



Nitrate and nitrite

Anuraga Jayanegara





Outline

- A. Introduction
- B. Structure
- C. Uptake and assimilation of N
- D. Factors affecting nitrate accumulation
- E. Analytical methods
- F. Biological effects
- G. Treatment for nitrate poisoning

A. Introduction



Nitrate and nitrite

- Occasionally forages accumulate nitrates in quantities that are toxic to some farm animals
- Influencing factors: drought, shade, use of herbicides, and application of nitrogenous fertilizers
- Nitrates are not very toxic, but they are readily converted by bacteria in the alimentary tract into nitrites, which are much more toxic

B. Structure

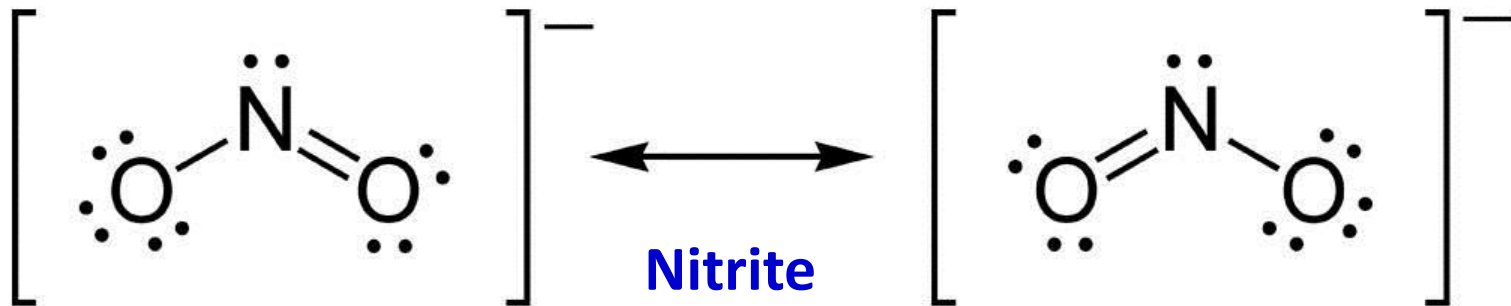
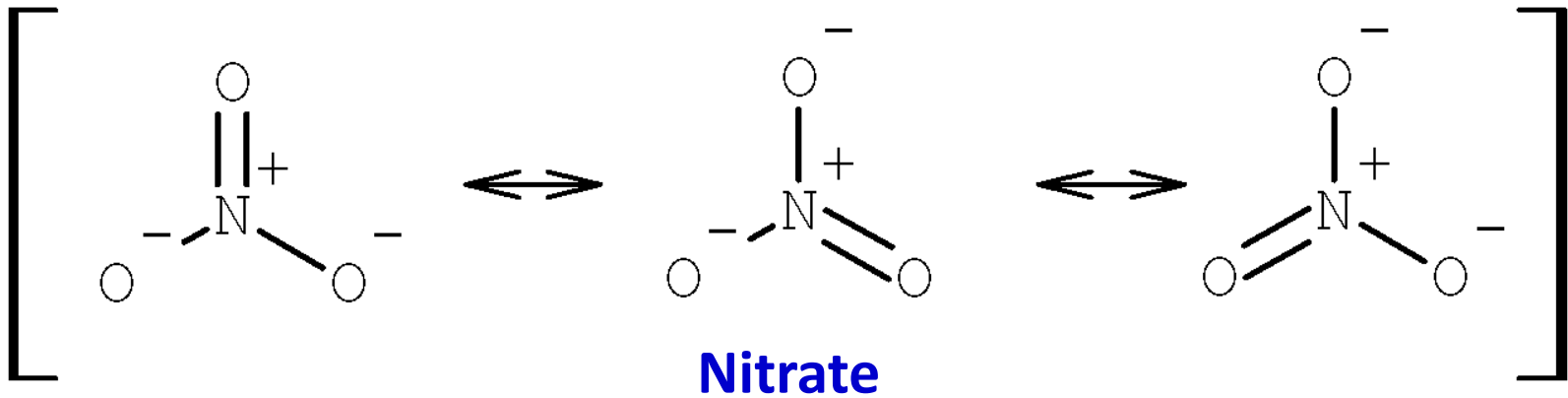
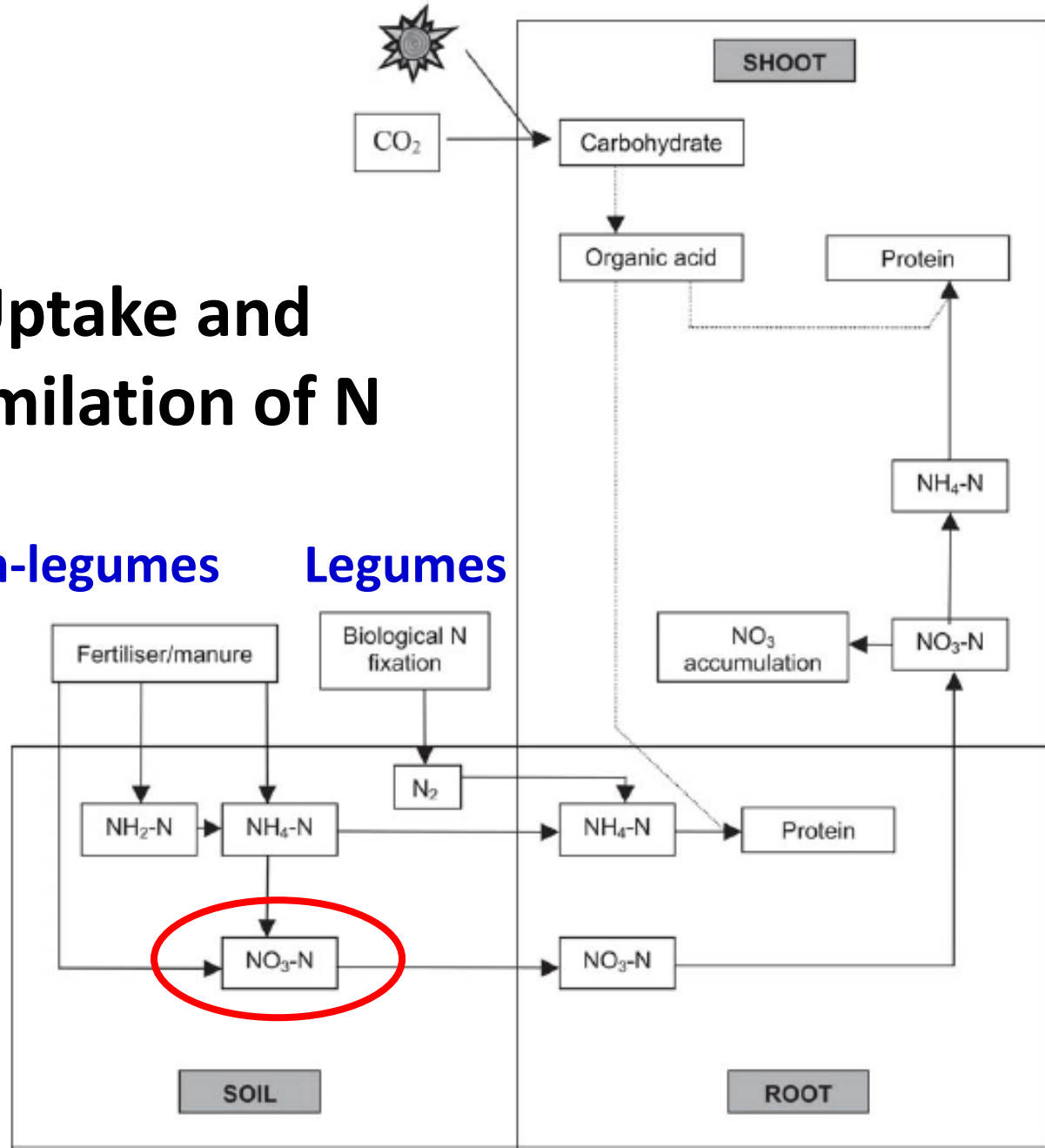


