Neth. J. agric. Sci. 30 (1982) 105-113

Nitrate poisoning in cattle. 7. Prevention

J. H. Geurink[1], A. Malestein[2], A. Kemp[1], A. Korzeniowski[3] and A. Th. van 't Klooster[2]

[1] Centre for Agrobiological Research, Wageningen, Netherlands
[2] Zootechnical Institute, State University, Utrecht, Netherlands
[3] Zootechnical Institute, Cracow, Poland

Accepted: 24 September 1981

Key-words: cattle, nitrate poisoning, methemoglobin formation, prevention, wolfram, tungsten

Summary

The results of 44 feeding experiments carried out with dry or lactating cows have been summarized in this paper. The rations consisted of hay, pre-wilted grass silage of freshly mown grass with and without concentrates.

A summary of the results of 291 methemoglobin (MHb) curves showed that as the nitrate content of the roughage increased the moment at which the highest MHb concentration occurred was delayed. It was also found that as the MHb peak increased more time was required to reduce MHb to normal values of 2-3 %.

The dry matter intake of preserved grass, such as hay and pre-wilted silage, was much more rapid than the intake of freshly mown grass. The rate of roughage intake effects the rate of nitrate intake and the formation of MHb in the blood. If nitrate content of the roughage is known, the relationship developed between nitrate intake and the formation of MHb in the blood can be used to calculate the amount of roughage that may be consumed per meal without causing symptoms of nitrate poisoning in the animal.

Preserved grass such as hay and pre-wilted silage, with a content up to some 0.75 % NO_3^- in the dry matter, may be supplied ad libitum. Preserved grass with a higher nitrate content should be supplied at a limited amount. Indoor feeding of fresh grass with op to 1.50 % NO_3^- in the dry matter may be supplied ad libitum. Under grazing conditions grass with a content of up to 2 % NO_3^- in the dry matter may be taken in ad libitum.

Nitrate poisoning after consumption of nitrate-rich roughage may also be prevented by inhibiting the nitrate reduction in the rumen by a daily dose of tungsten (wolfram). However, before such an application of tungsten against nitrate toxicity can be recommended in practice, all potential hazards should be carefully examined.

Introduction

After consumption of nitrate by ruminants, the nitrate is partly reduced by rumen micro-organisms to nitrite and ammonia. At high nitrate intake, nitrite will accumulate in the rumen fluid. This nitrite is rapidly absorbed from the rumen into the blood, where it converts HbO_2 (oxyhemoglobin) into MHb (methemoglobin) (Gamgee, 1868) and leads to low blood pressure (Ashbury & Rhode, 1964; Assali & Brinkman, 1973). Malestein et al. (1980) found that the supply of nitrite to cows in partu caused a drop in maternal blood pressure, whereas heart rate and respiration rate increased. These experiments showed clearly that the oxygen capacity of the blood decreased after nitrite treatment and that the oxygen supply to the foetus was adversely affected after nitrate intake of pregnant cows, especially because of the lower oxygen transfer via the placenta. Van Broekhoven & Stephany (1978) found that after ruminants consumed nitrate, the nitrosamine concentration in the rumen fluid increased at the same rate as the nitrite concentration increased, though on a much lower level. In milk-producing cows a positive relation was also established between nitrate intake and the nitrate and nitrite concentrations in the milk (Kemp et al., 1978).

In the Netherlands nitrate poisoning of cattle occurs occasionally, when ruminants are fed on preserved grass such as hay and pre-wilted grass silage, also when fed indoors on fresh grass, and sometimes after consuming Cruciferae such as turnips. In modern, intensive grassland management, heavy nitrogen dressings are applied as fertilizer and organic manure. The present use of nitrogen fertilizer on grassland is about 250 kg N per ha per year, varying upwards to some 600 kg. For maximum grass production the recommendation is 350-400 kg N, including nitrogen from organic manure. However, if much more nitrogen is applied than required for maximal production of grass, the nitrate content under certain conditions may rise to 6 % NO_3^- in the dry matter (Kemp et al., 1976). Detailed information of a great number of farms during 8 years showed that at a fertilizer level of some 400 kg N per ha per year, nitrate contents were regularly too high. In 20 % of the grass samples the NO_3^- content was higher than 0.75 % in the dry matter (J. A. Keuning and H. G. van der Meer, personal comm., 1980).

