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Effects of Antibacterial Agents on In Vitro Ovine Ruminal Biotransformation of the Hepatotoxic Pyrrolizidine Alkaloid Jacobine

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Ingestion of pyrrolizidine alkaloids, naturally occurring plant toxins, causes illness and death in a number of animal species. *Senecio jacobaea* pyrrolizidine alkaloids cause significant economic losses due to livestock poisoning, particularly in the Pacific Northwest. Some sheep are resistant to pyrrolizidine alkaloid poisoning, because ovine ruminal biotransformation detoxifies free pyrrolizidine alkaloids in digesta. Antibacterial agents modify ruminal fermentation. Pretreatment with antibacterial agents may account for some animal variability in resistance to pyrrolizidine alkaloid toxicosis, and antibacterial agents can also be used for characterizing ruminal pyrrolizidine alkaloid-biotransforming microflora. The objective of this study was to evaluate the effects of antibacterial agents on biotransformation of a predominant *S. jacobaea* pyrrolizidine alkaloid, jacobine, in ovine ruminal contents. Ovine ruminal jacobine biotransformation was tested in vitro with 20 independent antibacterial agents. Low amounts of rifampin and erythromycin prevented jacobine biotransformation. Chlortetracycline, lasalocid, monensin, penicillin G, and tetracycline were slightly less effective at inhibiting jacobine biotransformation. Bacitracin, crystal violet, kanamycin, and neomycin were moderately inhibitory against jacobine biotransformation. Brilliant green, chloramphenicol, gramicidin, nalidixic acid, polymyxin B SO_4, sodium azide, streptomycin, sulfisoxazole, and vancomycin had little to no effect on jacobine biotransformation. The antibiotics that were most effective at inhibiting biotransformation were those that are active against gram-positive bacteria. Therefore, gram-positive bacteria are most likely critical members of the jacobine-biotransforming consortia.

Toxic pyrrolizidine alkaloids (PA) are esters of necine ring structures (Fig. 1) and are found naturally in a variety of plant species (4, 23, 24). Some PA cause cirrhosis of the liver and some cause lung damage, leading to death (32). In the western United States, multimillion dollar livestock losses result from the poisoning of cattle and horses because of ingestion of tansy ragwort, *Senecio jacobaea* (10, 11, 34). Ingestion of plants which contain PA is also a worldwide problem, affecting animal and human populations, because of PA in various weeds, herbal remedies, and contaminated food and feed (17).

Although *S. jacobaea* PA are toxic for cattle, sheep are resistant to PA toxicosis (4, 5, 34). Several authors have concluded that hepatic factors in sheep convey resistance to that species, but new evidence from in vivo and in vitro studies has demonstrated that ovine ruminal bacterial biotransformation results in detoxification of ingested *S. jacobaea* prior to systemic absorption of PA (9, 10, 38). On the basis of thin-layer chromatography (TLC) and high-pressure liquid chromatography results, ovine ruminal *S. jacobaea* PA biotransformation involves, at the minimum, ester hydrolysis, releasing the dicarboxylic acid structure from the bicyclic, heterocyclic necine base. Similar hydrolysis, along with modification of the necine base, was seen for the single ester of heliotrine (19, 20). This hydrolytic transformation significantly decreases and may eliminate the toxicity of the compound (23, 24). Although the microorganisms that biotransform *S. jacobaea* PA have not been identified, they have been enumerated at approximately 3×10^7 to 5×10^7 per g of ovine ruminal contents, by a most-probable-number procedure (38). In the same ruminal contents, the bacterial flora biotransformed *S. jacobaea* PA at rates of approximately 25 mg/liter/h (38). However, in experimental conditions, the abilities of sheep ruminal contents to biotransform *S. jacobaea* PAs were variable, such that some animals were unable to transform the alkaloids quickly (1 or 2 days), while others transformed typical ruminal levels of PA (100 μg/ml) within 2 h. Since biotransformation of PA is mediated by bacteria, antibacterial agents (AA) are hypothesized to modify or eradicate ovine PA-biotransforming ability.

Four to six PA are found in *S. jacobaea* (33). Jacobine is the most persistent (33, 38), as well as being present in the largest amount in *S. jacobaea* collected in Oregon's Willamette Valley. Therefore, jacobine was selected as the most reliable index of ovine ruminal biotransformation of *S. jacobaea* PA for the present study.

