decreased kidney weights and renal tubular epithelial damage.
- Ochratoxin A has been shown to be teratogenic in rodents.
- **Chickens are very sensitive.** Decreased growth and enlarged kidneys occurred in chickens fed ochratoxin A at 1 ppm in the diet.

Interactions with Other Mycotoxins

- Ochratoxin may prevent fatty liver resulting from aflatoxin exposure (poultry). In the same study, however, the combination of ochratoxin and aflatoxin caused greater inhibition of growth when compared to aflatoxin alone.
- Doses of ochratoxin alone and penicillic acid alone, neither of which were lethal, caused 100% deaths when given simultaneously.

Mechanisms of Action

- Ochratoxin A may exert most of its effects as an inhibitor of protein synthesis. Ochratoxin inhibits protein synthesis by competition with phenylalanine in the phenylalanine-tRNA synthetase reaction.
- The kidney and spleen were more sensitive than the liver with regard to ochratoxin-induced protein synthesis inhibition.
- Ochratoxin A is immunosuppressive, causing decreased immunoglobulin production.
- Ochratoxins inhibit bovine carboxypeptidase A.
- Ochratoxin A is taken up by mitochondria and appears to inhibit several aspects of mitochondrial respiration.
- In the liver, ochratoxin A causes increased glycogen deposition in the cytoplasm of hepatocytes, and this increase is a sensitive indicator of ochratoxicosis.
- Low ochratoxin doses generally result in increased hepatic glycogen content, while high doses of the toxin cause decreased hepatic glycogen.
- In chickens fed ochratoxin at 2 to 8 ppm in the diet, there is a loss of collagen in the large intestine which may result in breaks and loss of integrity in the affected gut.
Ochratoxin A is a potent cause of decreased carotenoid pigmentation of chickens; the loss of yellow color from the fat of chickens decreases marketability. Even at 1 mg/gm (1 ppm) of diet the egg shells of chickens are stained; at higher concentrations, there are reductions in egg production, egg shells are poor or rubbery and the kidneys are damaged. The probability of hemorrhage may be increased by a multifactorial coagulopathy affecting factors VIII, V, and X in chickens. At 2 ppm and greater (dose related) in birds there is a decrease in breaking strength of bones and an increase in broken bones. Ochratoxin A has been associated with urinary tract cancers, but it is only weakly mutagenic. Ochratoxin A is a potent teratogen. Mice and/or rats exposed via their dams experienced malformations, fetal death, and occasional brain necrosis. Deformities also has been noted in hamsters and chickens, but not in pigs.

Absorption, Distribution, Metabolism and Excretion (ADME)

- Ochratoxins bind serum albumin (protein bound).
- Ochratoxin A is toxic in vivo and in vitro, suggesting that the molecule does not require metabolic activation to exert its effect. Pretreating an animal with phenobarbital to increase P-450 activity will cause an increase in the LD50 (lower sensitivity of the animal due to faster detoxification).
- The only metabolic reaction of ochratoxin A so far identified, is hydrolysis by carboxypeptidase A to break an amide bond. The reaction produces ochratoxin-alpha, which is much less toxic than the present compound. This reaction is especially prevalent in ruminant protozoa, and it is estimated that it allows cattle to tolerate up to 12 ppm of ochratoxin A in their feed.
- Ochratoxin A is believed to be hydrolyzed to a significant degree in the functional rumen.
- In spite of the ruminal destruction of some of the ingested ochratoxin, large doses reportedly result in some ochratoxin excretion in milk and may be associated with renal failure in cattle (see below).
- Whether differences occur among their cattle with regard to the ability of rumen flora to convert ochratoxin A to ochratoxin α, or whether other nephrotoxic mycotoxins may coexist, in hazardous concentrations, with ochratoxin A should be investigated.
- Ochratoxin A binds strongly to the proximal tubules of the kidney. Pigs exposed to ochratoxin A accumulate the toxin in the kidney, liver and muscle (in decreasing order) and these present the basis for some concern regarding residues (up to 67 ppb detected in kidneys) in human food products.
- The approximate half-life of ochratoxin A in swine tissues is 3 to 5 days, and little or no ochratoxin can be detected in kidney 30 days after exposure is terminated.
- In sheep, fetal blood concentrations are 100 to 400 times lower than maternal blood concentration.