Since the nitrogen requirement of a crop and the nitrogen supply from the soil are difficult to predict, a fertilizer regime directed at optimum grass production will regularly cause nitrate contents that are high enough to cause nitrate poisoning.

Prevention of nitrate poisoning in cattle consuming roughages with a high nitrate content should be aimed at preventing the formation of nitrite in the rumen and the formation of MHb in the blood. Crawford (1960) pointed out that limiting the amount and the frequency of nitrate intake were important in the prevention of nitrate poisoning. Kemp et al. (1977, 1978) and Geurink et al. (1979) showed that at least four factors are important in the formation of nitrite in the rumen fluid and of MHb in the blood.

1. The amount of nitrate consumed. As more nitrate is consumed per meal,

more nitrite is accumulated in the rumen fluid and a greater part of the Hb is converted into MHb.
2. Adaptation to a higher nitrate level by the rumen microbes. Equal daily doses of nitrate to cows will raise the nitrite content in the rumen fluid and the percentage of MHb in the blood during the first days, thereafter both factors remain more or less constant at the increased level. These increases are probably caused by changes in the activity of the reducing micro-organisms in the rumen.
3. The rate of nitrate intake. The rate of nitrate intake depends on the rate of roughage intake and its nitrate content. The higher the rate of nitrate intake, the more nitrate and nitrite is accumulated in the rumen fluid. The rate of intake of hay or pre-wilted grass silage is higher than intake rate of fresh grass.
4. The rate of release of nitrate from the roughage into the rumen fluid. As the nitrate in the roughage diffuses more rapidly in the rumen, more nitrite is accumulated and higher MHb percentages are formed in the blood. In preserved roughages like hay or pre-wilted grass silage the cell membranes are completely permeable and the nitrate in the cell can easily diffuse into the rumen fluid. In freshly harvested grass the release of nitrate into the rumen fluid is dependent on the mechanical damage to the plant cells (eating and ruminating) and is much slower, resulting in less nitrite accumulation in the rumen fluid.

This paper deals with different ways of preventing nitrate poisoning in cattle that have consumed nitrate-rich roughages. The experimental data were obtained during eight years of research on nitrate.

Experimental data

The results of 44 feeding experiments with dry or lactating Friesian cows are surveyed in this paper. The rations consisted of hay or freshly mown grass. A small number of the experiments included pre-wilted grass silage. In some experiments the lactating cows also received concentrates according to requirements. The rations of the experimental cows were always consumed within 120 min. During the experiments all the cows were permanently provided with a catheter in the jugular vein for obtaining blood samples for analyses of Hb and MHb. A number of cows were also provided with a rumen fistula for taking rumen fluid samples. This paper only includes results obtained from cows supplied with at least four meals of nitrate-rich roughage (nitrate-adapted animals; Kemp et al., 1977). Detailed information on sampling technique and chemical procedure was reported earlier (Kemp et al., 1977; Korzeniowski et al., 1980, 1981).

Results and discussion

Formation and reduction of methemoglobin

Fig. 1 shows the average trend in the MHb percentages in the blood after feeding cows roughages with various nitrate contents. Each curve is an average of a number of curves whose maximum MHb contents occurred in the range of 5-10; 10-15; 15-20; 20-25; 25-30; 30-40; 40-50; or over 50 %. In this way 291

