IN VITRO AND IN VIVO ALTERATIONS IN RUMINAL VOLATILE FATTY ACIDS BY ANTIMICROBIAL COMPOUNDS

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Introduction

The influence of rumen modifiers such as ionophores on rumen fermentation and ruminant performance has been well documented (Schelling, 1984). It is generally believed that the performance-enhancing effects of rumen modifiers can be attributed in part to altered microbial activity in the rumen, which results in an increased propionate production, a decreased methanogenesis as well as a decrease in the rate of protein degradation. However, many specific questions still remain unanswered.

In vitro techniques simulating ruminal fermentation have been used to study not only the mode of action of antimicrobial compounds, but also to find new rumen modifiers and to quantify their efficacies. Generally, they have proved to be excellent tools, but also some caution should be exercised. The purpose of this investigation was to study the relevance of batch culture fermentations as a predictor of the efficacy of antimicrobials to alter ruminal fermentation in vivo. The evaluation was mainly based on changes in volatile fatty acids (VFA) ratios in both in vitro and in vivo trials.

Materials and Methods

In vitro trials

Batch culture fermentations with concentrates as the substrate were conducted to assess the effects of antimicrobial compounds of different classes on the production of VFA. 2.5 ml unstrained ruminal fluid inoculum from sheep fed concentrates, grass cobs and hay (65:25:40) and 7.5 ml McDougall buffer were incubated at 39 °C with 150 mg ground concentrates in polyethylene tubes closed with rubbers stoppers equipped with bunsen valves. Compounds tested were monensin, lasalocid, maduramicin, salinomycin, penicillin G and chlortetracycline at concentrations of 2.5 and 25.0 μg/ml. All compounds were dissolved in 50 μl methanol; control tubes (0 μg/ml) contained an equivalent amount of the solvent. After 24 h fermentation, a sample of the incubation mixture was acidified and centrifuged. The supernatant was analyzed for VFA by gas chromatography using Chromosorb 101 as column packing, and L-lactic acid and ammonia enzymatically. Fermentations were always conducted in duplicate and repeated five times with ruminal fluid collected from different animals.

In vivo trials

Mature wethers fitted with permanent ruminal cannulas were utilized. They were individually penned and kept on floors. The animals were fed 600 g sheep concentrates by automatic feeders in four equal portions as well as 250 g grass cobs and 400 g chopped hay per day. Water and salt licks were always available. The experimental period consisted of a treatment period of 10 days as well as an adjustment and a posttreatment period each of 20 days. Compounds mentioned above were administered at 11.00 h as a 10% ethanol solution (50 ml) via the cannulas. Treatments were 12.5 ppm of the ionophores and 5.0 mg/kg body weight of penicillin G and chlortetracycline per day. Rumen fluid samples were taken at 10.00 h on the days indicated in figure 1 for the determination of VFA, L-lactic acid and ammonia.

Results

In vitro

All ionophores clearly enhanced dose-dependently the production of propionate at the expense of acetate and butyrate; the production of total VFA usually remained unaffected (table 1). Among the ionophores tested, salinomycin and maduramicin were most effective in enhancing propionate proportion ($p < 0.01$; paired t test); maduramicin tended to be slightly less potent than salinomycin. Monensin and lasalocid were

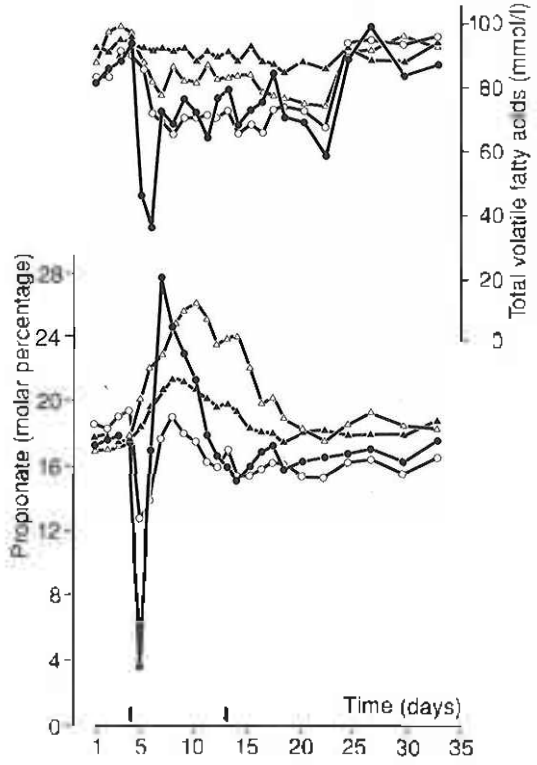

Figure 1. Mean effects of 12.5 ppm monensin (▲) and salinomycin (△) and 5.0 mg/kg b.w. chlortetracycline (○) and penicillin G (●) on in vivo ruminal molar proportion of propionate and total volatile fatty acid concentration in sheep (n = 4). The arrows on the time axis indicate the first and last day on which compounds were administered.

less effective than salinomycin and maduramicin ($p < 0.005$), but compared with the negative control they increased the molar proportion of propionate highly significant.

In contrast, both penicillin and chlortetracycline provoked reversed changes: each dose of those antibiotics reduced the total VFA levels, in particular propionate, so that the acetate-to-propionate ratio was markedly increased. Penicillin was more inhibitory than chlortetracycline ($p < 0.01$). Neither compound affected the L-lactate levels; effects on ammonia varied.

