Metabolism of Aflatoxin, Ochratoxin, Zearalenone, and Three Trichothecenes by Intact Rumen Fluid, Rumen Protozoa, and Rumen Bacteria

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The effect of rumen microbes on six mycotoxins (aflatoxin B_1, ochratoxin A, zearalenone, T-2 toxin, diacetoxyscirpenol, and deoxynivalenol) considered to be health risks for domestic animals was investigated. The mycotoxins were incubated with intact rumen fluid or fractions of rumen protozoa and bacteria from sheep and cattle in the presence or absence of milled feed. Rumen fluid had no effect on aflatoxin B_1 and deoxynivalenol. The remaining four mycotoxins were all metabolized, and protozoa were more active than bacteria. Metabolism of ochratoxin A, zearalenone, and diacetoxyscirpenol was moderately or slightly inhibited by addition of milled feed in vitro. The capacity of rumen fluid to degrade ochratoxin A decreased after feeding, but this activity was gradually restored by the next feeding time. Ochratoxin A was cleaved to ochratoxin α and phenylalanine; zearalenone was reduced to α-zearalenol and to a lesser degree to β-zearalenol; diacetoxyscirpenol and T-2 toxin were deacetylated to monoacetoxyscirpenol and HT-2 toxin, respectively. Feeding of 5 ppm (5 mg/kg) of ochratoxin A to sheep revealed 14 ppb (14 ng/ml) of ochratoxin A and ochratoxin α in rumen fluid after 1 h, but neither was detected in the blood. Whether such conversions in the rumen fluid may be considered as a first line of defense against toxic compounds present in the diet is briefly discussed.

Aflatoxin, ochratoxin A, and the *Fusarium* toxins zearalenone, T-2 toxin, diacetoxyscirpenol (DAS), and deoxynivalenol (DON) are considered to be potential health hazards for domestic ruminants. The degradation of ochratoxin A by ruminants was described by Hult et al. (3), who incubated it with the contents from the four stomachs of the cow. It was concluded that ochratoxin A was cleaved into the nontoxic ochratoxin α and phenylalanine by contents from all compartments except the abomasum. Kallela and Vasenius (4) studied the degradation of various amounts of zearalenone by bovine rumen fluid. After 48 h of incubation, the zearalenone content decreased by an average of 37.5%. No attempt was made to identify any metabolites. In the present study, we have further investigated the effect of the bacteria and protozoa in rumen fluid on the six mycotoxins mentioned above and the degree to which the presence of feed affects this interaction.

MATERIALS AND METHODS

Rumen fluid was taken from a fistulated sheep or cow 5 h after feeding, or otherwise as stated below. After filtration through cheesecloth to remove large feed particles, the fluid was used directly or separated into bacterial and protozoal fractions. To obtain the fractions, rumen fluid was centrifuged at 200 × *g* for 10 min. The pellet, which contained the protozoa and some bacteria, was diluted with buffer to the same volume as the supernatant, which contained the bulk of the bacteria. The rumen fluid was defaunated of protozoa by adding dioctyl sodium sulfosuccinate (OT)–1 mg of rumen fluid per ml, by the method of Orpin (11).

Mycotoxins (types and amounts are specified below) were incubated anaerobically under CO_2 in a shaking water bath at 38°C. The incubation contained 5 ml of buffer (1) and 5 ml

of rumen fluid or rumen microorganisms (see above). Incubation times are given below. Incubation was stopped by adding 1.5 ml of 1 M H_3PO_4. The toxins were extracted twice, each time with 25 ml of $CHCl_3$, except for the trichothecenes which were extracted twice, each time with 25 ml of ethyl acetate.

Ochratoxin A was analyzed according to Hult and Gatenbeck (2). A 5-ml portion of aflatoxin B_1 extract was diluted with 5 ml of hexane and injected through a Sep-Pak silica column (Waters Associates, Inc.). The column was washed with 5 ml of dry ethyl ether, and aflatoxin was eluted with 20 ml of 95% chloroform–5% methanol. The eluate was subsequently evaporated and separated on precoated thin-layer chromatography plates (Silica 60; Merck & Co., Inc.) by using 90% chloroform–10% acetone as the solvent. Aflatoxin was quantified by thin-layer chromatography fluorometry (excitation, 365 nm; emission, 450 nm). The zearalenone extract was filtered, evaporated, and dissolved in 2 ml of $CHCl_3$. The chloroform was injected through a Sep-Pak silica column. Zearalenone and the metabolites α- and β-zearalenol were eluted with 15 ml of 97% chloroform–3% ethanol, the eluate being subsequently evaporated and dissolved in CH_2Cl_2 for separation and quantification by high-pressure liquid chromatography. The conditions for this were as follows: column, μ-Porasil (Waters); mobile-phase, 1% ethanol and 1% acetic acid in water-saturated CH_2Cl_2; flow rate, 1.2 ml/min; and UV detection, 280 nm. The trichothecene extraction and clean-up procedures were partly adopted from T. Möller (personal communication). The extract was first filtered and evaporated. The dry extract was then dissolved in 50 ml of hexane and extracted with 25 ml of 15% methanol in 0.1% NaCl. The water phase was evaporated and dissolved in 5 ml of 20% methanol, which was injected through an activated Sep-Pak C-18 column. The Sep-Pak column was washed with 7 ml of 20% methanol, and the trichothecenes were eluted with 10 ml of 85% methanol.

