EFFECT OF A HAY AND A GRAIN DIET ON THE BIOAVAILABILITY OF OCHRATOXIN A IN THE RUMEN OF SHEEP

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ABSTRACT

The role of the rumen and its contents in the detoxification of ochratoxin A (OA) was studied in sheep. The first experiment established that very little conversion of OA to α ochratoxin (Oα) occurs systematically; 90 to 97% of the OA and metabolites was recovered as unaltered OA in the urine. Most of the small amount of Oα recovered was also in the urine. In this experiment, two sheep were fasted and another two fed normally, but feed intake had no significant effect. In the second experiment, two sheep fed hay and two fed grain were dosed with OA at .5 mg/kg of BW into the rumen via a cannula. Recoveries, in urine and feces, accounted for 58 to 70% of the administered OA, but almost all (> 97%) was in the form of Oα. About 76 to 92% of this Oα was in the urine. Although excretion patterns and pharmacodynamics tended to differ with different diets, one of the sheep fed grain had very low intake and the results were equivocal. In the third experiment, eight sheep (four fed hay, four fed grain) were given a single intraruminal dose of OA (.5 mg/kg of BW). The disappearance of OA from the rumen and the corresponding formation of Oα was much faster for hay-fed than for grain-fed sheep; the half-lives were .63 and 2.7 h for OA and .9 and 1.9 h for Oα, respectively. The maximum concentration of Oα occurred 1.4 h after dosing hay-fed sheep (Cmax = 220 μg/ml), compared with 7 h in the grain-fed sheep (Cmax = 88 μg/ml). The relative bioavailability of OA for sheep fed grain was 4.3 times greater than that for the sheep fed hay. The results demonstrate that the rumen of sheep has an important role in the detoxification of OA and that type of diet affects this metabolism. It, therefore, affects the bioavailability of OA and probably its toxicity to the animal.

Key Words: Ochratoxins, Ruminants, Diets, Hydrolysis, Bioavailability


Introduction

Degradation of ochratoxin (OA) to its alpha metabolite (Oα) is known to be the principal means of detoxification of OA (Chu et al., 1972; Chu, 1974). Pitout (1969) demonstrated that OA is hydrolyzed to Oα by enzymes such as carboxypeptidase A, which is secreted by the pancreas of the animal. Hydrolysis of OA in the small intestine may also contribute to the detoxification of OA, as indicated by Doster and Sinnhuber (1972). Hult et al. (1976) and Kiessling et al. (1984) reported that OA was degraded to Oα by ruminal microbes, particularly by ruminal protozoa. Little was known, however, about the importance of the rumen in the detoxification of OA until Sreemannarayana et al. (1988) reported that the bioavailability of OA in ruminant calves was only 27 to 36% of that in preruminant calves.
calves, indicating that the functional rumen may greatly reduce the toxicity of OA. Recent studies in our laboratory (Xiao et al., 1991) have also suggested that the type of diet is important: the rate of in vitro hydrolysis of OA was more rapid with ruminal digesta from sheep fed hay than with ruminal digesta from those fed grain. These observations are in agreement with the observation that the numbers and types of microorganisms (include the protozoa) in the rumen of sheep are affected by the composition of the diet (Nakamura and Kanegasaki, 1969; Eadie and Mann, 1970) and, as indicated above, that ruminal protozoa are able to hydrolyze OA more efficiently than ruminal bacteria. The bioavailability of OA (i.e., the amount of OA reaching systemic circulation, Shargel and Yu, 1985) and its toxic effects should therefore be lower in sheep fed hay than in sheep fed grain. In this study, the influence of diet on the hydrolysis of OA in the rumen of sheep and its subsequent bioavailability was evaluated. Experiments involving intravenous (i.v.) and intraruminal administration of OA were conducted in which the concentrations of OA and Oα in the rumen, semm, urine, and feces were monitored.

