Neth. J. agric. Sci. 28 (1980) 16-19

Nitrate poisoning in cattle. 5. The effect of tungsten on nitrite formation by rumen microbes

A. Korzeniowski*, J. H. Geurink and A. Kemp

Centre for Agrobiological Research, Wageningen, the Netherlands

Accepted: 16 July 1979

Key words: rumen, nitrate, nitrite, intoxication, tungsten, inhibition, nitrate reductase, molybdenum

Summary

Experiments performed in vitro on rumen fluid have shown an appreciable inhibition in nitrate reductase activity in rumen microbes achieved by addition of sodium tungstate. The rate of nitrite formation in rumen fluid was decreased by about 86 % if 20 μmol of Na_2WO_4 were added per litre, in the case of 100 and 500 μ moles of Na_2WO_4 per litre rumen fluid no nitrite accumulation was observed. This inhibitory effect was overcome in the case of elevated molybdenum levels. Introductory experiments performed on cows confirmed these in vitro findings. It is suggested that tungsten could be a very promising preventative of nitrite intoxication in ruminants.

Introduction

The application of large quantities of nitrogen fertilizers in intensive herbage production leads to elevated nitrate contents in fodder. This fodder nitrate is reduced in the rumen by microorganisms to nitrite and further to ammonia. In the case of high nitrate contents the rate of nitrite formation may exceed the rate of nitrite breakdown resulting in elevated nitrite concentration in the rumen fluid. This excessive nitrite, if produced in the rumen, is absorbed into the bloodstream and causes the following harmful effects:

a) conversion of blood hemoglobin into methemoglobin leading to anoxia;
b) serious drop in the blood pressure causing haemodynamic disturbances;
c) being a requisite for carcinogenic nitrosamine formation, nitrite raises the hazard of cancer to the animal and moreover to the consumer of animal products (Kemp et al., 1976, 1977; Geurink et al., 1979; Malestein et al., 1979, van Broekhoven, 1978).

* Visiting worker from the Institute of Zootechnics, Cracow, Poland.

For this reason there is an obvious interest in keeping under control the ruminal nitrite formation.

It is known that the formation of nitrite by the rumen microbes depends closely on the enzymatic activity of nitrate reductase, an enzyme which action is indispensably connected with the presence of molybdenum. It is the work of Tillman et al. (1965) who showed that sheep maintained on a molybdenum low diet had a diminished capacity of nitrate reduction. In spite of its scientific value, no practical use has been made of this finding due to difficulties in large scale preparation of molybdenum low diets. On the other hand, in studies about the mode of action of nitrate reductases in plants, an inhibition of the enzymatic activity has been achieved by addition of tungsten which forms with nitrate reductases an inactive tungsten analog (Heimer et al., 1969).

The objective of our work was to study whether a similar inhibition of nitrate reductase activity by tungsten could be achieved in rumen microbes. If so, this might lead to the development of a preventive measure against nitrite intoxication in ruminants.

Experiments and results

All the experiments were done in vitro on rumen fluid drawn from a hayfed rumen-fistulated cow. Immediately after receiving the rumen fluid was spiked with sodium tungstate (p.a.), alternatively with ammonium molybdate (p.a.) and finally with potassium nitrate (p.a.) up to 24 mmol/litre and incubated anaerobically at 38 °C. In the course of incubation samples were taken repeatedly and nitrite concentration was determined colorimetrically by the Griess reaction. Fig. 1 shows the time course of nitrite accumulation in rumen fluid in dependence of different tungsten additions. In rumen liquid with no tungsten added the rate of nitrite accumulation amounted up to 170 μmol l^{-1} h^{-1}, when 20 μmol of tungsten were added the accumulation was reduced to 23 μmol l^{-1} h^{-1} (86 % inhibition), and in the case of 100 and 500 μmol of tungsten no nitrite accumulation was observed.

According to the view (Hewitt, 1975) that the inhibitory action of tungsten results from the replacement of molybdenum by tungsten in the nitrate reductase protein, it could be expected that the molybdenum concentration plays a significant part in this process. Therefore a sequence of experiments was conducted in which the inhibitory action of tungsten was examined in the presence of elevated molybdenum concentrations. The experimental technique applied was the same as described previously. Three levels of molybdenum were investigated, viz 0.1, 4 and 20 μmol l^{-1}. Results of these experiments are shown in Fig. 2, the Y-axis represents the observed nitrite accumulation in dependence of the tungsten concentrations shown on the X-axis. It is seen that the inhibitory effect attained by 100 μmol of tungsten on a 0.1 μmol level of molybdenum is partly overcome by 4 μmol of molybdate and overcome completely by 20 μmol of molybdenum. The inhibitory effect of 500 μmol of tungsten remains intact at 4 μmol of molybdenum but is overcome almost completely by 20 μmol of molybdenum.