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TABLE 1. Degradation of ochratoxin, zearalenone, aflatoxin, DAS, T-2 toxin, and DON by sheep rumen fluid alone and sheep rumen fluid with feed addition[a]

Mycotoxin	Concn (mg/liter)[b]	Activity		% Inhibition by feed addition
		Rumen fluid (μg/ h × liter)	Rumen fluid + feed (μg/h × liter)	
Ochratoxin A	0.2	155 ± 10 (6)	110 ± 14 (5)	29
Ochratoxin A	4.7	517 ± 58 (4)	−[c]	−
Zearalenone	2.8	367 ± 83 (6)	281 ± 80 (6)	23
Aflatoxin B_1	0.2	0 (5)	−	−
DAS	5.0	845 ± 45 (6)	795 ± 96 (6)	6
T-2 toxin	20.0	1,730 ± 384 (4)	−	−
DON	2.5	0 (3)	−	−

[a] Activity is expressed as mean values ± standard deviation, and the number of determinations is given within parentheses. Incubation times were 0.5 to 3 h.

[b] Toxin concentrations have been adapted to detection limits, which explains the high T-2 toxin concentration used.

[c] −, Not determined.

The eluate was evaporated and transferred to a vial for derivatization. Separation and quantification were performed by gas chromatography with a flame ionization detector under the following conditions: glass column (2 mm by 6 ft [ca. 182.88 cm]) packed with 3% OV-17 on 100-200 mesh Gas-Chrom Q; nitrogen flow rate, 30 ml/min; injection block temperature, 250°C; temperature program from 190 to 260°C at 4°/min; initial temperature period, 6 min; and detector temperature, 300°C.

The recoveries and detection limits of the toxins were: ochratoxin A, 98% and 75 ng; aflatoxin B_1, 85% and 150 ng; zearalenone, 85% and 2 μg; DON, 43% and 2 μg; DAS, 82% and 2 μg; and T-2 toxin, 67% and 10 μg. The detection limits of the metabolites were: α-zearalenol, 0.5 μg; β-zearalenol, 1.0 μg; monoacetoxyscirpenol, 2 μg; and HT-2 toxin, 5 μg.

RESULTS

The ability of rumen microorganisms from sheep to degrade certain important mycotoxins was studied in vitro, and degradation rates are given in Table 1. A slight decrease in the amount of aflatoxin B_1 occurred within 30 min, but after this time no further reduction was evident. A similar effect was found with buffer alone. The degradation of ochratoxin A was measured at five different concentrations (0.24 to 4.6 mg/liter), and the activity was found to vary between 0.06 and 0.52 mg/h × liter of rumen fluid. Treatment of the values according to simple enzyme kinetics gives a maximum activity of 0.56 mg/h × liter and a concentration at half-maximal activity of 2.1 mg/liter.

The rumen fluid sampled 5 h after feeding was comparatively poor in feed constituents. Addition of milled feed of the same composition as that of the diet inhibited the degradation (Table 1). When rumen fluid was sampled at various times after feeding the sheep and tested for ochratoxin-degrading activity, the highest activity was found just before feeding, and the lowest (about one-third) was found 1 h afterwards, after which the activity increased until the next feeding (Fig. 1).

To find out whether toxin degradation was performed by protozoa or bacteria, rumen fluid was either fractionated by centrifugation or defaunated with OT. A majority of the activity against ochratoxin, zearalenone, DAS, and T-2 toxin could be ascribed to the protozoa (Fig. 2). Pretreatment with OT before analysis of the trichothecenes DAS and T-2 toxin

could not be performed, due to interference between OT and the toxins in the analysis method.