**Materials and Methods**

*Source of Animals and Preparation of Diets and Ochratoxin.* A total of 16 Suffolk female sheep ranging from 20 to 50 kg were used in the three experiments. They were obtained from the University flock. Two diets were fed to the sheep, a hay diet and a grain diet. The hay diet was mainly full-bloom alfalfa. Trace minerals and salts were offered free-choice to all sheep. The grain diet contained 45.6% barley, 45.6% wheat and 8.8% other ingredients. The grain-fed sheep were adapted to the diet by slowly increasing the amount of grain in the diet.

Ochratoxin A was produced by surface liquid fermentation with *Aspergillus ochraceus*. The liquid media was concentrated to yield a sample containing 1,300 mg of OA/kg of dry material. It did not contain any of the other commonly known mycotoxins. The OA used for dosing into the rumen was dissolved in 1 M phosphate buffer, pH 5.5. The toxin was administered via the cannula into the mid-part of the rumen using a 20-ml syringe attached to a soft plastic tube (30 cm × 1.5 mm i.d.). Detailed information on the preparation of the toxin and the nature of the diet and feeding regimen have been described (Xiao et al., 1991).

Animal care and experimental procedures were approved by the local animal care committee. Animals were cared for under the guidelines laid down by the Canadian Council on Animal Care.

**Experiment 1.** Four female Suffolk sheep weighing approximately 50 kg were injected i.v. with 0.2 mg of OA/kg of BW. Two of the four sheep were fasted for 24 h before the injection and fed 4 h after injection; the other two were injected 1 h after feeding. All sheep were fed hay ad libitum for a 7-h period each day and were kept in individual metabolism crates.

Blood was collected from a catheter that had been implanted in the jugular vein 1 d before administration of the toxin. A second such catheter was used for injection of the OA. Urine was collected from an indwelling cystic catheter, which was also placed 1 d before administration of the toxin. Feces were collected in fecal bags (Frohlich et al., 1987). Blood samples were collected at the following times after injection of OA: 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 h and then every 12 h up to 144 h. Urine and feces were collected every 4 h up to 12 h then every 12 h up to 144 h, with the volume or weight being determined. Blood samples were allowed to clot, and, after centrifugation, serum was separated and frozen at −20°C until it was analyzed for OA and Oα. Urine and feces samples were also stored at −20°C before analysis.

**Experiment 2.** Four female Suffolk sheep were fitted with a ruminal cannula (Komarek, 1981). Two were adapted to a hay diet and two to a grain diet for 15 d. The method of insertion of the ruminal cannulas, preparation of diets, and feeding regimens have been described (Xiao et al., 1991). Each sheep was given a single dose of OA (.5 mg of OA/kg of BW). The toxin was put into the rumen 1 h after feeding. The pH of ruminal fluid was determined as described by Xiao et al. (1991). Blood samples were collected as in Exp. 1, except that only a single catheter was implanted into the jugular vein. The experiment

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was repeated after 2 mo with the same sheep under the same conditions. The average weight of the sheep ranged from 50 to 70 kg. Feed consumption in one of the grain-fed sheep was only about 30% of that of the other sheep and the pH of the ruminal contents was much higher in the former sheep. The experiment therefore consisted of three treatment groups: Treatment 1, two hay-fed sheep, repeated twice; Treatment 2, one grain-fed sheep (low feed intake) repeated twice; and Treatment 3, one grain-fed sheep (normal intake), repeated twice.

Experiment 3. Eight female Suffolk sheep weighing approximately 20 kg were fitted with ruminal cannulas (Komarek, 1981). Fecal bags and blood and urinary catheters were not fitted to sheep. Compared with Exp. 2, these changes resulted in easier management of the sheep and reduced the stress on the sheep. Four of the sheep received hay and the other four received grain daily ad libitum from 0900 until 1600, after which the remaining feed was removed. Ochratoxin A (.5 mg/kg of BW) was administered as a single dose into the rumen via the cannula 1 h after feeding. Blood samples were collected using a 3.9 cm long 18G gauge needle into a syringe at 0, 6, 12, 24, 48, and 72 h after dosing. The blood samples were prepared as described above. Rumen samples were collected into a 25-ml syringe connected to a 50-ml syringe at 0, 1, 2, 3, 4, 6, 12, 24, 48, and 72 h after dosing and were placed on ice. Immediately after the measurement of pH, the rumen samples were filtered through cheesecloth and stored at -20°C until they were extracted for the assay of OA and Oa.

