CONTROL OF RUMINAL NITRATE REDUCTION BY SULPHUR COMPOUNDS

J. Takahashi

Department of Animal Science,
Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

Introduction

Rumen bacteria are assumed to reduce nitrate in an assimilatory manner as do plant cells and fungi (Prins et al., 1980). Nitrate must be reduced to nitrite primarily, and the nitrate reductase (EC 1.6.6.3) involved in the reaction is well known to be a molybdo-enzyme (Nason and Evans, 1953). The reducing step is of primary importance to the incidence of nitrate-nitrite poisoning in ruminants. It has been proposed that an inorganic combination of molybdenum and sulphur results in the formation of an insoluble complex salt (Ammerman and Goodrich, 1983) and it is therefore possible that S may inhibit nitrate reductase. In *in vitro* experiments, Takahashi et al., (1989) found that elemental sulphur decreased nitrite produced from nitrate by rumen microbial populations. In establishing a natural prophylactic for nitrate hazards in animal production, application of S containing compounds is valuable since it is a major element which constitutes several essential amino acids or biologically active tripeptide, glutathione.

The present paper deals with the inhibitory effects of S compunds on *in vitro* nitrate reduction by rumen micro-organisms.

Materials and Methods

Eight rumen fistulated Suffolk Down wethers, maintained on lucerne(*Medicago sativa*) cubes (50 g dry matter/kg body-weight$^{0.75}$ per d) were randomly divided in two equal groups for *in vitro* experiments. To obtain rumen fluid containing nitrate adapted microbial populations, each animal was given 0.55 g/kg body-weight$^{0.75}$ of $NaNO_3$ twice a day for 35 d via the fistula. Rumen fluid from each nitrate-adapted or nonadapted sheep was pooled and strained through four layers of muslin cloth. One volume of strained fluid was then added to four volumes of preheated (38°C) buffered mineral salt solution containing 10 mM $NaNO_3$, 10mM glucose and 40mM lactate with or without various amounts of the potential chemical inhibitors under investigation. The incubation was carried out anaerobically at 38°C for 24 h.

To test the inhibitory effects of inorganic and organic S compounds on nitrate reduction by the rumen microbial population adapted to nitrate, the following compounds were administered into separate incubation vessels: sulphide (1, 2, 3, 5, 8 and 10 mM $Na_2S{\cdot}9H_2O$), sulphite (1 and 10 mM Na_2SO_3), sulphate (1 and 10 mM Na_2SO_4) or L-cysteine (1 and 10 mM), DL-methionine (1 and 10 mM). After 24 h incubation, portions of culture media were collected for nitrate and nitrite determination.

To test direct effects of the inhibitors on nitrate reduction in nonadapted microbial populations, the anaerobic incubation was carried out for 24 h with or without administration of sulphide (1 and 10 mM $Na_2S{\cdot}9H_2O$) or L-cysteine (1 and 10 mM). After 24 h incubation, nitrate and nitrite were determined in each incubation medium.

Results

Figure 1 shows the effect of sulphide-S on nitrate reduction and nitrite formation in rumen fluid containing microbial populations adapted to nitrate. A marked inhibition of nitrate reduction was observed in rumen microbial populations adapted to nitrate as increasing amounts of sulphide-S was added. Consequently, nitrite formed was remarkably suppressed by the addition of sulphide-S. The addition of 5 mM sulphide decreased nitrite formation up to 90%. The addition of more than 8 mM sulphide completely suppressed nitrite formation by rumen microbes *in vitro*.

Figure 2 shows the degree of suppression of nitrite formation reduced from nitrate attributable to inorganic and organic S tested. Neither sulphate- nor sulphite-S affected nitrate reduction and nitrite formation. For S-containing amino

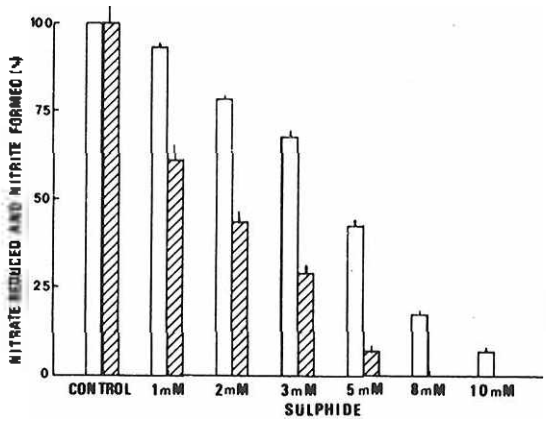

Figure 1. Effect of sulphide-sulphur on nitrate reduction and nitrite formation in rumen fluid contained microbial populations adapted to nitrate. The columns represent the means for five determinations. Vertical bars represent the standard errors of the means. □, Relative amount of nitrate reduced (control value indicates 140.00 ± 0.00 g N/ml); ▨, Relative amount of nitrite formed (control value indicates 103.20 ± 5.76 g N/ml).

