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Nitrate poisoning in cattle. 6. Tungsten (wolfram) as a prophylactic against nitrate-nitrite intoxication in ruminants

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Summary

The amount of nitrite formed in the rumen after supply of potassium nitrate was decreased considerably by tungsten, which was administered as sodium tungstate to cows per os and by rumen fistula in daily doses of up to 6.6 mg W per kg body weight. This effect of tungsten depends on the molybdenum content of the fodder and can be overcome in the case of high molybdenum levels.

The relationship between the nitrate intake of the animal, the dose of tungsten administered to the animal daily and the highest concentration of nitrite reached in the rumen is given as a mathematical equation as well as in the form of a triangular diagram. By means of this three-element relationship and as a result of kinetic studies on the action of tungsten, a dosage pattern of tungsten has been elaborated.

The effectiveness of tungsten as a protective against nitrate intoxication was proved on cows dosed repeatedly as well as with single doses of nitrate up to 500 mg NO_3^- per kg body weight. It is concluded finally that tungsten offers a high degree of protection against nitrate toxicity to ruminants.

Introduction

After consumption of nitrate by ruminants, rumen micro-organisms reduce it to ammonia, and nitrite is an intermediate in this reaction. The nitrite is partly absorbed into the blood-stream leading to methemoglobinemia and to a decrease in blood pressure. The formation of excessive nitrite in the rumen is a toxicological problem which has not been solved completely as yet. There is conclusive evidence

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that the total nitrate intake, the rate of intake and the type of fodder are playing a part. So prevention of nitrate poisoning may be focused on diminishing the nitrate intake, decreasing the rate of fodder intake or on changing of feeding systems (Kemp et al., 1976, 1977, 1978; Geurink et al., 1979; Malestein et al., 1980).

Another approach to the solution of this problem may be the depression of the nitrite formation rate by means of an inhibition of the enzymatic activity of nitrate reductase. Recently some efforts have been made aiming to control the formation of nitrite by rumen microbes in which use has been made of tungsten to inhibit the enzymatic activity of nitrate reductase (Korzeniowski et al., 1980). These in vitro experiments provided evidence that relatively low concentrations of sodium tungstate, 20-100 μmol per litre rumen fluid, are able to suppress strongly the rate of nitrite formation. Experiments done in vivo confirmed these findings.

Keeping in mind the strong inhibitory properties of tungsten against nitrite formation and the relatively low toxicity of tungstate to animals, tungsten looks very promising in preventing nitrite intoxication in ruminants. The purpose of the following experiments was to get more information about the use of tungsten for protection of cattle against nitrate poisoning.

Material and methods

The experimental procedure was focused on studying the rate of nitrite formation in rumen fluid in cows receiving different amounts of tungsten and molybdenum and dosed thereafter with different amounts of nitrate. All experiments were carried out on six rumen fistulated Friesian milking cows with a body weight of 520-585 kg. Throughout the study they were maintained indoors with free access to water. The daily ration consisted of 9 kg of hay low in nitrate given in two portions and supplemented with 6 kg of a pelleted commercial dairy concentrate given in three portions. During the whole preliminary and experimental period all the cows received regularly a daily dose of at least 120 mg NO_3^- per kg body weight. It can be assumed therefore that the rumen microbes were adapted to higher nitrate levels, a condition which had been found earlier to be essential in nitrate toxicity studies (Kemp et al., 1977). Nitrate was administered directly into the rumen through a fistula. Mostly potassium nitrate (pro analysi) was used but in cases of high nitrate doses it was replaced partly by an equivalent of the sodium salt. Before administration the aqueous nitrate solution was diluted to a concentration below 1 mol per litre and prewarmed to about 40 °C. Tungsten was administered to the cows either in the form of an aqueous solution of sodium tungstate (pro analysi) via the rumen fistula direct into the rumen, or as a pelleted concentrate applied per os containing 10 g W (as $Na_2WO_4 \cdot 2H_2O$) per kg concentrate.

On average the diet contained 1 mg of molybdenum per kg dry matter. In some experiments the influence of artificially elevated molybdenum levels in the fodder was examined. For this hay was sprayed with a solution of $(NH_4)_6Mo_7O_{24}$. Glycerol was added to this solution (100 g/litre), in order to avoid molybdenum losses in the form of dust.