Analytical Methods. Samples of serum, urine, feces, and ruminal fluid were extracted for OA and its metabolite, Oa, as described by Sreemannarayana et al. (1988). All samples except serum samples were subjected to reverse phase thin-layer chromatography cleanup (Frohlich et al., 1988). The HPLC analysis of OA was a modification of the procedure of Josefsson and Moller (1979). Before HPLC analysis the dry extracts were reconstituted in HPLC-grade methanol and were injected onto a 250-mm x 4.6-mm i.d. column containing 5 μm diameter C-18 bonded phase adsorbent and a 50-mm x 4.6-mm precolumn packed with CO:PELL C-18 ODS. The main column was maintained at 50°C. The mobile phase was methanol:water (70:30 vol/vol) acidified with a 1 M H3PO4 to pH 2.1 with a flow rate of 1.5 ml/min, except for the blood and urine samples. To facilitate the separation of OA and Oa from other interfering compounds, a two-solvent gradient system as used for blood and urine samples. Solvent A contained water adjusted to pH 2.1 with 1 M phosphoric acid, and solvent B consisted of methanol and isopropanol in the ratio of 90:10 (vol/vol). The percentage of solvent B was initially changed linearly from 60 to 70% over 3 min and then remained constant at 70% for the next 3 min. This was followed by two linear gradients in which solvent B changed from 75 to 85% over 2 min and from 85 to 60% over 1 min. During the last 2 min of the run and the 5-min equilibration period, the percentage of solvent B was 60%. The OA and Oa quantified fluorometrically with excitation at 333 nm and emission at 418 nm. All samples and standard were analyzed in duplicate. The retention times for Oa and OA were 4.4 and 8.9 min, respectively. The minimum detectable level for both OA and Oa was .05 ng/μl, and recoveries were 94%. Recoveries were estimated on the basis of recovery of OA from spiked samples of serum, urine, feces, and ruminal fluid.

Statistical and Pharmacokinetic Analysis. The concentration of OA in the serum as affected by time was subjected to pharmacokinetic analysis. The concentration-time data curve was fitted to a linear sum of exponential terms by use of an iterative, nonlinear, least squares regression technique as programmed in Nonlin (Metzler et al., 1973). The rate constants and coefficients from the computer-fitted function were used to calculate absorption, distribution, elimination, and disappearance half-lives of OA and Oa and the rate of formation of Oa. The area under the curve (AUC), which was used to estimate the relative bioavailability of both OA and Oa, was calculated according to the trapezoidal rule. Differences between dietary treatments were compared using the GLM procedure of SAS (1985).
Figure 1. Overall mean serum concentration of ochratoxin A (OA) in four sheep (two fed, two fasted) after intravenous injections of OA (2 mg/kg of BW). Each point represents mean ± SE (Exp. 1).

Results

Experiment 1. The four sheep (two fed and two fasted) seemed normal, except that urine production increased after the i.v. administration of OA. The concentration of OA in serum fell rapidly and toxin was not detectable 120 h after injection (Figure 1). No OA or other metabolites of OA were detected. The pharmacokinetic profile of OA showed that the disappearance of serum OA followed a bi-exponential decline. There were no differences (P > .05) in distribution or elimination of OA between fed and fasted sheep; half-life values ± SE were 1.5 ± 1 and 17.3 ± 1.4 h, respectively.