The primary objective was to determine the effects of common antibacterial treatments on biotransformation of jacobine in ovine ruminal contents in vitro. Specifically, those AA which were expected to inhibit PA biotransformation in ovine ruminal contents were tested on the bases of research by earlier workers with analogous alkaloids and antibiotic resistance properties of ruminal bacteria (13–15, 19, 20).

MATERIALS AND METHODS

Media. The in vitro assay buffer (a modified McDougall buffer) contained (in grams per liter): Na_2CO_3, 4; KCl, 0.57; Na_2HPO_4, 9.3; NaCl, 0.47; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.04; $(NH_4)_2SO_4$, 2.64, and resazurin, 0.002 (25). The assay buffer was made anaerobic by the Hungate technique

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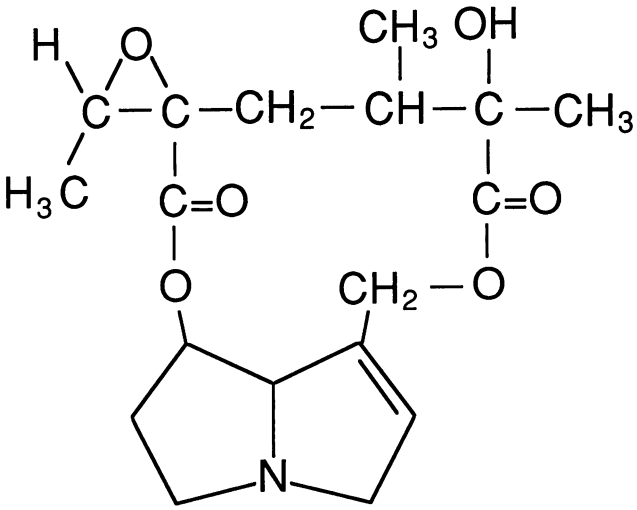

FIG. 1. Structure of jacobine.

(3, 8) (by being boiled under a continuous stream of oxygen-free CO_2), sealed, and stored in an anaerobic glovebox (atmosphere: CO_2-H_2, 95:5) until used. Immediately prior to usage, purified *S. jacobaea* PA (11) was added as a filter-sterilized, 10-mg/ml solution (dissolved in 1% [vol/vol] H_3PO_4 and then titrated to pH 7.0 with 10 M NaOH), for a final total PA concentration of 200 μg/ml. A separately prepared, PA-free, modified McDougall buffer was used for initial pretreatment of ruminal samples. This sample preparation buffer was the same as the assay buffer, except that $NaHCO_3$ (9.8 g/liter) replaced the Na_2CO_3, the oxygen-free gas phase was N_2-CO_2 (70:30), and no PA solution was added.

Antibiotic treatments. AA were dissolved or suspended in the in vitro anaerobic assay buffer immediately prior to use and serially diluted in the anaerobic glovebox to the appropriate concentrations. In initial experiments, each AA was tested with the 10-, 100-, and 1,000-μg/ml dose. In trials which showed complete inhibition at 10 μg/ml, a 1-μg/ml antibiotic concentration was also tested in subsequent experiments.

PA biotransformation assay. The PA biotransformation assay was an in vitro incubation of ovine ruminal contents with added PA. Three healthy, 75-kg rumen-cannulated ewes (maintained on 1.4 kg of Purina Sheep Checkers plus a grass and alfalfa hay mixture fed ad libitum) were sampled for ruminal contents at 3 h after feeding. The ewes had been fed 5% *S. jacobaea* pellets for 3 weeks, approximately 1 month prior to sampling. At the time of sampling, the ruminal contents were manually mixed inside the rumen, and then approximately 100 g of combined ruminal solids and liquor was removed and transported anaerobically to the laboratory. Mixed ruminal contents were anaerobically blended with 50 ml of sample preparation buffer at high speed for 1 min. Blended ruminal contents were transferred to the anaerobic glovebox and dispensed into prepared assay tubes (1 ml of blended ruminal material plus 3 ml of AA-treated buffer). The tubes were stoppered and incubated at 30°C and sampled at 24 and 48 h and 8 days. Aliquots of samples were anaerobically removed and frozen (−14°C) until analysis by TLC. Samples were analyzed less than 3 weeks following collection. The ruminal contents from the three ewes were assayed on separate occasions by using duplicate assay tubes.