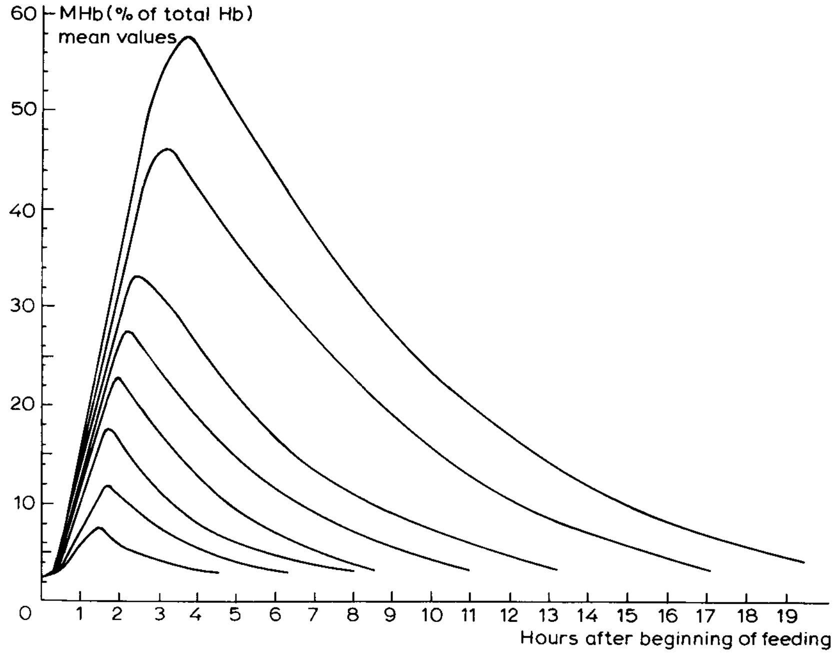

Fig. 1. Rate of formation and reduction of methemoglobin after feeding cows different amounts of nitrate.

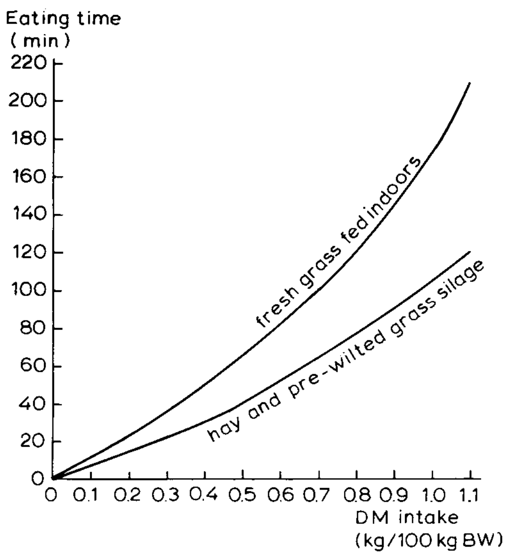

Fig. 2. Relationship between dry matter intake and eating time by cows fed freshly mown grass and preserved grass. BW = bodyweight.

curves were summarized.

The moment at wich the highest MHb concentration occurred was delayed as the concentration peak increased. MHb peaks between 5-10 % were found about 93 min after the beginning of feeding, with a range of 67 to 119 min. MHb peaks between 30-40 % occurred some 152 min after the beginning of feeding, with a range of 126 to 178 min. Peaks of over 50 % (average 57 %) averaged 223 min with a range of 209 to 237 min.

These ranges can be partly explained from differences in the intake rate of the nitrate-rich ration. The preceding clearly shows that within 2-3 hours after intake of the nitrate-rich roughage much increased MHb concentrations may occur in the blood. These concentrations have been associated with nitrate poisoning symptoms, such as discolouration of the mucous membranes (Kemp et al., 1976). Fig. 1 also shows that when the MHb peak is higher, more time is required to reduce MHb to normal values of 2-3 %. For instance, a maximum MHb value of 20 % will be reduced to a normal value some eight hours after the beginning of feeding, whereas about 20 hours will be required when the maximum MHb content is 60 %. In estimating the number of meals that can be supplied per 24 hours, the time required for the reduction of MHb must be known because when the next meal is supplied the MHb content will increase from the level that exists at the time of feeding (accumulation).

The rate of roughage intake

The rate of nitrate intake is dependent on the rate of roughage intake and the nitrate content of the roughage. Fig. 2 shows the relationship between dry matter intake and consumption rate of hay, pre-wilted grass silage and freshly mown grass fed indoors. This time was measured for adult cows which were fed twice daily. The relationship was not linear, for as more dry matter was taken in the intake rate decreased, which lenghtened the time for eating. To consume 1.1 kg dry matter per 100 kg bodyweight in the form of hay or pre-wilted grass silage about 2 hours were required. The rate of dry matter intake for grass was much slower, requiring about 3.5 hours for the intake of 1.1 kg dry matter per 100 kg bodyweight.

Compared with hay and pre-wilted grass silage about 50-70 % more time was required for the consumption of freshly mown grass, probably because of its much lower dry matter content.