Figure 1 Uptake and assimilation of nitrogen.

C. Uptake and assimilation of N

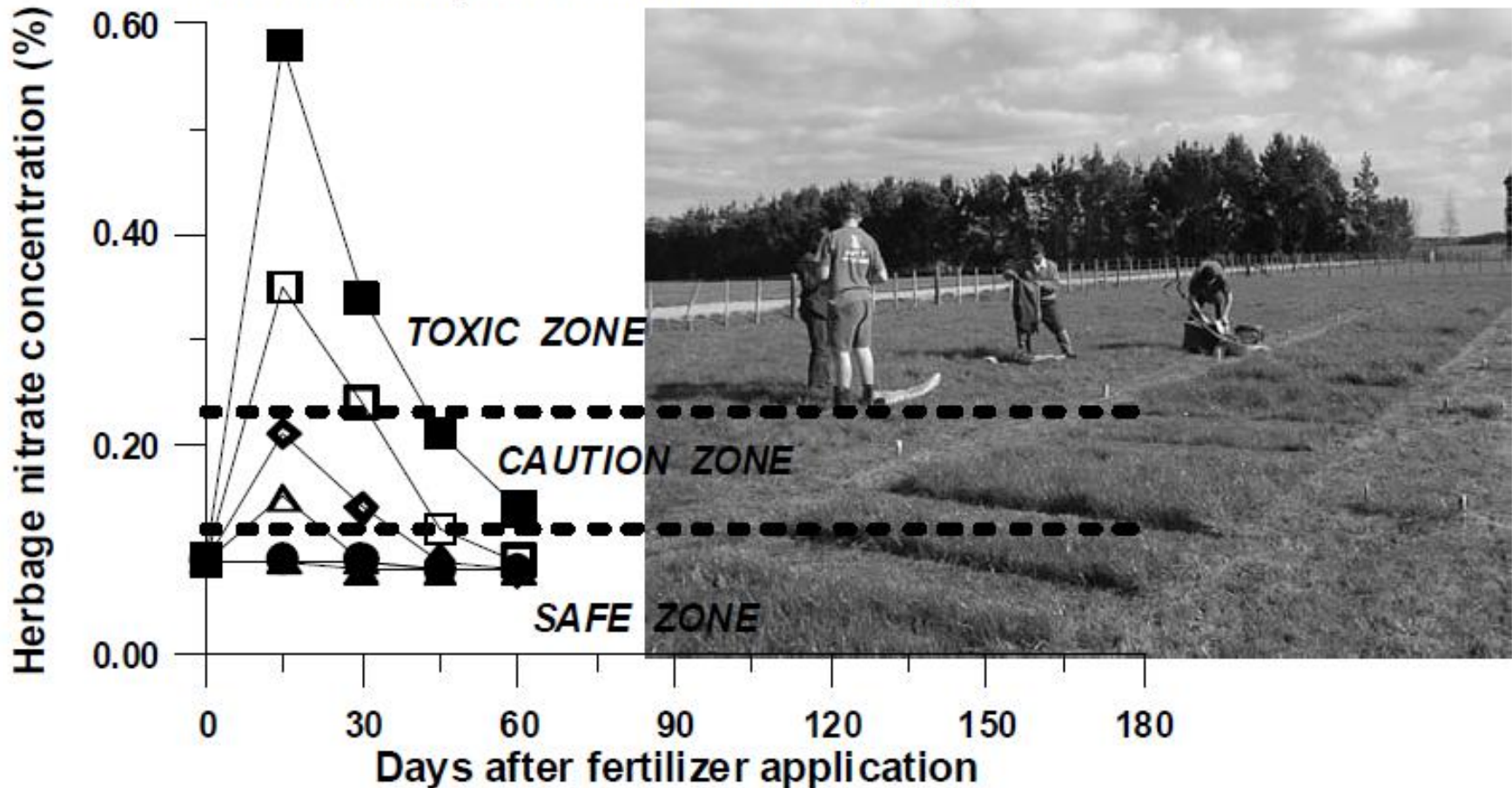
Non-legumes Legumes



D. Factors affecting nitrate accumulation

1. Nitrogen input

Figure 2 Effect of fertilizer nitrogen on nitrate nitrogen concentration in the pasture (■ 600 kgN/ha; □ 400 kgN/ha, ◇ 200 kgN/ha; △ 100 kgN/ha, ● 50 kgN/ha; ▲ 0 kgN/ha) (inset plate: Field experiment examining the effect of fertilizer nitrogen on nitrate concentration in pasture).





2. Plant species

- Differences in nitrate accumulation have been found between species and varieties
- Nitrate accumulation is less likely to occur in legume plants which are actively fixing N in their root nodules as ammonia
- In grasses, high concentration of nitrate is found in the year of sowing and it decreases with the age of the crop
- In general, forage crops which produce considerable amounts of leafy materials, such as ryegrass convert nitrate to organic N, whereas grain crops such as oat converts less nitrate to organic N, thereby resulting in nitrate accumulation



3. Growth stage

- In mature leaves, nitrate reduction is limited leading to the accumulation of nitrate, especially soon after N fertilizer application
- Nitrates normally accumulate in stems and conductive tissues
- Concentrations tend to be low in leaves because of high nitrate reductase enzyme levels