Just before nitrate was added, sampling of the rumen fluid was started and sam-

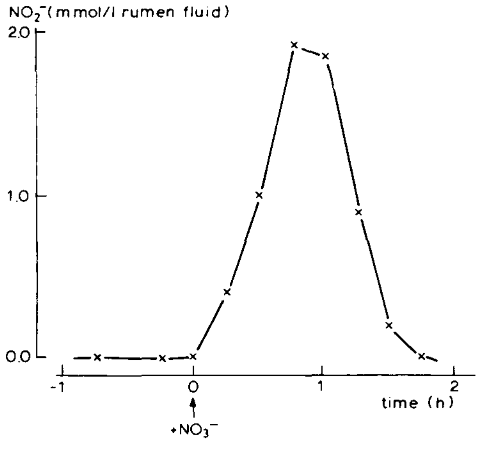

Fig. 1. Nitrite concentration response in the rumen to a single intraruminal dose of 120 mg NO_3^- per kg body weight.

ples were taken every 15 minutes. This was done by means of a perforated tube, pressed through the fistula almost to the bottom of the ventral rumen sack. The sampling tube was provided on its whole length with numerous holes, 1 mm in diameter, so the liquid sample drawn from the rumen was of limited particle size. To preserve samples, 40 ml of rumen fluid was shaked immediately with 10 ml of a saturated solution of lead acetate and stored at + 3 °C and analysed within a fortnight for nitrate and nitrite as described previously (Kemp et al., 1977). The nitrite concentrations found in the rumen fluid were plotted on a graph against time as shown (as an example) in Fig. 1. The highest nitrite content found in this way has been chosen as an index which is used in this paper to express the capacity of nitrite formation in the rumen.

Results and discussion

Effect of a single tungsten dose on the nitrite formation rate in the rumen
In this experiment cows were dosed successively once or twice daily with intraruminal single doses of potassium nitrate amounting to 120 mg NO_3^- per kg body weight. After every nitrate dose applied, the highest nitrite concentration in the rumen was determined as described earlier in this paper. These peak values of nitrite concentration are shown in Fig. 2. At time t = 0 marked on the time axis of this graph, the animals received single intraruminal doses amounting 5.6 or 11.3 mg of W per kg body weight given in the form of sodium tungstate. This resulted in a distinct, transitory suppression of the nitrite formation capacity in the rumen. A lag phase is seen in the action of tungsten, the inhibition effect arises gradually and the strongest effect is reached about 24 hours after administration of tungsten. About 72 hours after tungsten administration, the inhibition effect is over. A twofold increase of the tungsten dose, does hardly increase the inhibition effect.

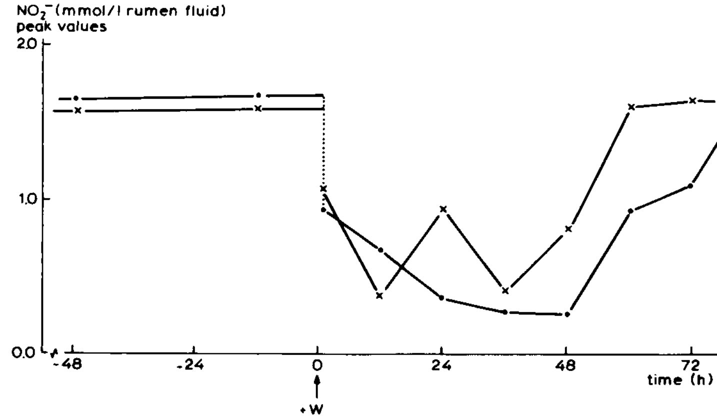

Fig. 2. Effect of a single intraruminal dose of sodium tungstate on the highest nitrite concentration reached in the rumen after 120 mg NO_3^- per kg body weight had been applied: X, single dose of 5.6 mg W per kg body weight; ●, single dose of 11.5 mg W per kg body weight.

The effect and after effect of repeatedly administered tungsten doses on the nitrite formation rate in the rumen

The aim of these experiments was to investigate the influence of tungsten applied in multiple doses onto the ruminal nitrite formation capacity. It is seen in Fig. 3 that beginning at time = 0 a cow received regularly twice daily an intraruminal dose of 2.2 mg of W per kg body weight.