Cumulative excretion data (Table 1) showed that most of the recovered OA and metabolites (90 to 98%) were excreted as unchanged OA into the urine. Approximately 2.4% of the injected OA was excreted as OA in the urine and 4% in the feces. No intact OA was detected in the feces.

Experiment 2. No overt illness due to OA treatment was observed. One of the sheep fed grain was stressed somewhat because of irritation by the urinary catheter, as a result, it reduced its feed consumption to 30% of that of the other sheep. This resulted in a higher pH in the rumen compared to that in the rumen of the other grain-fed sheep. The pH values 2 h after feeding, in the rumens of grain-fed sheep with normal intake, sheep fed grain with 30% of normal intake, and sheep fed hay were 5.8, 6.5, and 6.9, respectively, with corresponding intakes of 1.5, .5, and 2.0 kg feed/d. After dosing, urine volume tended to increase in all sheep.

The average OA concentrations in serum at different times for sheep fed grain (normal intake), a reduced level of grain, or hay from both trials of the experiment are shown in Figure 2. The results suggest that the disappearance of OA from serum, as in Exp. 1,

TABLE 1. CUMULATIVE EXCRETION OF OCHRATOXIN A (OA) AND ITS METABOLITE (α OCHRATOXIN) BY SHEEP AFTER INTRAVENOUS INJECTION OF 2 mg OA/kg OF BODY WEIGHT (EXP. 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>OA Urine (mg)</th>
<th>OA Feces (mg)</th>
<th>OA Recovery of dose (%)</th>
<th>Ocα Urine (mg)</th>
<th>Ocα Feces (mg)</th>
<th>Ocα Recovery of dose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>2</td>
<td>5.13</td>
<td>0</td>
<td>.17</td>
<td>.12</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>2</td>
<td>5.70</td>
<td>0</td>
<td>.20</td>
<td>.41</td>
<td>60.7</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>1.69</td>
<td></td>
<td>.16</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cumulative excretion of α ochratoxin expressed in terms of OA equivalents as calculated from the molecular weight of OA/molecular weight of Ocα(403/256) x mg of Ocα.
followed a biphasic decline. Small secondary peaks were found after the initial absorption phase, suggesting that biliary recycling may have occurred. The absorption and elimination of OA for all treatment groups followed the same pattern and was not influenced by the dietary treatment (P > .05). The half-life values ± SE for absorption, distribution, and elimination of OA were 2.6 ± .1, 3.1 ± .1, and 33.8 ± 1.2 h, respectively.

Although the time to reach peak serum concentrations of OA (T\text{max}) was not significantly different for dietary treatment (grain or hay; overall T\text{max} = 4.2 ± .3 h), the concentrations (C\text{max}) were different. The relative bioavailability of OA in the serum expressed as AUC was approximately three times greater for sheep fed grain than for sheep fed hay or a small amount of grain (30%) (P < .05). The corresponding AUC (0 to 120 h) ± SE for these three groups were 7,232 ± 443, 1,853 ± 154, and 2,730 ± 403, respectively. Table 2 gives the cumulative excretion of OA and of OA in the urine and feces of sheep. Most of the administered OA (90 to 99%) that was recovered was converted to OA, and most of this compound was excreted in the urine. The total amount of OA excreted in the urine was also substantially greater in grain-fed sheep (.70 mg for normal intake, .25 mg for 30% of normal intake) than in hay-fed sheep (.09 mg) (P < .05).

Experiment 3. This experiment was conducted to confirm the results of Exp. 2. All sheep survived without overt illness or abnormal ruminal pH or feed uptake. The pH ± SE of the rumen contents of sheep fed hay or grain were 6.9 ± .1 and 5.6 ± .1, respectively.