TLC of PA. Thawed samples (0.5 ml) were transferred to 1.5-ml microcentrifuge tubes, and 0.05 ml of 5 M NaOH was added. After mixing, 0.5 ml of methylene chloride was added, and the samples were vortexed to form an emulsion. The aqueous and solvent layers were resolved by centrifugation (16,000 × *g*, 5 min), and the methylene chloride layer was transferred to fresh tubes and evaporated to dryness under a vacuum. The residue was suspended in 0.025 ml of methylene chloride, and 4 μl was spotted onto HP-KF silica gel TLC plates (Whatman International, Maidstone, England). Chromatograms were developed in chloroform-methanol-propionic acid (72:18:10) solvent. Alkaloid-containing spots were visualized by sequential spraying with Dragendorff spray reagent (Sigma Co., St. Louis, Mo.) and 50-g/liter sodium nitrite solution. Standards of purified *S. jacobaea* PA and chromatographically pure jacobine were placed on each TLC plate (11). The R_f for jacobine was 0.81. Minor changes in the molecule, such as reduction of the epoxide, resulted in a changed, usually larger, R_f and (in some cases) differences in the color reaction. Lack of PA spots, compared with PA standards, was indicative of more significant transformation, such as hydrolysis of the lactone from the necine base or possibly modification or degradation of the necine base itself. These more significant changes represent significant reductions, if not elimination, of the alkaloid's toxicity (23, 24). Only lack of PA spots, and not just a changed R_f, was used to indicate jacobine transformation in this project. The detection limit was 3 μg/ml.

RESULTS

Animal variability. Ruminal contents from each ewe were assayed for PA biotransformation, without AA, prior to testing with AA. Jacobine dropped to the TLC detection limit for ewe 1 and ewe 2 by 9 h of incubation, but 12 to 20 h was required for PA biotransformation in ruminal contents of ewe 3. Because of the eightfold dilution of ruminal contents for this assay, these numbers indicated disappearance of jacobine from whole ruminal contents within approximately 1.1 h (ewes 1 and 2) to 1.5 to 2.5 h (ewe 3); this PA biotransformation was probably faster than would occur for intact ruminal contents, because of the stimulating effect of dilution in this experimental protocol (21). The variation among ewes was also seen in the subsequent AA assay results (Table 1), because the slowing of PA transformation, due to AA treatments, was more evident in the ruminal contents from ewe 3, compared with those in the other ewes.

Effects of AA. The minimum PA transformation-inhibiting concentration (MPTIC) was defined as the lowest concentration of AA which prevented jacobine biotransformation as determined by the presence of a jacobine spot on the TLC plate. The MPTICs of each antibiotic for the three sampling periods are in Tables 1 to 3. In general, the tables are grouped by the ability of AA to prevent biotransformation of jacobine from the least inhibitory groups to the most inhibitory on the basis the results for ewes 1 and 2.

The earliest results, at 24 h of incubation, demonstrated acute effects of AA on jacobine biotransformation (Table 1). On the basis of the results from ewes 1 and 2, erythromycin and rifampin were inhibitory at the lowest tested concentrations (<1 μg/ml), and many of the AA (chlorotetracycline, tetracycline, crystal violet, kanamycin, ionophores, penicillin G, and bacitracin) were inhibitory at 10 μg/ml. Neomycin was inhibitory at 100 μg/ml. The remaining AA (brilliant

TABLE 1. Ratings for antibacterial treatments, based on inhibition of PA biotransformation after 24 h

Antibacterial treatment	24-h MPTIC[a]		
	Ewe 1	Ewe 2	Ewe 3
Brilliant green	1,000	1,000	ND
Chloramphenicol	1,000	1,000	<10
Gramicidin	1,000	>1,000	<10
Nalidixic acid	1,000	1,000	<10
Polymyxin B sulfate	1,000	1,000	<10
Sodium azide	1,000	>1,000	100
Streptomycin	1,000	>1,000	<10
Sulfisoxazole	>1,000	>1,000	<10
Vancomycin	1,000	1,000	<10
Neomycin	100	100	<10
Chlortetracycline	10	10	<1
Crystal violet	10	100	1
Kanamycin	10	<10	<10
Lasalocid	10	1	10
Monensin	10	10	1
Penicillin G	10	10	10
Bacitracin	<10	<10	<10
Tetracycline	<10	<10	<10
Erythromycin	<1	<1	<1
Rifampin	<1	<1	<1

[a] Values indicate the lowest concentrations for antibacterial agents at which TLC spots for jacobine were still present after 24 h of incubation. <, the lowest concentration tested was inhibitory; >, the highest concentration tested was not inhibitory; ND, not determined.