In vivo

The major results of the in vivo trial are presented in figure 1 (for reasons of clarity only the main treatments are shown). In all cases ionophores increased the propionate proportions significantly, whereas total VFA levels were not. The efficacy of salinomycin to change VFA levels markedly exceeded those of monensin and lasalocid ($p < 0.025$). While the propionate-enhancing response to lasalocid was similar to that of monensin, the response to maduramicin clearly exceeded that of monensin or lasalocid, but remained slightly below the response to salinomycin. L lactate and pH remained unifluenced; ammonia levels tended to drop after ionophores.

The nonionophores, however, adversely affected rumen fermentation. Both compounds, particularly penicillin, reduced total VFA levels ($p < 0.01$) (fig. 1). Penicillin induced a sudden, large decrease in propionate levels, reaching a minimum value after one day. Thereafter, the propionate concentration rose rapidly above pretreatment levels culminating on the third day of the treatment, whereafter a gradual decline set in and normal levels were achieved in about 4 days (i.e., prior to the end of the treatment period). Only during the first 3 days of the treatment were L-lactate levels elevated after penicillin (about tenfold); rumen ammonia levels were significantly reduced during the whole treatment period. After chlortetracycline only a transient decline in propionate was observed; L-lactate and ammonia levels tended to increase and decrease, resp.

Discussion

This study not only confirms the previously demonstrated favourable influence of ionophores on rumen fermentation but also shows that batch culture fermentations are reliable tools to quantify the efficacy of ionophores to change rumen fermentation in vivo, because a positive relationship was observed between in vitro and in vivo changes in the VFA proportions. Very little information is available on the relative efficacy of iononphores, but Negaraja et al. (1987) also reported that salinomycin is more potent in enhancing propionate production than monensin or lasalocid. A similar difference in efficacy has been observed in performance studies (Merchen and Berger, 1985). Interesting enough, the efficacy of maduramicin to alter the in vitro and in vivo VFA composition is slightly lower than that of salino-

TABLE 1. EFFECTS OF ANTIMICROBIAL COMPOUNDS ON RUMINAL VFA PARAMETERS IN VITRO. MEANS ± SEM (N=6) ARE PRESENTED

Item	μg/ml	Propionate (molar %)	Acetate: propionate ratio	Total VFA (mmol/l)
Control	0	19.9 ± 0.8	3.23 ± 0.15	92.7 ± 2.4
Monensin	2.5	22.4 ± 1.1[b]	2.78 ± 0.17[c]	89.3 ± 2.5
	25.0	23.6 ± 1.1[b]	2.58 ± 0.16[c]	87.1 ± 3.9
Lasalocid	2.5	22.0 ± 1.1[b]	2.85 ± 0.14[c]	95.6 ± 2.7
	25.0	23.7 ± 1.1[b]	2.59 ± 0.14[c]	91.6 ± 2.9
Maduramicin	2.5	23.1 ± 1.2[b]	2.72 ± 0.17[c]	93.8 ± 3.0
	25.0	25.1 ± 1.1[b]	2.45 ± 0.14[c]	88.0 ± 3.6
Salinomycin	2.5	23.4 ± 1.1[b]	2.67 ± 0.15[c]	91.1 ± 3.9
	25.0	24.9 ± 0.9[b]	2.39 ± 0.11[b]	85.4 ± 3.7
Penicillin G	2.5	14.0 ± 1.1[b]	5.37 ± 0.51[b]	81.3 ± 3.5
	25.0	12.1 ± 0.4[c]	6.48 ± 0.45[c]	78.9 ± 3.6
Chlortetracycline	2.5	17.4 ± 0.7[b]	3.84 ± 0.18[c]	87.4 ± 3.5
	25.0	16.7 ± 0.6[c]	4.11 ± 0.15[c]	83.6 ± 3.1[b]

*Means with superscripts are significantly different from control (a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$).

mycin. In contrast, the optimum dosage of maduramicin to prevent and to control coccidiosis in poultry is about tenfold below that of salinomycin. Our present laboratory studies with about 40 ionophores confirm that the relative efficacy of ionophoric anticoccidials and that of rumen modifiers clearly differ.

The detrimental effects of penicillin and chlortetracycline on rumen fermentation in vitro demonstrate that batch cultures can also be used to predict adverse influences on rumen fermentation in vivo. Some of the differences between in vitro and in vivo, however, become apparent e.g. the transient changes in the propionate and lactate levels in sheep after penicillin are absent in vitro. We used high doses of antimicrobials as such doses are therapeutically relevant and would be more likely to elicit a response. However, as the response to a high dose might be different, in nature and magnitude, from that of a lower dose, we also tested 5- and 10-fold lower doses of penicillin in vivo, but found similar changes although of a smaller magnitude. Further work is required to explain the observed adaptation phenomena.

In conclusion, these experiments show that simple batch culture fermentations are satisfactory models – at least for ionophores and some therapeutics – to predict alterations in ruminal fermentation in vivo.

(Key world: Antimicrobials, Rumen Fermentation, Sheep)

Literature Cited

Merchen, N.R. and L.L. Berger. 1985. Effect of salinomycin level on nutrient digestibility and ruminal characteristics of sheep and feedlot performance of cattle. J. Anim. Sci. 60: 1338-1346.

Nagaraja, T.G., M.B. Taylor, D.L. Harmon and J.E. Boyer. 1987. In vitro lactic acid inhibition and alterations in volatile fatty acid production by antimicrobial feed additives. J. Anim. Sci. 65:1064-1076.

Schelling, G.T. 1984. Monensin mode of action in the rumen. J. Anim. Sci. 58:1518-1527.