Our results show that DAS, T-2 toxin, ochratoxin A, and zearalenone were degraded by rumen microorganisms, but the protozoa are invariably more active than the bacteria (Fig. 2). In the presence of ochratoxin A and zearalenone, the bacterial fraction is almost inactive. Only minor differences were observed between rumen fluid from sheep and cattle in the rate of mycotoxin metabolism. Aflatoxin B_1 and DON were not degraded by rumen microorganisms. Ochratoxin A was cleaved primarily into ochratoxin α and phenylalanine, as described by Hult et al. (3). More than 90% of the zearalenone was degraded to zearalenol, and about twice as much α-zearalenol as β-zearalenol was formed. DAS was rapidly and completely deacetylated to monoacetoxyscirpenol (Fig. 3A). T-2 toxin was similarly deacetylated to HT-2 toxin (Fig. 3B).

The faunal composition of protozoa in the rumen can vary with the dietary composition (8). A fistulated sheep was, therefore, fed a high-concentrate ration (hay plus concentrate, 3:7 [wt/wt]) for 12 days. The ability of the rumen fluid from this sheep to degrade ochratoxin A was compared with that of the same sheep fed a normal ration (hay plus concentrate, 5:4 [wt/wt]). Ability to degrade fell by about 20% after feeding with the high-concentrate ration.

Ochratoxin A was added to the diet at two concentrations (2 and 5 ppm [2 and 5 mg/kg]) and fed to a fistulated sheep. The 2-ppm concentration ration was given for 4 days, followed by the 5-ppm concentration for 2 days. Rumen fluid and blood were sampled at various times after feeding and analyzed for ochratoxin content. When feeding 2 ppm, no ochratoxin A was found in rumen fluid samples half an hour or more after feeding. With 5 ppm in the diet, rumen concentrations were 29 and 14 ppb (29 and 14 ng/ml) at 0.5 and 1 h after feeding, respectively. Ochratoxin α was found in all samples. No ochratoxin A or ochratoxin α was found in blood samples taken 1 h after feeding.

DISCUSSION

The metabolism of ingested material by the ruminal microbes may be considered as a first line of defense against toxic materials present in the diet. On the other hand, ruminants may be at a disadvantage if substances become toxic as a result of the action of ruminal microbes.

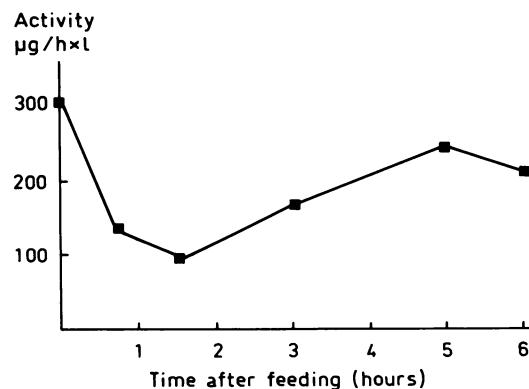Our results indicate very mixed effects when different

FIG. 1. Degradation of ochratoxin A by sheep rumen fluid sampled at various times after feeding. Incubation time was 0.5 h, and initial concentration of ochratoxin A was 0.2 mg/liter.
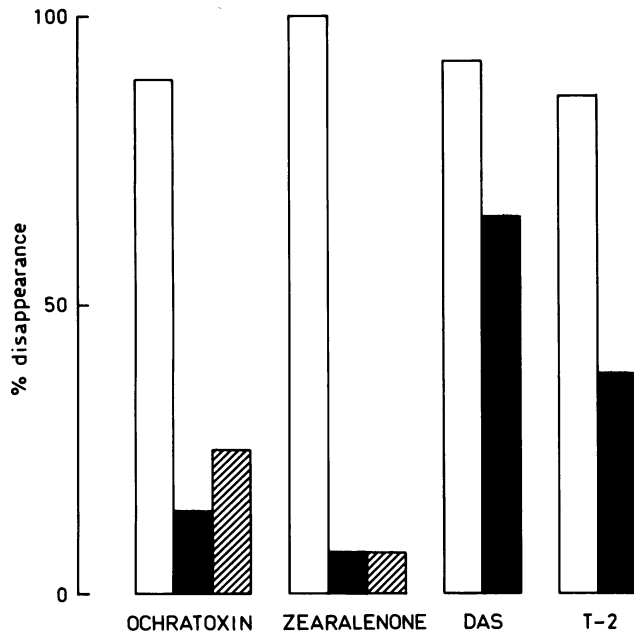
FIG. 2. Disappearance of ochratoxin, zearalenone, and trichothecenes in protozoa (□), bacteria (■), and rumen fluid treated with OT (▨). Rumen fluid from sheep was used. The results are expressed as the percentage of the disappearance in intact rumen fluid. Incubation times and initial concentration were as follows: ochratoxin A, 4 h and 0.2 mg/liter; zearalenone, 3 h and 2.8 mg/liter; DAS, 0.5 h and 5 mg/liter; and T-2 toxin, 0.5 h and 20 mg/liter.

mycotoxins are exposed to the rumen microorganisms. The protozoa are invariably more active than the bacteria (Fig. 2). In the presence of ochratoxin A and zearalenone, bacteria are almost inactive. Only minor differences were observed in the rate of mycotoxin metabolism between rumen fluid from sheep and cattle.