TABLE 3. Ratings for antibacterial treatments, based on inhibition of PA biotransformation after 8 days

Antibacterial treatment	8-day MPTIC[a]		
	Ewe 1	Ewe 2	Ewe 3
Brilliant green	>1,000	>1,000	>1,000
Chloramphenicol	>1,000	1,000	1,000
Gramicidin	>1,000	>1,000	>1,000
Nalidixic acid	>1,000	>1,000	>1,000
Sodium azide	>1,000	>1,000	1,000
Sulfisoxazole	>1,000	>1,000	1,000
Kanamycin	1,000	1,000	1,000
Polymyxin B sulfate	1,000	1,000	1,000
Streptomycin	1,000	>1,000	>1,000
Vancomycin	1,000	1,000	1,000
Bacitracin	100	100	<10
Crystal violet	>100	>100	100
Lasalocid	100	100	10
Neomycin	100	100	100
Penicillin G	100	100	100
Erythromycin	10	10	10
Chlortetracycline	10	1,000	100
Monensin	10	1,000	10
Tetracycline	10	100	100
Rifampin	<1	<1	<1

[a] Values indicate the lowest concentrations for antibacterial agents at which TLC spots for jacobine were still present after 8 days of incubation. <, the lowest concentration tested was inhibitory; >, the highest concentration tested was not inhibitory.

green, chloramphenicol, gramicidin, nalidixic acid, polymyxin B SO_4, sodium azide, streptomycin, sulfisoxazole, and vancomycin) were inhibitory only at 1,000 μg/ml or not at all. In general, the ruminal contents of ewe 3 were considerably more sensitive to the AA, because jacobine biotransformation for that animal already occurred at a slower rate. Therefore, the resultant MPTICs were lower for most AA with ewe 3.

TABLE 2. Ratings for antibacterial treatments, based on inhibition of PA biotransformation after 48 h

Antibacterial treatment	48-h MPTIC[a]		
	Ewe 1	Ewe 2	Ewe 3
Brilliant green	1,000	1,000	>1,000
Chloramphenicol	1,000	1,000	1,000
Gramicidin	>1,000	>1,000	100
Nalidixic acid	1,000	1,000	100
Polymyxin B sulfate	1,000	1,000	100
Sodium azide	>1,000	>1,000	100
Streptomycin	1,000	>1,000	1,000
Sulfisoxazole	>1,000	>1,000	10
Vancomycin	1,000	1,000	100
Bacitracin	100	ND	<10
Crystal violet	>100	100	100
Kanamycin	100	100	<10
Neomycin	100	100	<10
Chlortetracycline	10	10	10
Lasalocid	10	1	10
Monensin	10	10	10
Penicillin G	10	10	10
Tetracycline	10	100	<10
Erythromycin	<1	10	10
Rifampin	<1	<1	<1

[a] Values indicate the lowest concentrations for antibacterial agents at which TLC spots for jacobine were still present after 48 h of incubation. <, the lowest concentration tested was inhibitory; >, the highest concentration tested was not inhibitory; ND, not determined.

By the second day of incubation (48 h), PA biotransformation occurred for the lower concentrations of three AA which had prevented jacobine disappearance earlier (Table 2). This occurred for ruminal contents from ewes 1 and 2 when bacitracin, crystal violet, and kanamycin were used, increasing the MPTIC from 10 to 100 μg/ml. Results for the remaining treatments were the same as those at 24 h. The MPTIC for ewe 3 approached the values for the other ewes, indicating that the lower values at 24 h were due to slowing of the biotransformation rate at low AA concentrations rather than to bactericidal activity.

The results for day 8 indicated that the earlier MPTIC concentrations of most of the AA were bactericidal for critical PA-biotransforming bacteria, because jacobine persisted in most of the tubes where biotransformation had not already occurred (ewes 1 and 2 [Table 3]). Exceptions were seen for erythromycin, increasing the MPTIC to 10 μg/ml; lasalocid and penicillin G, increasing the MPTIC to 100 μg/ml; and kanamycin, increasing the MPTIC to 1,000 μg/ml. For the case of erythromycin, the antibiotic itself was visualized by the TLC procedure as four distinct spots. Those spots did not decrease in intensity with incubation, so the change in MPTIC for that AA was due to changes in the bacterial population (adaptation) rather than detoxification of erythromycin. The other AA did not appear on the TLC, so this observation was not possible. The changes of MPTIC for those AA may have been due to population adaptation or modification or detoxification of AA. In addition, the results for ewe 3 generally caught up with those for ewes 1 and 2. This effect for ewe 3 indicated that low concentrations of AA (below the MPTIC for ewes 1 and 2) slowed the rate of jacobine biotransformation in the ruminal contents of ewe 3, while higher concentrations of AA were bactericidal for the critical PA-biotransforming bacteria when used above the