Restricted feeding of nitrate-rich roughage

In earlier papers, Kemp et al. (1978) and Geurink et al. (1979) demonstrated the relationship between the nitrate intake and the formation of MHb in the blood of cows fed on rations consisting of different roughages. For maintaining a normal MHb content in the blood while feeding hay, a maximum of 3 to 5 g NO_3^- per 100 kg bodyweight could be consumed per meal. The variation in maximum nitrate amounts was caused by differences in the rate of nitrate intake. A value of 20 % MHb was attained after a nitrate intake of 9 to 14 g, whereas an intake of 15 to 21 g NO_3^- per 100 kg bodyweight resulted in 50 % MHb in the blood. This relation between nitrate intake and MHb formation also applied to pre-wilted grass silage (unpublished data). Based on the effect of herbage preservation on the premeability of the cell membranes this was to be expected. Maintaining normal MHb contents in the blood, roughly twice as much nitrate can be taken in with fresh grass as with preserved roughages. This difference is caused partly by a slower dry matter intake of fresh grass compared with hay or pre-wilted silage (Fig. 2), causing a lower rate of nitrate intake. Also there is a considerably slower rate of release of nitrate from the fresh plant cells into the rumen fluid (Geuring et al, 1979). In feeding freshly mown grass indoors to maintain a normal MHb content, a maximum of 6 to 12 g NO_3^- per 100 kg bodyweight may be consumed per meal, dependent on the rate of nitrate intake. A nitrate intake per meal of 18 to 28 g may increase the MHb to 20 %, whereas an intake of 30 to 40 g of NO_3^- per 100 kg bodyweight per meal may increase MHb in the blood to 50 % (Kemp et al., 1978; Geuring et al., 1979).

Fig. 3 shows the amount of hay and pre-wilted silage that may be safely supplied per meal based on the relationship between nitrate intake and the formation of MHb in the blood. The figure has been divided into three parts: a nitrate intake level at which poisoning symptoms do not occur; a nitrate intake level at which slight to clear clinical symptoms occur; and a nitrate intake level at which severe symptoms occur and usually lead to death.

To maintain a normal MHb content in the blood while feeding hay or pre-wilted silage containing 5 % NO_3^- in the dry matter, no more than 0.06 kg dry matter per 100 kg bodyweight may be supplied per meal (3 g NO_3^- intake per 100

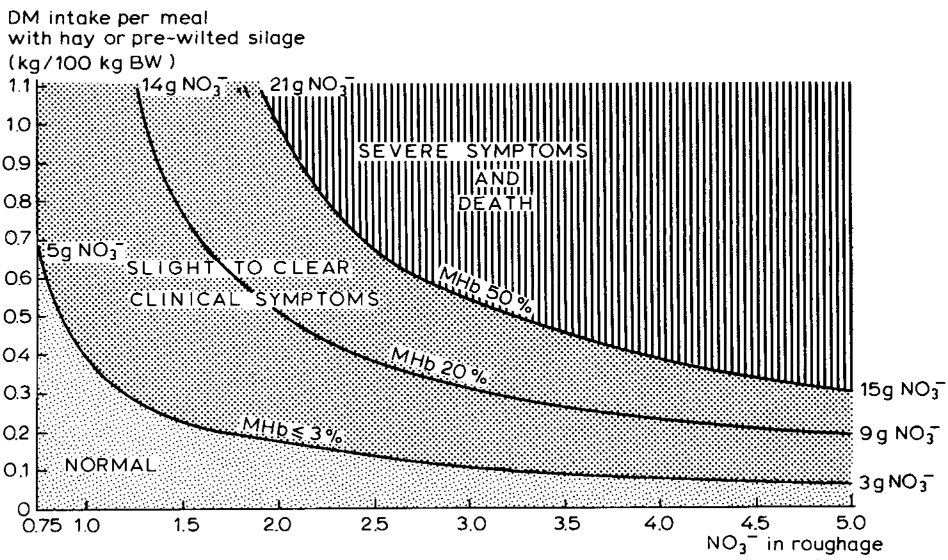

Fig. 3. The nitrate content (x-axis) and the dry matter intake (y-axis) of preserved grass in relation to the formation of methemoglobin. BW = bodyweight.

kg bodyweight). Feeding 0.2 kg dry matter per 100 kg bodyweight of the same roughage would result in about 20 % MHb in the blood. An intake of 0.3 kg dry matter per 100 kg bodyweight will lead to a MHb level of 50 % and usually cause death of the cow. When the nitrate content is 0.75 % in the dry matter, a maximum of 0.66 kg dry matter per 100 kg bodyweight may be consumed per meal and still maintain a normal MHb content (intake of 5 g NO_3^- per 100 kg bodyweight). Fig. 2 shows that about 60 min will be required for that amount of intake. Under these conditions the nitrate intake rate is so slow that nitrite does not accumulate in the rumen. Therefore hay and pre-wilted silage with a nitrate content up to 0.75 % in the dry matter may be supplied ad libitum without increasing MHb content in the blood. A meal of hay and pre-wilted silage with greater than 0.75 % NO_3^- in the dry matter can be fed no more than every 60 min. The amount per feeding can be determined from Fig. 3.