Figure 3 shows the average ruminal concentration of OA in sheep fed hay or grain after being dosed with OA. The disappearance of OA from the rumen followed a monoeXponential decline that was more rapid for hay-fed sheep than for grain-fed sheep, and, as shown in Table 3, the corresponding half-life for disappearance of OA from the rumen was 4.2 times faster in sheep fed hay (.63 h) than in those fed grain (2.67 h) (P < .001). The total amount of OA in the rumen as determined by AUC for the rumen data (Table 3) was 4.3 times greater in sheep fed grain than in those fed hay (P < .01). The bioavailability of OA, as determined by AUC for the serum data (Table 3), was also 4.3 times greater in sheep fed the former than in sheep fed the latter diet.

![Figure 3. Mean ruminal concentration of ochratoxin A (OA) in sheep given a single intraruminal dose of OA (0.5 mg/kg of BW). Hay: hay-fed sheep (values represent mean ± SE of four sheep); Grain: grain-fed sheep (values represent mean ± SE of four sheep) (Exp. 3).](image)

TABLE 2. CUMULATIVE EXCRETION OF OCHRATOXIN A (OA) AND ITS METABOLITE, α OCHRATOXIN (OA), BY SHEEP GIVEN A SINGLE INTRARUMINAL DOSE OF OA (.5 mg/kg OF BODY WEIGHT) (EXP. 2)

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>n</th>
<th>Time periods</th>
<th>Cumulative excretion, mg\textsuperscript{a}</th>
<th>OA</th>
<th>OA</th>
<th>Recovery of dose, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Grain\textsuperscript{b}</td>
<td>1</td>
<td>2</td>
<td>.70</td>
<td>.07</td>
<td>22.28</td>
<td>1.89</td>
</tr>
<tr>
<td>Grain\textsuperscript{c}</td>
<td>1</td>
<td>2</td>
<td>.25</td>
<td>.06</td>
<td>16.63</td>
<td>3.72</td>
</tr>
<tr>
<td>Hay</td>
<td>2</td>
<td>2</td>
<td>.09</td>
<td>.15</td>
<td>12.02</td>
<td>3.86</td>
</tr>
<tr>
<td>SE</td>
<td>1</td>
<td></td>
<td>.10</td>
<td>.09</td>
<td>1.76</td>
<td>1.55</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Cumulative excretion of OA expressed in terms of OA equivalents according to: molecular weight of OA/molecular weight of OA (403/256) × mg of OA.

\textsuperscript{b} Feed intake was normal.

\textsuperscript{c} Feed intake was 30% of normal.
in the hay-fed sheep, whereas in the grain-fed sheep it reached a corresponding maximum concentration of 88 ng/ml at 7 h after dosing. After reaching the peak concentration Oα disappearance followed a bi-exponential decline in hay-fed sheep, whereas it followed a mono-exponential decline in grain-fed sheep. Overall, the amount of Oα formed, in terms of AUC, was 1.5 times greater in sheep fed hay than in those fed grain (P < .05). The half lives for the formation of Oα in the rumen of sheep fed hay or fed grain were .9 and 1.93 h, respectively. The half-life for the disappearance of Oα from the rumen was not affected by dietary treatment (P = .2).

Discussion

This study demonstrates that the rumen contents of sheep are able to hydrolyze efficiently OA and that the diet is an important factor affecting this hydrolysis and, therefore, the bioavailability of OA in sheep. In Exp. 1, involving i.v. injection of OA (.2 mg/kg BW), the disappearance of OA followed a bi-exponential decline, which suggests a two-compartment open model for the pharmacokinetics of OA, which is similar to that reported by Galtier et al. (1979). The disappearance half-life (half-life distribution) of OA from the

![Figure 4. Mean ruminal concentration of α ochratoxin (Oα) in sheep after a single intramrinal dose of ochratoxin A (.5 mg/kg of body weight). Hay: hay-fed sheep (values represent mean ± SE of four sheep); Grain: grain-fed sheep (values represent mean ± SE of four sheep) (Exp. 3).]