TABLE 4. Approximate ruminal concentrations for AA used as feed additives or in veterinary therapeutics[a]

AA	24-h MPTIC[b] (μg/ml)	Peak ruminal amt (μg/ml)	Species	Purpose
Bacitracin	<10	0.3–0.7	Bovine	Production[c]
		0.7–2.3	Bovine	Prevent liver abscess
Chlortetracycline	<1–10	0.2–0.7	Bovine	Production
		3.2	Bovine	Prevent shipping fever
		6.9–19	Bovine	Prevent anaplasmosis
		9.5	Ovine	Prevent abortion
Lasalocid	1–10	0.6–1.9	Bovine	Production
		8.3	Bovine	Control coccidiosis
		1.8–8.4	Ovine	Prevent coccidiosis
Monensin	1–10	0.5–3.3	Bovine	Production
		1.1	Caprine	Prevent coccidiosis
Neomycin	<10–100	30	Bovine	Treat dysentery and enteritis
		30	Ovine	Treat dysentery and enteritis
Tetracycline	<10	38	Bovine	Treat enteritis and pneumonia

[a] Compiled from references 2 and 27, with calculations based on ruminal size amounting to 108 liters for a 900-kg bovine or 8.4 liters for a 70-kg ovine or caprine, based on calculations from reference 16.
[b] Summarized from Table 1.
[c] Production is used to indicate usage for production improvement, including increased rate of weight gain and increased feed efficiency.

MPTIC. Finally, variability between ewes 1 and 2 appeared to increase by 8 days. This increase in variability has also been seen for pure cultures with long incubation times, indicating adaptation to, or detoxification of, the AA (14).

DISCUSSION

These experiments were performed in a manner similar to that used to obtain an MIC (1), and the concentration distribution for many of the AA corresponded to the ranges used for MIC with selected ruminal bacteria (39). However, these experiments were different from MIC determination, because jacobine biotransformation may involve multiple bacterial species (19, 20); rumen bacterial suspensions, instead of pure cultures, were used; three sample intervals, rather than a single endpoint, were used; and jacobine transformation, rather than complete inhibition of cell growth (40), was used as the test condition. A reduction in the rate of jacobine biotransformation, as opposed to preventing that process, indicated a capability to adapt to, or detoxify, the AA or that the AA caused a slowing of biotransformation, rather than bactericidal activity. Because of these differences from MIC, the MPTIC was used to describe the results. The MPTIC reflects the effects of AA on the PA-biotransforming population rather than a single bacterial species. Therefore, if a chain of events, involving multiple species, is needed for PA biotransformation, then the interruption of any step in that chain of events will prevent PA biotransformation.

Earlier efforts to isolate and identify PA detoxifying activity have been described for the nonmacrocyclic PA helotrine and lasiocarpine (19, 20, 31). Nonmacrocyclic PA are less toxic and more easily biotransformed compared with macrocyclic PA (20, 24). Ruminal biotransformation of *S. jacobaea* macrocyclic PA, such as jacobine, was not seen during early experiments by others (35, 36), but subsequent work has been more successful (38). The improved results are probably due to a combination of factors, such as utilization of ruminal solids (21), anaerobic technique, blending, attention to diurnal effects (uniform sampling times), differences in animal diets, preexposure to PA, and individual differences among animals. Individual differences among ewes, which shared housing and feed, suggest that the ability to degrade PAs is not as contagious as nitrotoxin degradation (7). Sources of individual differences were not tested, but may have included rumination or feeding behavior, ruminal flow rates, and effects of cannulation on ruminal motility.