To investigate changes in ruminal nitrite formation capacity caused by this tungsten application, in arbitrarily taken intervals single doses of 120 mg NO_3^- (as KNO_3) per kg body weight had been applied intraruminally and thereafter the highest ruminal nitrite concentration was determined as described earlier. The peak values of ruminal nitrite concentration found are plotted against time in Fig. 3. As in the case of a single tungsten dose (Fig. 2), the nitrite formation capacity is being reduced gradually from a value of about 1.9 mmol NO_2^- per litre and a steady stat of about 0.1 mmol is reached during a period of about 48 hours.

Cessation of tungsten administration results, as seen in Fig. 4, in a sluggish restoration of the nitrite formation capacity in the rumen to about the same level as prior to tungsten treatment. This process lasted 96 hours at least. The after-effect observed may be explained either by a prolonged tungsten action in the rumen or by an inactivation of the nitrate reducing rumen bacteria. The latter is less probable because ruminal inoculation of the experimental cows with rumen contents drawn from a tungsten free, nitrate adapted cow did not exert any influence on the after effect.

Protective value of tungsten against heavy nitrate doses

These experiments were performed on cows treated permanently with 6.6 mg W per kg body weight daily. Two animals were dosed with single doses of nitrate, 240 and 500 mg NO_3^- per kg body weight respectively. Fig. 5 shows the time course of the ru-

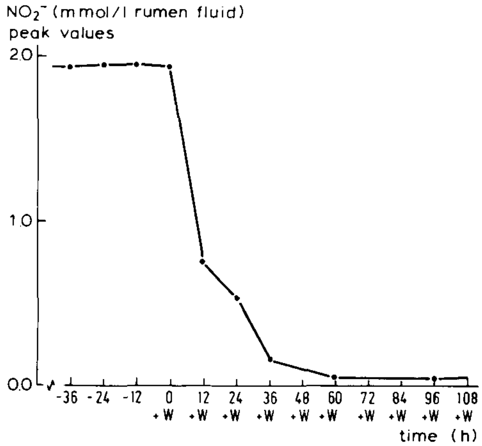

Fig. 3. Effect of repeatedly applied intraruminal doses of 2.2 mg W per kg body weight on the highest nitrite concentration reached in the rumen after doses of 120 mg NO_3^- per kg body weight had been applied.

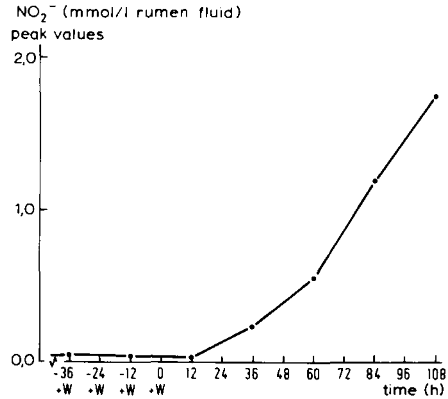

Fig. 4. After effect of repeatedly applied intraruminal doses of 2.2 mg W per kg body weight on the highest nitrite concentration reached in the rumen after doses of 120 mg NO_3^- per kg body weight had been applied.

minal nitrite concentration induced by the nitrate doses mentioned above. Results of another nitrate loading test are shown as an example in Fig. 6. It demonstrates the ruminal nitrite response after a tungsten treated cow (6.6 mg W/kg) was dosed at hourly intervals with five subsequent nitrate doses, a situation more similar to the uptake of nitrate from nitrate rich fodder under practical conditions. To spare animals, no experiments have been done with high nitrate doses on cows which have not been protected with tungsten.

In general results obtained in both experiments (Fig. 5 and 6) are similar and comparable. The nitrite response curves observed in tungsten protected cows which were dosed heavily with nitrate are rather flat and show at the end a characteristic peak. But the most important feature are the low peak values of nitrite concentration being a result of the inhibitory action of tungsten. This is demonstrated clearly if one compares nitrite response curves in Fig. 5 and 6 with the curve in Fig. 1.