<table>
<thead>
<tr>
<th>TABLE 3. PHARMACOKINETIC PARAMETERS IN THE RUMEN CONTENTS AND SERUM FOR OCHRATOXIN A (OA) AND α OCHRATOXIN (Oα) AFTER INTRARUMINAL ADMINISTRATION OF OA (.5 mg/kg OF BODY WEIGHT) TO SHEEP FED DIETS CONTAINING GRAIN OR HAY (EXP. 3)</th>
</tr>
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<tbody>
<tr>
<td>Item</td>
</tr>
<tr>
<td>OA</td>
</tr>
<tr>
<td>( T_{1/2} ) disappearance in rumen(^a)</td>
</tr>
<tr>
<td>AUC, serum(^b)</td>
</tr>
<tr>
<td>Oα</td>
</tr>
<tr>
<td>( T_{1/2} ) formation in rumen(^c)</td>
</tr>
<tr>
<td>( T_{1/2} ) disappearance in rumen(^d)</td>
</tr>
<tr>
<td>C_{\text{max}} rumen(^e)</td>
</tr>
<tr>
<td>( T_{\text{max}} ) rumen(^f)</td>
</tr>
<tr>
<td>AUC, rumen(^g)</td>
</tr>
<tr>
<td>AUC, serum(^h)</td>
</tr>
</tbody>
</table>

\(^a\)Half-time (h) for disappearance of OA or Oα from the rumen.

\(^b\)Area under the concentration-time curve for OA or Oα (ng/ml x h) in the serum.

\(^c\)Half-life (h) for formation of Oα in the rumen.

\(^d\)The maximum concentration (ng/ml) of Oα found in the rumen.

\(^e\)The time (h) to reach maximum concentration of Oα in the rumen.

\(^f\)Area under the concentration-time curve for Oα (ng/ml x h) in the rumen.
serum of sheep was 1.5 h. This value is similar to those obtained in previous studies with cattle (1.8 h; Sreramnarayana et al., 1988), rats (2.1 h; Galtier et al., 1979), and rabbits (1.9 h; Galtier and Alvinerie, 1981), but not with chickens (.5 h; Galtier and Alvinerie, 1981) or pigs (8.0 h; Galtier and Alvinerie, 1981). These data suggest that OA is rapidly distributed between the central peripheral compartments of most animals. The elimination of OA, which was expressed as the elimination half-life, was 17.3 h. Among different species there are side differences in the serum half-life of OA. The values, as summarized by Kuiper-Goodman and Scott (1989), were 510 h in the rhesus monkey, 72 to 120 h in the pig, 77 h in the preruminant calf, 55 to 120 h in the rat, 24 to 39 h in the mouse, 8.2 h in the rabbit, 6.7 h in the quail, and 4.1 h in the chicken. The prolonged elimination half-life obtained with the first few classes of animals strongly suggested significant reabsorption/redistribution via enterohepatic circulation. The much shorter distribution half-life observed in the current study together with the small amount of OA excreted as Ox into the urine and feces of the injected sheep suggest the bilary recycling was limited in sheep.

The results of the experiments in which OA was administered intraruminally suggest that the rumen contents of sheep may play an important role in the detoxification of OA. Cumulative excretion data from Exp. 2 indicated that about 90 to 99% of the toxin was converted to the nontoxic metabolite, Ox. Cumulative excretion data showed that only .5 to 3% of the original dose of OA was found in the urine. The amount of OA reaching the circulatory system was influenced by the type of diet. The systemic exposure of OA, expressed in terms of AUC (0 to 120 h), was found to be only 25.6% as extensive in sheep fed hay as in those fed grain. The effects of diet on the hydrolysis of OA and consequent reduction in the bioavailability of OA was most clearly shown in Exp. 3. The results demonstrated that after receiving a single oral intraruminal dose of OA the rate of disappearance of OA from the rumen was 4.2 times faster in sheep fed hay than in sheep fed grain, and the relative bioavailability of OA for sheep fed grain was 4.3 times greater than for sheep fed hay. It was also shown that the rate of formation of the OA metabolite, Ox, was much greater in the rumen of sheep fed hay than in the rumen of those fed grain. The time to reach the maximum concentration of Ox was five times shorter in sheep fed hay than in those fed grain. The disappearance of Ox from the rumen of hay-fed sheep followed a biexponential decline. The reason for this is not clear, but it may have been due to the attachment of Ox to the microbes in sheep fed hay.