Among AA which were tested in this project, the 24-h MPTIC was above expected peak ruminal concentrations of bacitracin and lasalocid (Table 4). However, the MPTIC ranges for chlortetracycline and monensin overlapped the expected peak ruminal concentrations. Therefore, when these antibiotics are used as feed additives for growth promotion, they may inhibit PA biotransformation. The expected peak ruminal concentration of bacitracin, when used as prophylaxis, is also below the MPTIC. Prophylactic and therapeutic levels of chlortetracycline, lasalocid, monensin, neomycin, and tetracycline are also near, or higher than, the levels that prevented PA biotransformation. In some cases, the higher 8-day MPTIC indicates that PA-transforming bacteria might adapt to the AA, but this possibility needs additional investigation.

Addition of AA to rumen bacterial suspensions is expected to influence various subpopulations, including bacteria which directly modify PA; bacteria which, as a result of their metabolic activities, indirectly enhance PA biotransformation; and bacteria which compete in some way against PA transformation. These influences are expected to occur in vivo, as well as in vitro. In those cases in which AA had little or no effect on jacobine biotransformation, it is unlikely that the AA was detrimental to detoxification, and it is possible that the AA enhanced that process by inhibiting competitive subpopulations. The positive effects of selected AA on PA biotransformation are under investigation, both as methods to isolate PA-biotransforming bacteria and as potential methods to improve PA detoxification in vivo. The negative effects of other AA on jacobine biotransformation could be

TABLE 5. Antibacterial activity of selected AA for ruminal bacteria[a]

AA	Resistance or sensitivity to AA[b]												
	Gram-negative species						Gram-positive species						
	Megasphaera elsdenii	*Prevotella ruminicola*	*Selenomonas ruminantium*	*Ruminobacter amylophilus*	*Succinivibrio dextrinosolvens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	*Eubacterium ruminantium*	*Eubacterium limosum*	*Lachnospira multiparus*	*Butyrivibrio fibrisolvens*	*Ruminococcus albus*	*Ruminococcus flavefaciens*
Streptomycin	r	r	v	v	r	s	r	s	r	r	v	r	r
Polymyxin B SO_4	r	v	s	r	s	s	r	r	r	sa	s	s	s
Kanamycin	r	r	s	v	s	r	r	r	r	r	s	s	v
Vancomycin	r	s	v	v	s	s	s	s	s	r	s	s	s
Neomycin	r	sa	v	r	s	s	r	r	r	r	v	r	s
Lasalocid	r	sa	r	r	r	sa	s	s	NT	s	s	s	s
Monensin	r	sa	r	r	r	sa	s	s	s	s	s	s	s
Bacitracin	v	s	v	v	s	s	v	s	s	s	s	s	s
Chlortetracycline	v	sa	s	sa	s	s	s	s	s	s	s	s	s
Erythromycin	s	s	v	s	s	s	s	s	s	s	s	s	s
Chloramphenicol	s	s	s	s	s	s	s	s	s	s	s	s	s
Tetracycline	s	sa	s	s	s	s	s	s	s	s	s	s	r
Penicillin G	sa	sa	sa	sa	s	s	sa	s	s	s	sa	s	s

[a] Compiled from references 2, 6, 12–15, 26, 28–30, and 39.

[b] Resistance (r), sensitivity (s), or sensitivity followed by adaptation (sa) was based on evaluations in the cited references by using inhibition zones (antibiotic disk studies) or MICs. Results which conflicted within or among references are indicated as variable (v). NT, not tested.

due either to selective toxicity against bacteria which are beneficial for jacobine biotransformation or to more general toxicity against the overall ruminal population. At the highest concentrations, some of the AA probably inhibited many bacterial activities, but at the lower concentrations, near the MPTIC, checks of fermentation acids by gas liquid chromatography indicated that most, if not all, fermentation activity remained similar in the amounts of total products (data not shown) for most AA. This was expected, because the lower amounts of AA were often similar to, or less than, levels that are used in veterinary therapy or feed additives (Table 4).