Fig. 4 shows the amount of dry matter in the form of freshly mown grass that may be fed per meal indoors. To maintain a normal MHb content in the blood while feeding freshly mown grass containing 5 % NO_3^- in the dry matter, no more than 0.12 kg dry matter per 100 kg bodyweight may be supplied per meal (6 g NO_3^- intake per 100 kg bodyweight). Feeding 0.4 kg dry matter per 100 kg bodyweight of the same freshly mown grass would result in some 20 % MHb in the blood, whereas an intake of 0.6 kg dry matter of this herbage may increase values upwards to 50 % MHb. With freshly mown grass containing 1.50 % NO_3^- in the dry matter, a normal MHb content in the blood is maintained if no more than 0.8 kg dry matter per 100 kg bodyweight is supplied per meal (intake of 12 g

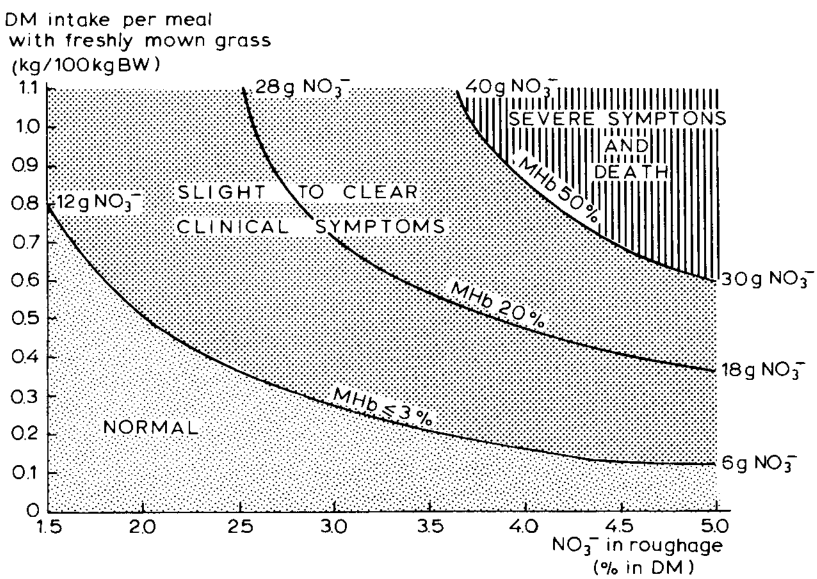

Fig. 4. The nitrate content (x-axis) and the dry matter intake (y-axis) of freshly mown grass in relation to the formation of methemoglobin. BW = bodyweight.

NO_3^- per 100 kg bodyweight). Fig. 2 shows that about 120 min will be required for that amount of intake. Under these conditions the nitrate intake rate is so slow that nitrite does not accumulate in the rumen.

Therefore freshly mown grass with a nitrate content up to 1.50 % NO_3^- in the dry matter may be supplied ad libitum without increasing MHb content in the blood. A meal of freshly mown grass fed indoors with greater than 1.50 % NO_3^- in the dry matter can be fed no more than every 120 min. The amount per feeding can be determined form Fig. 4.