The present results and those of a previous study in our laboratory (Xiao et al., 1991) indicate that diet influences the pH and probably the type of microflora that developed in the rumen. Diets that promote rapid fermentation in the rumen result in rapid production of VFA, which is usually associated not only with a reduction in pH of the rumen, but also with a change in the microbial population, partially the ruminal protozoa (Jouany et al., 1988). Presumably, diet affected the population of a protozoa (Eadie and Mann, 1970), and, as a result, the rate of hydrolysis of OA (Kiesling et al., 1984) was reduced in sheep fed grain compared with those fed hay. From a practical view, OA-contaminated grain should be fed to animals consuming a diet containing a high proportion of hay rather than grain. Under these conditions, OA would be efficiently hydrolyzed in the rumen and, therefore, its toxic effects should be greatly reduced. Research however, needs to be carried out to establish relationships among percentage of grain in the diet, relative rates of hydrolysis of OA, and corresponding toxic effects of OA to the animal.

Although diet may affect the rate of hydrolysis of OA and therefore amount taken up by the systemic circulation, it did not affect the half-life for overall absorption, distribution, and elimination of OA. Also, the very long persistence of OA in the rumen of grain-fed sheep and Ox in both grain-fed and hay-fed sheep would suggest either that direct absorption of Ox across the rumen epithelium either does not occur or that it is a slow process. In contrast to these results, Ox, but not OA, seemed to be rapidly eliminated from the blood of sheep; in all cases the concentration of Ox in urine was high relative to OA. These data indicate that Ox is cleared at a sufficiently rapid rate via the
kidney to prevent its accumulation in the blood. Finally, the toxicity of OA to the ruminant was not established in this study. Nevertheless, a single small dose of OA seemed to increase the volume of urine. Continued administration of a high concentration of OA probably would result in a more severe problem and presumably would affect the ruminal epithelia.

The extent of hydrolysis of OA in the lower part of the gastrointestinal tract was not determined in the current study. Hydrolysis was probably not significant, because a previous study (Sreemannarayana et al., 1988) demonstrated that a functional rumen in calves serves as a primary and major site for the detoxification of ingested OA which, if inactive, as in the preruminant, or circumvented, as shown in the current study by i.v. administration of OA, will permit excessive systemic accumulations of the toxin. This conclusion is also supported by unpublished data from our laboratory. These studies demonstrated that OA is rapidly hydrolyzed by digesta from the cecum and large intestine of rats, in vitro, but there is very little hydrolysis of OA with digesta from the stomach or small intestine. The cecum and large intestine, nevertheless, probably would not have significantly contributed to the hydrolysis of OA in the current study because any of the OA that escaped hydrolysis in the rumen would have been absorbed, mainly in the upper portion of the gastrointestinal tract (Kumagai and Aibara, 1982). Further definitive studies need to be conducted to establish the degree of detoxification of OA in the digestive system of nonruminants and the role of biliary recycling.

Implications

The current study demonstrated that very little ochratoxin A was hydrolyzed to α-ochratoxin when it was administered i.v. to fed or fasted sheep, whereas considerable hydrolysis of ochratoxin A occurred when it was administered intraruminally. The disappearance of ochratoxin A from the rumen and the corresponding appearance of α-ochratoxin was much faster in sheep fed hay than in those fed diets containing grain. The corresponding concentration of ochratoxin A in the blood (bioavailability) was also much lower in hay-fed than in grain-fed sheep. The results show not only that the rumen of sheep has an important role in the metabolism of ochratoxin A, but also that the type of diet is of great importance.

Literature Cited

BIOAVAILABILITY OF OCHRATOXIN IN RUMINANTS