Many of the AA have been tested with pure cultures of ruminal bacteria (Table 5). These AA can be grouped as ruminal broad spectrum (bacitracin, chlortetracycline, erythromycin, chloramphenicol, tetracycline, and penicillin G), anti-gram positive (the ionophores monensin and lasalocid), and species specific, which do not exhibit a clear pattern of rumen antibacterial activity. These activities vary somewhat from patterns for human therapies, which are based on realistic serum concentrations (not digesta levels) of antibiotics (18). The ruminal broad-spectrum antibiotics were the most inhibitory to PA biotransformation, except chloramphenicol, which is not inhibitory in the mixed rumen ecosystem, and streptomycin, which does not inhibit most ruminal bacteria. The ionophores inhibited PA biotransformation at approximate levels (1 to 10 μg/ml) which inhibit gram-positive but not gram-negative ruminal bacteria (26, 28, 29, 37, 39, 40). Crystal violet, which inhibited PA biotransformation, generally inhibits gram-positive bacteria (18). Sodium azide, which generally inhibits gram-negative bacteria (22), was less inhibitory for PA biotransformation. On the basis of the pattern of inhibition, these data suggest that gram-positive type bacteria are critical for PA biotransformation in the ovine rumen. This result will be helpful for additional research in isolating PA-transforming bacteria, as well as research with animal resistance to PA toxicity. For example, treatments which increase relative numbers of gram-positive bacteria might improve PA biotransformation in vivo, while treatments which inhibit PA biotransformation can be avoided. Tests of the effects of AA as feed additives on PA detoxification in the rumen remain to be performed. Additional investigation with AA which did not inhibit PA biotransformation is also under way.

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REFERENCES

1. **Anhalt, J. P., L. D. Sabath, and A. L. Barry.** 1980. Special tests: bactericidal activity, activity of antimicrobics in combination, and detection of β-lactamase production, p. 478–484. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
2. **Bennett, K. (ed.).** 1991. Compendium of veterinary products, 1st ed., p. 10–516. North American Compendiums, Inc., Port Huron, Mich.
3. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25:**1324–1328.
4. **Cheeke, P. R.** 1984. Comparative toxicity and metabolism of pyrrolizidine alkaloids in ruminants and nonruminant species. Can. J. Anim. Sci. **64**(Suppl.)**:**201–202.
5. **Cheeke, P. R.** 1988. Toxicity and metabolism of pyrrolizidine alkaloids. J. Anim. Sci. **66:**2343–2350.
6. **Chen, M., and M. J. Wolin.** 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Appl. Environ. Microbiol. **38:**72–77.
7. **Cheng, K. J., R. C. Phillippe, G. C. Kozub, W. Majak, and J. W. Costerton.** 1985. Induction of nitrate and nitrite metabolism in bovine rumen fluid and the transfer of this capacity to untreated animals. Can. J. Anim. Sci. **65:**647–652.
8. **Costilow, R. N.** 1981. Biophysical factors in growth, p. 66–78. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of

methods for general bacteriology. American Society for Microbiology, Washington, D.C.