4. Drought

- Nitrate concentration is high after a short period of drought for two reasons
- (1) during the drought period the nitrate concentration builds up in the soil and most of the N is taken up in this form
- (2) the moisture stress during the drought period causes dry matter yield depression, thereby resulting in less reduction of nitrate to organic N



5. Sunlight

- Sunlight affects nitrate accumulation through its direct effect on nitrate reduction and indirect effect on dry matter yield
- Nitrate reduction occurs in young leaves and requires light as an energy source --> shaded plants or cloudy weather lack sufficient energy to convert nitrate to amino acids



6. Plant stress factors

- Plant stress factors, such as hail, light frost, or plant disease can damage plant leaf area and reduce photosynthetic activity
--> increase nitrate hazard in animals

E. Analytical methods



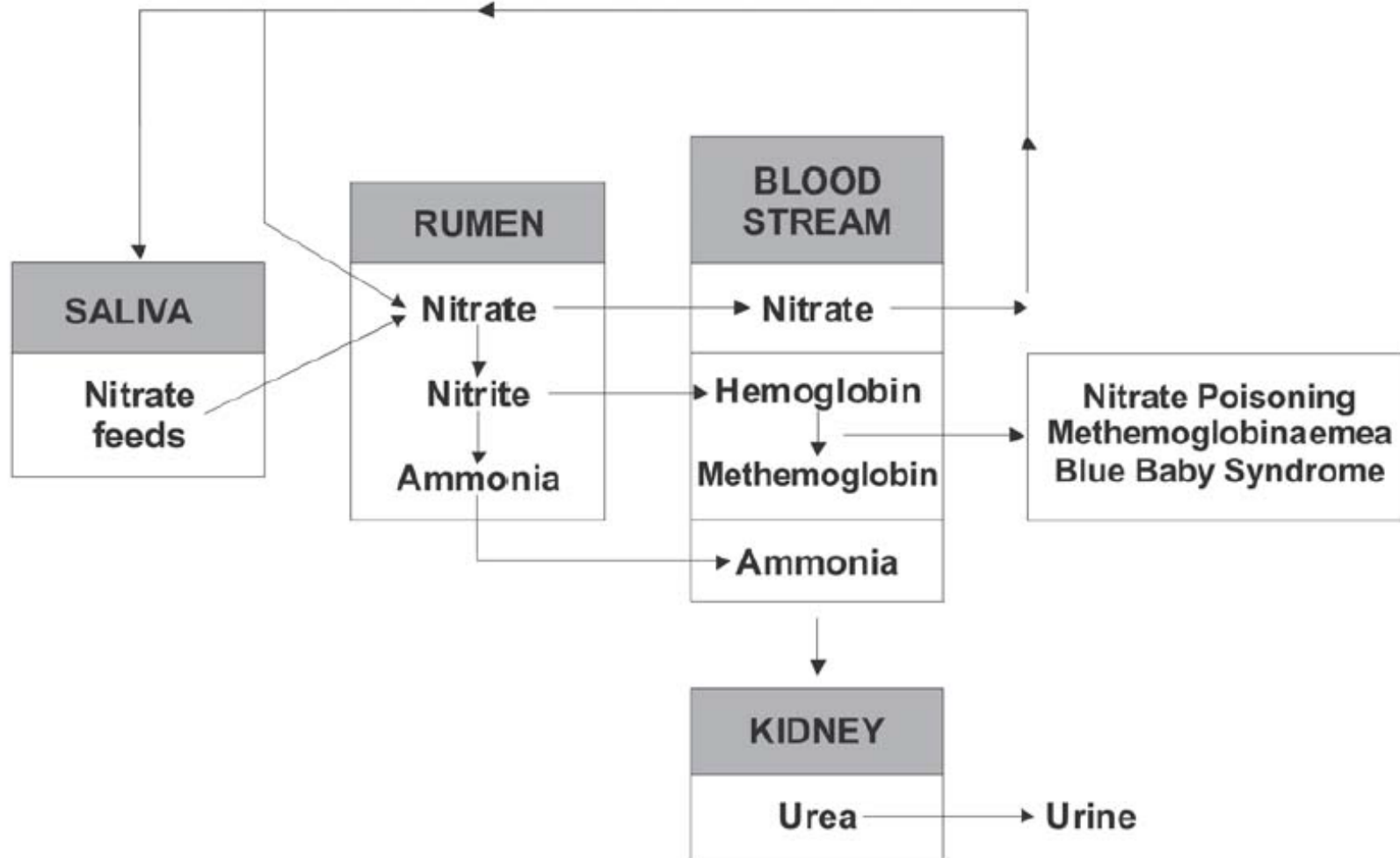
Methods

1. Extraction with distilled water
2. Reduction reaction from nitrate to nitrite
3. Reaction to form color (from nitrite only)
4. Measurement of absorbance using a spectrophotometer
5. Nitrate content is calculated by difference

[More detailed](#)

F. Biological effects

Figure 3 Nitrate pathway in plants and ruminants (Kvasnicka & Krystal 1996).