Relationship between the nitrate dose, the tungsten dose and the ruminal nitrite concentration

Fig. 7 shows as an example the influence of increasing tungsten doses applied to the animal daily on the highest nitrite concentration in the rumen after the animals were dosed with different single doses of nitrate administrated intraruminally as KNO_3. This graph demonstrates convincingly the effectiveness of tungsten in suppression of nitrite formation in the rumen. No difference was found in the action of tungsten either applied orally as sodium tungstate incorporated into a concentrate or infused intraruminally as an aqueous solution of sodium tungstate.

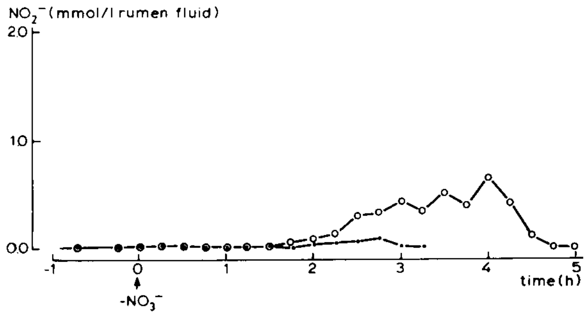

Fig. 5. Nitrite concentration response in the rumen at a single intraruminal dose of nitrate administered to a cow receiving daily intraruminally 6.6 mg W per kg body weight: ●, 240 mg NO_3^- per kg body weight; ○, 500 mg NO_3^- per kg body weight.

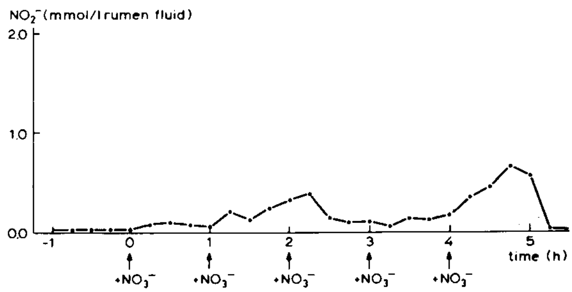

Fig. 6. Nitrite concentration response in the rumen after intraruminal administration of five successive nitrate doses of 120 mg NO_3^- per kg body weight given at hourly intervals to a cow receiving daily 6.6 mg W per kg body weight.

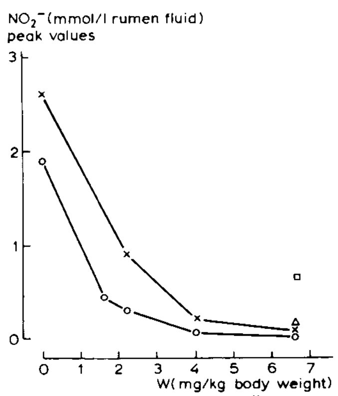

Fig. 7. Relation between the daily tungsten dose and the highest nitrite concentration in the rumen after different single doses of nitrate had been applied. ○, 120 mg NO_3^- per kg body weight; ×, 180 mg NO_3^- per kg body weight; △, 240 mg NO_3^- per kg body weight; □, 500 mg NO_3^- per kg body weight.
All measurements have been made on cows equilibrated with the daily tungsten doses for some days.

Analysing data of 44 experiments performed in the range from nil to 6.6 mg of tungsten daily and from 60 mg to 500 mg of nitrate per kg body weight it was found that the following equation roughly outlines the nitrite-nitrate-tungsten relationship

$$\log NO_2^- = 0.0033\ NO_3^- - 0.25\ W - 0.19$$

$\log NO_2^-$ is the common logarithm of the highest ruminal nitrite concentration expressed in mmol per litre rumen fluid; NO_3^- is the single nitrate dose expressed in mg of NO_3^- per kg body weight; W is the daily tungsten dose expressed in mg of W per kg body weight.

It should be emphasized that this relationship applies to animals adapted to nitrate and equilibrated with the tungsten dose under consideration during a period of at least 3-4 days. The not-linear relationship found between the nitrate dose and the highest ruminal nitrite concentration may be explained by a rise of nitrate concentration, on the one hand hastening the formation of nitrite, and on the other hand resulting in nitrite reductase inhibition and suppression of the breakdown of the nitrite being generated. Fig. 8 shows the above mentioned nitrite-nitrate-tungsten relationship in the form of a triangular diagram. Assuming a level of maximum nitrite concentration acceptable in the rumen, by use of this diagram the required tungsten dose can be found in relation to the single dose of nitrate uptake by the animal. Inversely, the expected ruminal nitrite concentration can be found in this diagram if nitrate and tungsten doses are known. The presented three-component relationship was derived for a dietary molybenum concentration equal to 1 mg of Mo per kg dry matter.

