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Effect of nitroethane administration on ruminal VFA production and specific activity of methane production

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ABSTRACT

Oral administration of 72 mg nitroethane/kg body weight d^{-1} to ewes reduced ($P<0.05$) the specific activity of ruminal methane production (SARMP) by 26, 69 and 29% on days 1, 2 and 5 of treatment, respectively. Administration of 24 mg nitroethane/kg body weight d^{-1} reduced ($P<0.05$) SARMP by 26 and 42% on days 1 and 2 but not on day 5 (13% reduction) of treatment. Rumen VFA production was unaffected by nitroethane administration. These results demonstrate that nitroethane reduces ruminal methanogenesis in vivo without redirecting the flow of reductant generated during fermentation to propionate and butyrate.

KEY WORDS: methane, nitroethane, rumen, fermentation

INTRODUCTION

Ruminal methanogenesis is an inefficient process that results in the loss of 2 to 14% of the gross energy consumed by ruminants (Johnson and Johnson, 1995). Ruminant microbiologists have long attempted to develop strategies to reduce energetic losses associated with ruminal methane production (Van Nevel and Demeyer, 1996) but these were mostly unsuccessful because inhibition of methanogenesis also inhibited microbial interspecies hydrogen transfer reactions considered beneficial to fermentation (Miller, 1995). Recently, we reported that select nitrocompounds markedly inhibited ruminal methanogenesis *in vitro* without an apparent inhibition in microbial interspecies hydrogen transfer (Anderson et al., 2003). Considering that these nitrocompounds also exert bactericidal activity against some important foodborne pathogens (Jung et al., 2003), it seems reasonable to propose that nitro-supplementation may be

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developed into a feed additive that not only reduces costs associated with ruminal methane emissions but also to control zoonotic pathogens. Presently, we examine the potential of nitroethane to reduce methane production in vivo.

MATERIAL AND METHODS

Fifteen mature ewes maintained on a Bermuda grass hay:cracked maize diet (9:1) and supplemental minerals were randomly allocated (n=5 per treatment) to one of the following treatments: 0, 24 or 72 mg nitroethane/kg BW per day for 5 days. Treatments were administered as the sodium salt of nitroethane (Majak et al., 1986) *via* oral gavage of two equal sized potions given at morning (08:00) and afternoon (16:00) meals. Rumen fluid collected *via* stomach tube 2 h after administration of the morning meal 3 days before initiation of treatment (pre-treatment) and on days 1, 2 and 5 of treatment was transferred immediately upon collection into serum vials which were then capped and returned to the lab for determinations of volatile fatty acid concentrations and specific activity of methane production (SARMP). Each serum vial was filled completely (approximately 60 ml) before capping to minimize contact of air with the fluid. Volatile fatty acid concentrations were measured by gas chromatography (Hinton et al., 1990). Determination of SARMP was accomplished by combining, in 18×150 crimp top tubes, 5 ml rumen fluid from each ewe with 5 ml anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground lucerne (to pass a 4 mm screen). The tubes were capped and incubated 3 h at 39°C under a H_2:CO_2 (50:50 mix) atmosphere. At the end of the incubation period, concentrations of hydrogen and methane present in the headspace of the incubations were determined *via* gas chromatography. Protein concentrations of the rumen fluid incubations were measured by the modified Lowry procedure (Sigma-Aldrich, St. Louis, MO, USA). Tests for treatment effects were performed using an analysis of variance procedure of Statistix® 8 Analytical Software (Tallahassee, FL) and means were further separated using a Tukey's multiple range test.

RESULTS AND DISCUSSION

Consistent with observations from earlier *in vitro* studies (Anderson et al., 2003), oral administration of nitroethane to sheep significantly reduced the SARMP (Table 1). Reductions in the SARMP observed on day 2 of treatment indicate that the effect of nitroethane may be dose dependent. Pretreatment activities did not differ between the allotted groups (mean±SD; 0.055±0.006 μmol CH_4/mg protein h^{-1}). Oral administration of nitroethane had no effect ($P>0.05$) on amounts or molar proportions of volatile fatty acids produced (Table 2). This observation indicates that unlike that typically observed with other methane inhibition strategies, reductant (electrons) generated during ruminal digestion were

Table 1. Effect of oral nitroethane administration of specific activity of methane production (SARMP) in ovine rumen contents *in vivo*

Amount of nitroethane administered, mg/kg BW d^{-1}[a]	SARMP µmol CH_4/mg protein per h		
	day 1	day 2	day 5
0	0.046[b]	0.052[b]	0.048[b]
24	0.034[c]	0.030[c]	0.042[b,c]
72	0.026[c]	0.016[d]	0.034[c]
P value	0.0003	0.0001	0.0144
SEM	0.002	0.003	0.03

[a] administered (n=5 per treatment) as the sodium salt of nitroethane in two equal sized portions, at 08.00 and 16.00

[b,c,d] means were further separated using a Tukey's multiple range test and values within columns with unlike superscripts differ ($P<0.05$)

Table 2. Effect of oral nitroethane administration of volatile fatty acid accumulation in ovine rumen contents *in vivo*

Amount of nitroethane administered, mg/kg BW d^{-1}[b]	Volatile fatty acid conc'n, µmol/ml[a]								
	day 1			day 2			day 5		
	C2	C3	C4	C2	C3	C4	C2	C3	C4
0	49.8	11.8	5.7	68.2	16.6	6.4	55.9	14.9	5.7
24	49.9	12.9	6.6	58.1	14.3	6.2	54.9	14.5	6.0
72	44.6	11.6	5.4	56.5	14.8	6.9	53.8	14.8	5.4
P value	0.08	0.50	0.42	0.14	0.58	0.85	0.87	0.97	0.68
SEM	1.70	0.84	0.65	4.12	1.61	0.81	2.91	1.18	0.44

[a] C2, acetate; C3, propionate; C4, butyrate; [b] administered (n=5 per treatment) as the sodium salt of nitroethane in two equal sized portions, at 08:00 and 16:00

not directed to the production of the more reduced volatile fatty acids, propionate and butyrate (Table 2). The fate of the electrons that otherwise would have been used to reduce carbon dioxide to methane is not yet known but it is attractive to hypothesize that they may have been consumed by anabolic cell processes (i.e. microbial cell growth) or via other reductive processes. The magnitude of the reductions in SARMP were less on day 5 than on the first two days of treatment thus suggesting a ruminal adaptation to nitroethane. At least one ruminal microbe (*Denitrobacterium detoxificans*) is known to metabolize nitroethane, coupling its reduction to the oxidation of hydrogen or formate (Anderson et al., 2003), although this bacterium is not typically a predominant member of the rumen microflora. Evidence suggests that the populations of nitro-reducing bacteria like *D. nitrobacterium* can be enriched *in situ via* feeding of nitroethane (Majak et al.,

1986) and if concentrations of nitro-reducing bacteria were high enough then it is reasonable to expect that they may be able to consume reducing equivalents at the expense of methanogenesis. This possibility will be examined in future experiments by correlating H_2 consumption with nitroethane reduction in separate *in vitro* incubations where nitroethane is included as a substrate.

CONCLUSIONS

Oral nitroethane administration to sheep reduced the SARMP. Further studies are needed to determine if the effect of nitroethane on SARMP translates into long-term reductions in whole animal methane emissions and improvements in energy utilization.

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