The capacity of rumen fluid to degrade ochratoxin A varied according to the interval between feeding and sampling. Whether this is an effect of feed inhibition, or a variation in the population of protozoa, is not known, since a

similar variation in the number of protozoa in rumen fluid has been observed by Michalowski and Muszyński (7). Addition of milled feed to the sheep rumen fluid in vitro inhibited rumen fluid activity moderately with zearalenone and ochratoxin A as substrates and only slightly with DAS (Table 1).

Whether the observed metabolic effects on four of the six mycotoxins studied can be considered as evidence of a rumen defense mechanism must be evaluated from case to case. Conversion of ochratoxin A into ochratoxin α is, without question, a detoxification process, because ochratoxin α is nontoxic as regards renal effects in pigs (12). Deacetylation of DAS and T-2 toxin probably would also reduce toxicity, because the products monoacetoxyscirpenol and HT-2 toxin are less active inhibitors of protein synthesis than are their mother compounds (9). Kallela and Vasenius (4) showed that the amount of zearalenone was decreased by ruminal digestion and concluded that the estrogenic effect of the toxin was most likely reduced too. In the present study, we have shown that the decrease in zearalenone was the result of a reduction to zearalenol, mainly α-zearalenol. This product has three to four times as much estrogenic activity as does the parent compound (1a). Whether the reduction of zearalenone to zearalenol involves an increase or a decrease in toxicity depends on which mode of action is applied to the zearalenone. According to Kiang et al. (5), zearalenone and zearalenol compete with estradiol at its cytosolic receptor sites. In this case, the microbial metabolism of zearalenone to zearalenol gives a product with toxicity at least as high for the host animal as that of zearalenone itself. The reduction of zearalenone to zearalenol also increases the polarity, which may influence not only excretion but also the uptake from the intestines into the blood stream. An additional mechanism for zearalenone action was suggested by Kiessling and Pettersson (6) and Olsen et al. (10). They showed that the conversion of zearalenone to zearalenol in the liver was catalyzed by a hydroxysteroid dehydrogenase, which is normally involved in the metabolism of steroids. In other words, the presence of zearalenone may lead to a disturbance of the normal steroid metabolism. If a combination of liver metabolism and the receptor site competition is applied to the action of zearalenone, the conversion of zearalenone to zearalenol in
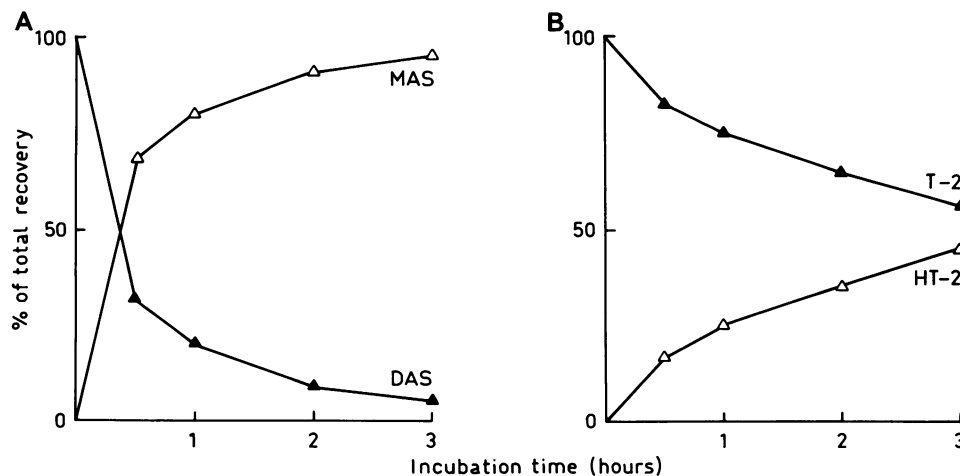

FIG. 3. Degradation of trichothecenes by sheep rumen fluid. Formation of monoacetoxyscirpenol from DAS (A), and formation of HT-2 toxin from T-2 toxin (B). DAS (25 μg) and T-2 toxin (100 μg) were added to the incubations.

the rumen should lead to a product which is less toxic to the host animal.

For the microbe itself, increased water solubility normally means a detoxification due to an increased rate of excretion, but for the host the same process is double edged. Increased polarity not only facilitates excretion, but also changes the uptake from the intestines into the blood stream. Thus, no single process can explain the processing of mycotoxins in the ruminants from the point of view of the ruminant.

ACKNOWLEDGMENTS

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