Influence of elevated dietary molybdenum levels on the action of tungsten

As found earlier in our in vitro experiments (Korzeniowski et al., 1980), molybdenum showed a marked effect on the action of tungsten in rumen fluid. A similar interaction between molybdenum and tungsten has been found in experiments performed on cows. Results of these experiments are shown in Fig. 9. In comparison to animals fed a diet containing 1 mg of molybdenum per kg dry matter, the inhibitory

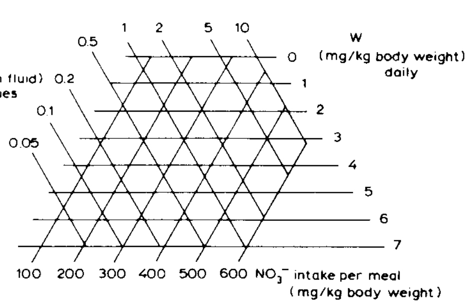

Fig. 8. Relation between the nitrate dose, daily tungsten dose and the highest nitrite concentration reached in the rumen.

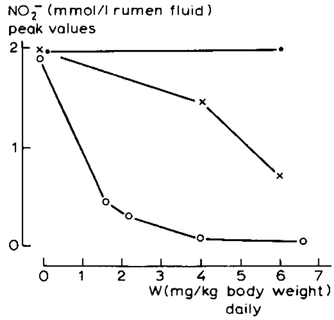

Fig. 9. Relation between the daily tungsten dose and the highest nitrite concentration reached in the rumen after doses of 120 mg NO_3^- per kg body weight had been applied to cows which were fed on rations with different molybdenum concentrations: ○, 1 mg Mo per kg dry matter; ×, 6 mg Mo per kg dry matter; ●, 11 mg Mo per kg dry matter.

properties of tungsten are considerably reduced in the case of 6 mg of molybdenum per kg dry matter of the diet. With 11 mg of molybdenum in the diet even a dose of 6 mg of tungsten did not exert any detectable influence on the rate of nitrite formation in the rumen. Although three levels of molybdenum have been examined only, it may be concluded that elevated molybdenum levels require a sharp increase of the tungsten dose to achieve an effective suppression in nitrite formation. Whether increased tungsten doses required to compensate for elevated molybdenum levels would exert any adverse effects to the animal, is a matter for further research.

Adverse effects of tungsten

If the utility of tungsten as a prophylactic against nitrate toxicity has to be considered, all potential hazards should be carefully examined. For combating nitrate toxicity by tungsten, may be called a substitution of one poison by another one. In general, the tungstate ion is considered as relatively harmless (Kazantzis, 1979) and toxicological data about it is rather scarce. In the guinea pig the lethal dose of sodium tungstate given orally is said to be 990 mg/kg (Spector, 1956). In 70-day experiments sodium tungstate incorporated in a diet fed to rats showed a 100 % mortality by a concentration of 2 % W (Kinard & Van De Erve, 1941). In our experiments during periods of one month cows received total doses up to 120 mg W per kg body weight and no visible symptoms occurred. Nevertheless, an application of tungsten in practice should be preceded by studies on the eventual potential danger to the animal, to the consumer of animal products and to the environment.

In our opinion the following aspects are of importance.

– *Inhibition of sulphite oxidase.* Though no adverse effects were found in tungsten treated rats (Cohen et al., 1973), an increased susceptibility to bisulphite toxicity was seen as a result of sulphite oxidase inhibition.

– *Inhibition of xanthine oxidase.* This enzyme also exhibits tungsten inhibition (Higgins et al., 1956; Owen et al., 1968), nevertheless as yet it is difficult to draw a conclusion from literature data whether this process may be detrimental to cattle.

– *Influence on cellulose digestion.* Molybdenum is said to stimulate cellulose digestion (Church, 1975). The interaction between tungsten and molybdenum suggests that tungsten may lead to disturbances there. Even if this is so, the effect was imperceptible in our experiments.