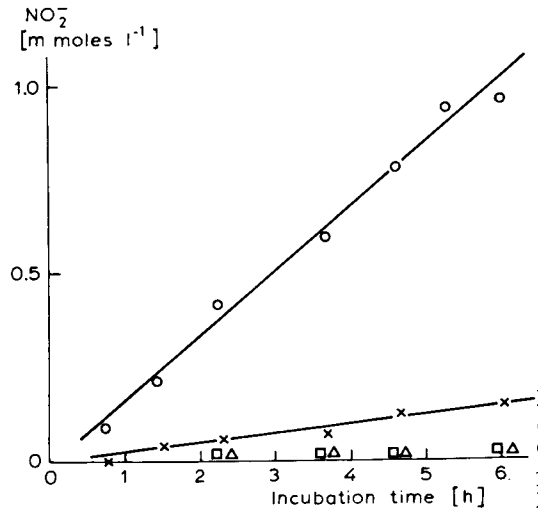

Fig. 1. The influence of sodium tungstate on nitrite accumulation in rumen fluid. ○, no Na_2WO_4; ×, 20 μmol of Na_2WO_4 per litre; △, 100 μmol of Na_2WO_4 per litre; □, 500 μmol of Na_2WO_4 per litre.

Conclusions

It is concluded that sodium tungstate suppresses the rate of nitrite formation in rumen liquid under in vitro conditions. It was found that this inhibitory effect of tungsten depends closely on the molybdenum content in rumen liquid and in the case of elevated molybdenum concentrations higher levels of tungsten are needed than in low molybdenum media.

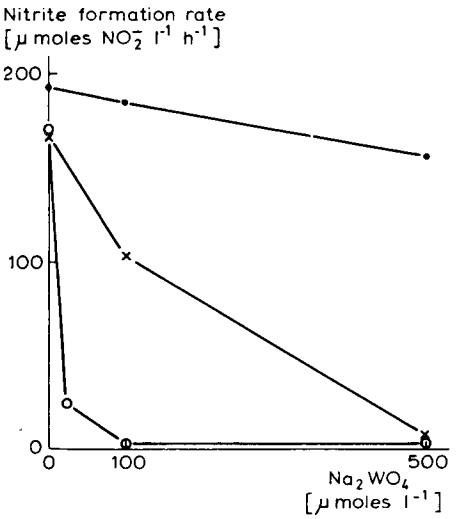

Fig. 2. The influence of tungstate and molybdate on the rate of nitrite accumulation in rumen fluid. ○, 0.1 μmol of Mo per litre; ×, 4 μmol of Mo per litre; •, 20 μmol of Mo per litre.

The strong inhibition of nitrate reductase activity by tungsten on the one hand, and the relatively low toxicity of this metal (Patty, 1963) on the other hand, suggest that tungsten could be a very promising preventative of nitrite intoxication in ruminants. Introductory experiments performed on cows confirmed the in vitro findings mentioned before. For instance intraruminal doses of 2 g of tungsten daily in the form of sodium tungstate highly suppressed the rate of nitrite accumulation in the rumen.

Acknowledgement

We thank Dr B. W. Veen of our Institute for his valuable suggestion on the possible inhibition of nitrate reductase activity in rumen fluid by tungsten.

References

Broekhoven, L. W. van & R. W. Stephany, 1978. Environmental aspects of N-nitroso compounds. IARC Scientific Publications: 19.

Geurink, J. H., A. Malestein, A. Kemp & A. Th. van 't Klooster, 1979. Nitrate poisoning in cattle. 3. The relationship between nitrate intake with hay or fresh roughage and the speed of intake on the formation of methemoglobin. *Neth. J. agric. Sci.* 27: 268-276.

Heimer, Y. M., J. L. Wray & P. Filner, 1969. The effect of tungstate on nitrate assimilation in higher plant tissues. *Pl. Physiol.* 44: 1197-1199.

Hewitt, E. J., 1975. Assimilatory nitrate-nitrite reduction. *A. Rev. Pl. Physiol.* 26: 73-100.

Kemp, A., J. H. Geurink, R. T. Haalstra & A. Malestein, 1976. Nitrate poisoning in cattle. 1. Discolouration of the vaginal mucous membrane as aid in the prevention of nitrate poisoning in cattle. *Stikstof* 19: 40-48.

Kemp, A., J. H. Geurink, R. T. Haalstra & A. Malestein, 1977. Nitrate poisoning in cattle. 2. Changes in nitrite in rumen fluid and methemoglobin formation after high nitrate intake. *Neth. J. agric. Sci.* 25: 51-62.

Kemp, A., J. H. Geurink, A. Malestein & A. Th. van 't Klooster, 1978. Grassland production and nitrate poisoning in cattle. *Proc. 7th Meet. Eur. Grassland Fed.* (Gent).

Malestein, A., J. H. Geurink, G. Schuyt, A. J. H. Schotman, A. Kemp & A. Th. van 't Klooster, 1979. Nitrate poisoning in cattle. 4. The effect of nitrite dosing during parturition on the oxygen capacity of maternal blood and the oxygen supply to the unborn calf. (In press.)

Patty, F. A. (Ed.), 1963. Industrial hygiene and toxicology. Interscience Publishers, Vol. 2: 1161-1163.

Tillman, A. D., G. M. Sheriha & R. J. Sirny, 1965. Nitrate reduction studies with sheep. *J. Animal Sci.* 84: 1140-1146.