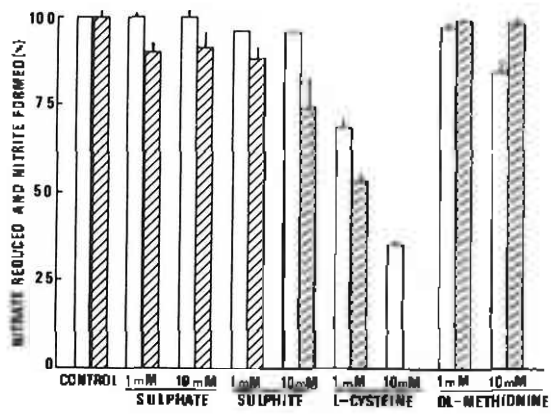

Figure 2. Effects of inorganic and organic sulfurs on nitrate reduction and nitrite formation in rumen fluid contained microbial population adapted to nitrite. The columns represent the means for five determinations. Vertical bars represent the standard errors of the means. □, Relative amount of nitrate reduced (control value indicates 140.00 + 0.00 g N/ml); ▨, Relative amount of nitrite formed (control value indicates 106.00 ± 2.12 g N/ml).

acids, DL-methionine proved to be inefficient in inhibiting microbial reduction of nitrate. The addition of L-cysteine into the medium however, significantly ($P < 0.01$) lowered nitrate reduction and nitrite formation with an evident dose-dependent effect. The extent of the depression of nitrite formation was 50% at 1 mM ($P < 0.01$) and 100% at 10 mM ($P < 0.01$) L-cysteine concentrations.

Figure 3 shows the inhibitory effects of sulphide and L-cysteine on nitrite formation by rumen microbial populations nonadapted to nitrate. The effect of sulphide and L-cysteine on the inhibition of nitrite formation in nitrate-adapted and nonadapted organisms is approximately the same. However, the effect on the inhibition of nitrate reduction in nonadapted organisms is greater than that in nitrate-adapted organisms.

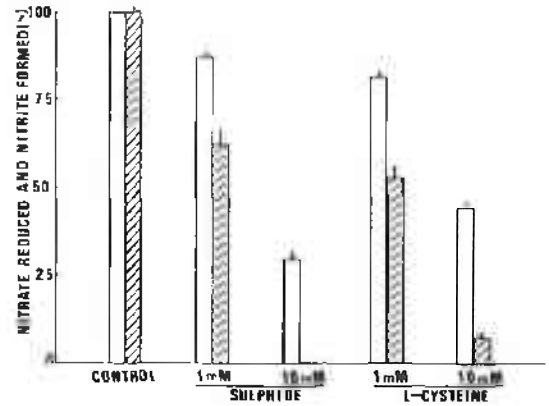

Figure 3. Effects of sulfide and L-cystein on nitrate reduction and nitrite formation in non-induced rumen microbial population with nitrate. The columns represent the means for five determinations. Vertical bars represent the standard errors of the means. □, Relative amount of nitrate reduced (control value indicates 140.00 ± 0.00 g N/ml); ▨, Relative amount of nitrite (control value indicates 69.81 ± 1.59 g N/ml).

Discussion

In *in vivo* physiological concentrations (< 1

*m*M sulphide), sulphide absorption may be extremely rapid. Consequently, undissociated sulphide can be directly absorbed across the rumen epithelium, although the turnover rate is variable depending on the N:S ratio in the rumen. It is probable that the undissociated sulphydryl ions or H_2S absorbed from rumen can become a metabolic poison for the animal due to sulphide overdose. In terms of partial recovery of VFA production reduced by nitrate (Takahashi et al., 1989), it is unlikely that the sulphide added, even at 10 mM, will adversely affect the microbial fermentation in vitro. Therefore, a marked depression in nitrate reduction by the addition of sulphide indicates that the elemental S degraded from sulphide inhibits the activity of nitrate reductase due to the interference resulting from the incorporation of Mo into the enzyme. In an early study of nitrate reductase in wild type *Neurospora crassa* (5297a), Nason and Evans (1953) observed that the -*SH* group played a significant part in the stability of this enzyme and showed the small inhibitory effects of L-cysteine at a higher concentration (1 mM) on enzyme activity. Although the participation of the -*SH* group in the activity and synthesis of nitrate reductase (Azoulay et al., 1969) cannot be ruled out in the present work, it seems reasonable to assume that the sulphide S generated from L-cysteine by L-cysteine hydrogen sulphide lyase (EC 4.4.1.1.) of rumen bacterial origin interferes with the nitrate reduction. Currently, the interference of undergraded S in nitrate reductase activity from rumen microbial origin has not yet been established.

Therefore, the significant suppressing effect of L-cysteine on nitrite formation by rumen microbes is of particular interest when considering the dietary hazards of nitrate to livestock.

(**Key Words:** Cysteine, Nitrite, Nitrate Reduction)

Literature Cited

Ammerman, C.B. and R.D. Goodrich. 1983. Advances in mineral nutrition in ruminants. J. Anim. Sci. 57:519-533.

Azoulay, E., J. Puig and M.L. Martins Rosado De Sousa. 1969. Regulation de la synthese de la nitrate-reductase chez *Escherichia coli* K 12. Annales de l'Institute Pasteur, Paris 117:474-485.

Nason, A. and H.J. Evans. 1953. Triphosphopyridine nucleotide-nitrate reductase in *Neurospora*. J. Biol. Chem. 202:655-673.

Prins, R.A., W. Cline-Theil, A. Malestein and G.H.M. Counotte. 1980. Inhibition of nitrate reduction in some rumen bacteria by tungstate. Appl. Environ. Microbiol. 40:163-165.

Takahashi, J., N. Johchi and H. Fujita. 1989. Inhibitory effects of sulphur compounds, copper and tungsten on nitrate reduction by mixed rumen micro-organisms. Brit. J. Nutr. (in press).