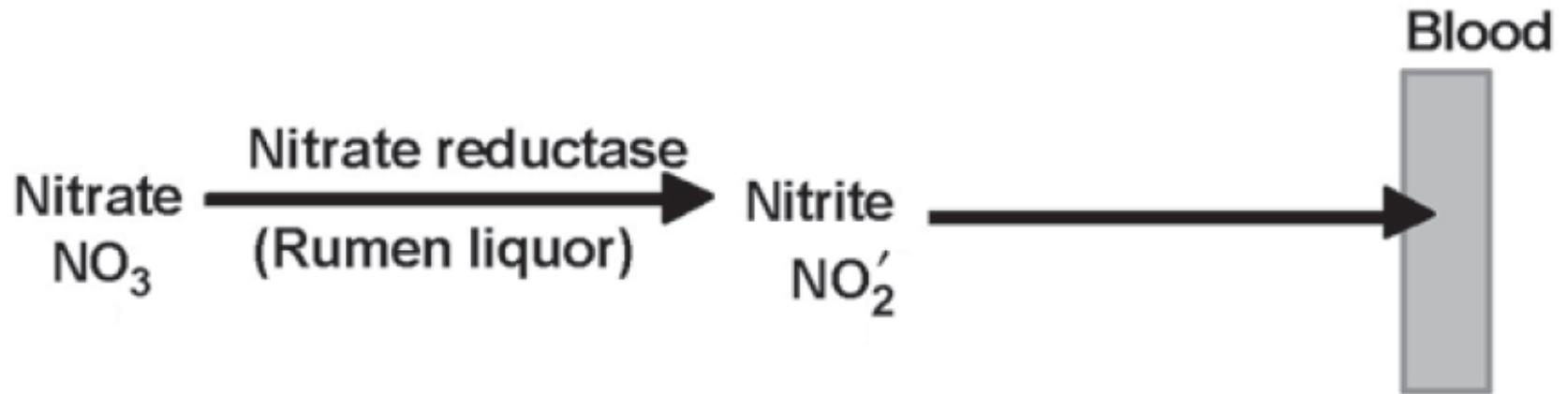


Fig. 1. Mechanism of action of nitrate.



- Nitrites pass from GIT into the blood --> combine with hemoglobin in the red blood cells to form methemoglobin, a compound that is incapable of taking up and transporting oxygen
- Clinical signs of nitrite poisoning: oxygen deficiency, general weakness, fall in blood pressure, heart rate increases, muscle tremors develop
- Clinical signs begin to appear when methemoglobin reaches 30-40% within the blood and moves on to convulsions followed by death at 80-90%
- Young animals and babies are particularly at risk --> small volume of blood --> requires only a small amount of nitrite to convert all the hemoglobin to methemoglobin --> blue baby syndrome



- Nitrite may interfere with progesterone synthesis, which is the primary hormone involved in supporting pregnancy --> abortus problem
- 7.6-9.0 g nitrate per 100 kg body weight is lethal to animals --> pasture with a nitrate content $>0.3\%$ is likely to be toxic to animals with a live body weight of 300 kg



Other biological effects:

- [Takahashi et al. \(1998\)](#)
- [Sar et al. \(2004\)](#)
- [Van Zijderveld et al. \(2010\)](#)
- [Van Zijderveld et al. \(2011\)](#)

G. Treatment for nitrate poisoning



1. Immediately remove the animal from high nitrate feed source and offer plenty of water
2. A high carbohydrate, low nitrate feed should be offered as this will decrease rumen nitrate concentration and also decrease the rumen pH, which slows down the reduction of nitrate to nitrite in the rumen
3. Methylene blue treatment --> treated intravenously --> methylene blue is thought to act as an electron acceptor for the methemoglobin reductase enzyme in blood, thereby speeding the reconversion of methemoglobin to hemoglobin



Thank you for your attention!