9. **Craig, A. M., L. L. Blythe, E. D. Lassen, and M. L. Slizeski.** 1986. Resistance of sheep to pyrrolizidine alkaloids. Isr. J. Vet. Med. **42:**376–384.
10. **Craig, A. M., C. J. Latham, L. L. Blythe, W. B. Schmotzer, and O. A. O'Connor.** Submitted for publication.
11. **Craig, A. M., G. Sheggeby, and C. E. Wicks.** 1984. Large scale extraction of pyrrolizidine alkaloids from tansy ragwort (*Senecio jacobaea*). Vet. Hum. Toxicol. **26:**108–111.
12. **Dennis, S. M., T. G. Nagaraja, and E. E. Bartley.** 1981. Effects of lasalocid or monensin on lactate-producing or -using rumen bacteria. J. Anim. Sci. **52:**418–426.
13. **El Akkad, I., and P. N. Hobson.** 1966. Effect of antibiotics on some rumen and intestinal bacteria. Nature (London) **209:**1046–1047.
14. **Fulghum, R. S., B. B. Baldwin, and P. P. Williams.** 1968. Antibiotic susceptibility of anaerobic ruminal bacteria. Appl. Microbiol. **16:**301–307.
15. **Henderson, C., C. S. Stewart, and F. V. Nekrep.** 1981. The effect of monensin on pure and mixed cultures of rumen bacteria. J. Appl. Bacteriol. **51:**159–169.
16. **Hungate, R. E.** 1966. The rumen and its microbes, p. 154–155. Academic Press, Inc., New York.
17. **Huxtable, R. J.** 1979. New aspects of the toxicology and pharmacology of pyrrolizidine alkaloids. Gen. Pharmacol. **10:** 159–167.
18. **Katstrup, E. K., B. R. Olin, S. I. Connell, and S. K. Hebel (ed.).** 1991. Drug facts and comparisons. J. P. Lippincott Co., St. Louis.
19. **Lanigan, G. W.** 1971. Metabolism of pyrrolizidine alkaloids in the ovine rumen. III. The competitive relationship between heliotrine metabolism and methanogenesis in rumen fluid *in vitro*. Aust. J. Agric. Res. **22:**123–130.
20. **Lanigan, G. W.** 1976. *Peptococcus heliotrinreducans*, sp. nov., a cytochrome-producing anaerobe which metabolizes pyrrolizidine alkaloids. J. Gen. Microbiol. **94:**1–10.
21. **Majak, W., K. J. Cheng, and J. W. Hall.** 1982. The effect of cattle diet on the metabolism of 3-nitropropanol by ruminal microorganisms. Can. J. Anim. Sci. **62:**855–860.
22. **Mallmann, W. L., W. E. Botwright, and E. S. Churchill.** 1941. The selective bacteriostatic effect of slow oxidizing agents. J. Infect. Dis. **69:**215–219.
23. **Mattocks, A. R.** 1968. Toxicity of pyrrolizidine alkaloids. Nature (London) **217:**723–728.
24. **Mattocks, A. R.** 1981. Relation of structural features to pyrrolic metabolites in livers of rats given pyrrolizidine alkaloids and derivatives. Chem. Biol. Interactions **35:**301–310.
25. **McDougall, E. I.** 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. **43:**99–109.
26. **Muir, L. A., and A. Barreto, Jr.** 1979. Sensitivity of *Streptococcus bovis* to various antibiotics. J. Anim. Sci. **48:**468–473.
27. **Muirhead, S. (ed.).** 1990. Feed additive compendium, vol. 28. Miller Publishing Co., Minnetonka, Minn.
28. **Nagaraja, T. G., and M. B. Taylor.** 1987. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. Appl. Environ. Microbiol. **53:**1620–1625.
29. **Newbold, C. J., R. J. Wallace, N. D. Watt, and A. J. Richardson.** 1988. Effect of the novel ionophore tetronasin (ICI 139603) on ruminal microorganisms. Appl. Environ. Microbiol. **54:**544–547.
30. **Purser, D. B., T. J. Klopfenstein, and J. H. Cline.** 1965. Influence of tylosin and aureomycin upon rumen metabolism and the microbial population. J. Anim. Sci. **24:**1039–1044.
31. **Russell, G. R., and R. M. Smith.** 1968. Reduction of heliotrine by a rumen microorganism. Aust. J. Biol. Sci. **21:**1277–1290.
32. **Schultze, A. E., J. G. Wagner, S. M. White, and R. A. Roth.** 1991. Early indications of monocrotaline pyrrole-induced lung injury in rats. Toxicol. Appl. Pharmacol. **109:**41–50.
33. **Segall, H. J.** 1978. Pyrrolizidine alkaloids derived from *Senecio jacobaea*. Toxicol. Lett. **1:**279–284.
34. **Sharrow, S. H., and W. D. Mosher.** 1982. Sheep as a biological control agent for tansy ragwort. J. Range Man. **35:**480–482.
35. **Shull, L. R., G. W. Buckmaster, and P. R. Cheeke.** 1976. Factors influencing pyrrolizidine (*Senecio*) alkaloid metabolism: species, liver sulfhydryls and rumen fermentation. J. Anim. Sci. **43:**1247–1253.
36. **Swick, R. A., P. R. Cheeke, H. S. Ramsdell, and D. R. Buhler.** 1983. Effect of sheep rumen fermentation and methane inhibition on the toxicity of *Senecio jacobaea*. J. Anim. Sci. **56:**645–651.
37. **Van Nevel, C. J., and D. I. Demeyer.** 1988. Manipulation of rumen fermentation, p. 387–443. *In* P. N. Hobson (ed.), The rumen microbial ecosystem. Elsevier Applied Science Publishers, New York.
38. **Wachenheim, D. E., S. B. Scott, L. L. Blythe, and A. M. Craig.** 1991. Ruminal biodegradation of pyrrolizidine alkaloids in vitro, and most probable numbers of ruminal pyrrolizidine alkaloid degrading bacteria, abstr. Q-85, p. 290. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
39. **Wang, C.-L., B. B. Baldwin, R. S. Fulghum, and P. P. Williams.** 1969. Quantitative antibiotic sensitivities of ruminal bacteria. Appl. Microbiol. **18:**677–679.
40. **Watanabe, K., J. Watanabe, S. Kuramitsu, and H. B. Maruyama.** 1981. Comparison of the activity of ionophores with other antibacterial agents against anaerobes. Antimicrob. Agents Chemother. **19:**519–525.