– *Influence on copper metabolism.* According to ties which exist between molybdenum and the metabolism of copper, it may be expected that tungsten acting as a molybdenum antagonist may affect the copper status of the animal. Indeed, such interaction has already been demonstrated (Bremner, 1979). Nevertheless further research is needed on tungsten induced copper metabolism disturbances in cattle under practical condition.
– *Residual problems.* Radioisotope studies (Mullen et al., 1976) revealed a rather low absorption of radiotungsten by the dairly cow; during the 84-hour period after dosing, 79 % of the orally administered dose was recovered, with 64 % recovered in the faeces, 14.6 % in the urine and 0.4 % in milk. According to data compiled from literature (Stokinger, 1963), tungsten tends to be deposited in the bone and spleen with lesser amounts going to the kidney and liver and possibly the skin. Authors agree that much of the absorbed tungsten is rapidly excreted in the urine (Aamodt, 1973; Kazantzis, 1979). The results quoted were obtained mostly by radiotungsten studies and no data on the concentration of tungsten in tissues and body fluids were found. In our experiments a cow receiving for three weeks a daily dose of 2.2 mg W/kg body weight excreted 0.22 mg W/litre milk, in the case of a daily dose of 6.6 mg W/kg body weight the tungsten concentration in milk increased to 1.4 mg W/litre.
– *Environmental problems.* Almost all the tungsten administered to animals will accumulate in the soil fertilized with manure originating from tungsten treated animals. There a tungsten inhibition of molybdenum containing enzymes in soil and root microbes may be expected as well as an inhibition in the higher plant. The effects of increased tungsten concentration in soil have been investigated by Quin et al. (1976). The authors conclude that it is highly unlike that tungsten in amounts exceeding 1500 times the concentration of soil molybdenum will seriously affect pasture growth. It is noteworthy to mention that tungsten inhibition may be reversed by addition of more molybdenum.

Conclusions

1. Tungsten applied as sodium tungstate has been shown to be an effective inhibitor of nitrite formation in the rumen of cattle.
2. The effective dose of tungsten depends on the amount of nitrate the animal is dosed with and on the level of nitrite admissible in the rumen. This dose-response relationship is given by the equation $\log NO_2^- = 0.0033\, NO_3^- - 0.25\, W - 0.19$ and in addition presented as a triangular diagram (Fig. 8). It was estimated for a dietary molybdenum concentration equal to 1 mg Mo per kg dry matter.
3. Increased molybdenum concentration in the ration suppresses the action of tungsten and more tungsten is needed to achieve the same inhibition in nitrite formation. It is an open question whether increased tungsten doses would cause any harmful effects to animals receiving molybdenum rich feed. In the light of the competitive W – Mo interaction rather a beneficial effect of tungsten could be expected, i.e. an increased tolerance of cattle to higher dietary concentrations of molybdenum.

4. No difference was found in action of tungsten applied either orally as sodium tungstate incorporated into a concentrate or infused intraruminal as an aqueous solution of sodium tungstate.
5. Kinetic studies on the action of tungsten revealed that to achieve a steady state effect, tungsten should be administerd repeatedly once daily at least.
6. There is a lag phase between the time when the first tungsten dose is given and the onset of the inhibitory action. Administering a loading dose of tungsten hardly seems to shorten this lag phase. Therefore, if use is to be made of tungsten in the prevention of nitrate-nitrite intoxication, feeding of nitrate rich fodder should be preceded by administration of tungsten in due time.
7. In the course of one month experiments no adaptation of ruminal microbed to tungsten was observed. This means that there is no need to increase the tungsten dose during a long period of tungsten application in order to secure a constant inhibition effect.
8. Though no adverse effects have been found in our experiments, the eventual application of tungsten in practice should be anticipated by long term toxicological investigations.
9. Results from in vitro experiments and studies on nitrite formation and breakdown in the rumen indicate that the elevated nitrate concentration in the rumen on the one hand hastens nitrite formation, whilst on the other hand nitrite breakdown is suppressed due to the inhibitory action of nitrate against the activity of nitrite reductase. This results in a very rapid increase of the ruminal nitrite concentration.

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