In feeding turnips the acceptable level of nitrate intake is somewhat higher than with preserved grass, but distinctly lower than with fresh grass. When working with a nitrate content in the roughage as in Figs. 3 and 4, it should be realized that there may be considerable variation in the nitrate contents within one load. When an average content of 0.75 % NO_3^- is established in a load of baled hay, the contents in the separate bales may vary from about 0.60 to 1.00 % (Kemp et al., 1978). This means that the individual animals may sometimes be fed on roughage with contents above 0.75 % NO_3^- in the dry matter and that with feeding ad libitum, the MHb contents in the bood may rise. It is to be expected that the rate of intake of grazing animals will be slower than with two meals a day indoors, and because of this the grazed grass may contain some 2.0 % NO_3^- in the dry matter without increasing the MHb content in the blood. Probably this also explains why under Dutch conditions nitrate poisoning in grazing cattle hardly occurs, despite much increased nitrate contents regularly occurring in the herbage. About two times more nitrate can be safely taken in with fresh grass than with preserved roughages. This means that when the nitrate content in the herbage is too high, the grass should be preferably grazed or fed indoors instead of preserving it for hay or pre-wilted silage. Differences in grass species may also affect the relationship between nitrate intake and MHb formation. Previous papers (Kemp et al., 1978; Geurink et al., 1979) demonstrated that nitrate supplied in feshly mown cocksfoot caused a higher MHb percentage than an equal dose of nitrate supplied in freshly mown ryegrass. This difference between cocksfoot and ryegrass cannot presently be explained.

Discolouration of the vaginal membrane

The formation of MHb in the blood is associated with a change in the colour of the blood from red to chocolate-brown, which is reflected in the discolouration of the mucous membranes from pink to gray-brown. Because these discolourations already occur with MHb contents higher than 20 %, whereas severe clinical symptoms only occur abouve 50 %, nitrate poisoning can be detected in an early stage. Kemp et al. (1976) clearly demonstrated that the discolouration of the mucous membranes can also be used as a reliable criterion in deciding whether or not preventive measures are to be taken.

Preventing nitrite formation in the rumen by dosing with small amounts of sodium tungstate

Nitrite formation in the rumen after comsumption of nitrate-rich forage can also be avoided by inhibition of the enzymatic activity of nitrate reductase. Molybdenum is known to be indespensable for the action of nitrate reductase enzyme. Recently, Korzenowski et al (1980 and 1981) demonstrated, both in vitro and in vivo, that the addition of sodium tungstate to the rumen fluid could considerably inhibit the nitrate reductase acticity in the rumen and result in considerably less nitrite formation from nitrate-rich forage.

However, for practical application of tungstate as a prophylactic against nitrate toxicity, alle potential hazards should be carefully considered. In general, tungstate ion is considered as relatively harmless. Nevertheless, practical application of tungsten should be preceded by studies on the possible dangers to the animal, to the consumer of animal products, and to the environment. Suggestions for further research in this field have been given by Korzeniowsky et al. (1981).

References

Ashbury, A. C. & E. A. Rhode, 1964 Nitrite intoxication in cattle: The effects of nitrite on blood pressure. *Am. J. vet. Res.* 25: 1010-1013.

Assali, N. S. & C. R. Brinkman, 1973. The role of circulatory buffers in fetal tolerance to stress. *Am. J. Obst. Gynecol.* 117: 643.

Broekhoven, L. W. van & R. W. Stephany, 1978. Environmental aspects of N'nitroso compounds. *IARC scient. Publ.* 19: 461-463.

Crawford, R. F., 1960. Some effects of nitrate in forage in ruminant animals. Ph. D. thesis, Cornell University, Ithaca, N.Y.: 1-156.

Gamgee, A. 1868. Researches on the blood. On the action of nitrites on blood. *Phil. Trans. Roy. Soc. Lond.*: 589.

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 of fresh roughage and the speed of intake on the formation of methemoglobin. *Neth. J. agric. Sci.* 27: 268-276.

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 Fedn* (Gent).

Korzeniowsky, A., J. H. Geuring & A. Kemp, 1980. Nitrate poisoning in cattle. 5. The affect of tungsten on nitrite formation by rumen microbes. *Neth. J. agric. Sci.* 28: 16-19.

Korzeniowsky, A., J. H. Geuring & A. Kemp, 1981. Nitrate poisoning in cattle. 6. Tungsten (wolfram) as a prophylactic against nitrate-nitrite intoxication in ruminants. *Neth. J. agric. Sci.* 29: 37-47.

Malestein, A., J. H. Geurink, G. Schuyt, A. J. Schotman, A. Kemp & A. Th. van 't Klooster, 1980. Nitrate poisoning in cattle. 4. The affect of nitrite dosing during parturition on the oxygen capacity of maternal blood and the oxygen supply to the unborn calf. *Vet. Quarterly* 2 (3, July) 149-159.