Signs

- One disease syndrome described in swine was termed endemic porcine nephropathy. Porcine nephropathy was initially recognized in Denmark and Sweden, but cases have been seen in Iowa swine in several instances.
- Although high doses in swine may cause gastrointestinal effects as well as liver and lymphoid damage, much more common is the chronic renal failure syndrome.
- Acute exposure to ochratoxin A generally causes anorexia, polydipsia, dehydration and depression. Continued intake of toxic levels causes reductions in rate of gain and feed efficiency. Diarrhea may occur.
- Primary clinical signs include:
  - Polydipsia
  - Polyuria
  - Dilute urine
  - Reduced gain and feed efficiency
  - Cattle may be similarly, but much less commonly affected as compared to swine.
- Human endemic nephropathy in the Balkan countries (Yugoslavia, Bulgaria, Romania) of Europe (termed Balkan Endemic Nephropathy) has been associated with ochratoxin contamination of cereals and swine tissues. Other mycotoxins and other risk factors are also being investigated.
- Areas of high incidence of Balkan Nephropathy also have high rates of urinary tract cancer.
- Renal, hepatic, and mammary gland tumors were noted in laboratory rodents in response to dietary ochratoxin A exposure.
- In turkeys, one bite of ochratoxin containing feed causes immediate feed refusal, although this does not occur similarly in the chicken.
- In poultry, reduced egg production and egg shell quality may be seen.

Clinical Pathological Findings and Lesions

- Changes vary with the severity and duration of the syndrome.
- Acute toxicosis.
  - Clinical pathology.
    - Increased PCV and hemoglobin.
    - Increased blood urea nitrogen, total protein, and the activities of lactic dehydrogenase, isocitric dehydrogenase and
aspartate aminotransferase.
- Increasingly dilute urine, with increases in urine glucose and protein, and increases in urinary enzymes such as leucine aminopeptidase.
- Lesions vary with the dose and duration of exposure.
- Gross lesions in acutely poisoned animals may include:
  - Dehydration.
  - Enteritis.
  - Gastric and intestinal hyperemia.
  - Pale, swollen kidneys.
- Microscopic lesions in the acute form may include:
  - Proximal tubular (especially the P3 segment) necrosis and dilation.
  - Focal areas of intestinal mucosal damage in the lower intestine.
  - Prolonged exposure of animals to low levels of ochratoxin A causes a primary problem of nephropathy.
  - Exposure to high doses of ochratoxin A causes nephrosis, enteritis, lymphoid necrosis and hepatic necrosis.
  - A similar nephropathy in cattle fed ochratoxin-contaminated feedstuffs was also reported in Iowa.
  - Birds may have visceral gout and nephrosis.
- Gross lesions after chronic exposure may include:
  - Few lesions except for greyish white kidneys that are firm, and may have cystic dilation at the cut cortical surface.
  - Potential urinary tract tumors.
- Microscopic findings in the chronic form may include:
  - Mild degenerative changes in the tubular epithelium of the kidneys with prominent dilated tubules and interstitial fibrosis, and tubular atrophy. Basement membranes may be thickened and sclerosis of the glomeruli may be seen. The tubular epithelial nuclei become enlarged and nuclei become enlarged and necrotic. Thickened basement membranes may occur and dilated tubules with flat tubular epithelial cells may be seen.
  - Birds may have urate crystals and nephrosis with more chronic effects than in the acute form. Other lesions encountered experimentally were mentioned with regard to Mechanisms of Action (above).

**Diagnosis**

- Change of feed prior to the onset of illness.
- Feed may be moldy but this can be difficult to recognize due to differences between different areas of the feed and due to inability to recognize mold damaged feed after milling.
- Polydipsia, polyuria.
- Appropriate lesions, especially in the kidneys.
- Poor egg production with poor, stained, or rubbery shells in chickens.
- Detection of ochratoxin in the feed in toxic concentrations.
- Detection of ochratoxin-alpha in kidney, liver and skeletal muscle.
- Urine and feces may also be analyzed for ochratoxin.

**Treatment**

- Best to avoid in the first place by properly drying grains and storing feeds. The use of mold inhibitors can also be of value.
- In acutely exposed animals, such as dogs, appropriate measures to evacuate the gastrointestinal tract may be used with adsorbents and saline cathartics as indicated to remove ochratoxin containing feedstuffs.
  - **Note** - Not likely for animals to be presented with only a known recent exposure to ochratoxin, but might know of recent ingestion of mold-damaged grain or feed.
- Supportive care should be similar to that for other causes of renal failure.
Citrinin

Citrinin is probably a mycotoxin in search of a disease. In other words, the toxin has been recognized for several years, has known toxicity, primarily affecting the kidneys but is not known to actually cause clinical toxicosis.

![Citrinin Structure]

**Toxicity**

- Generally the toxicity of citrinin is low enough that concentrations found in naturally contaminated feedstuffs do not approximate those necessary to cause experimental toxicosis.
- LD50s range from 19 to 110 mg/kg in various species, using various routes of administration.

**Significance**

- May interact and coexist with ochratoxin and might potentiate the toxicity of the latter.
- May also coexist with other toxins such as patulin and oxalic acid.

**References**

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