A review of factors affecting and prevention of pasture-induced nitrate toxicity in grazing animals

N.S. BOLAN and P.D. KEMP
Institute of Natural Resources, Massey University, Palmerston North
N.S.Bolan@massey.ac.nz

Abstract

Although plant roots take up nitrogen (N) both as ammonium (NH_4^+) and nitrate (NO_3^-) ions, under most soil conditions uptake of NO_3^- dominates. Once absorbed by the plant roots, the NO_3^- is reduced to NH_4^+, which is subsequently assimilated into organic compounds. However, when the rate of uptake exceeds the rate of NO_3^- reduction, accumulation of NO_3^- in plants occurs. Ruminant animals with high NO_3^- levels in their diets accumulate nitrite (NO_2^-). Nitrite is absorbed into the blood and combines with hemoglobin to form methemoglobin. This condition is known as nitrate poisoning (i.e., methemoglobinaemea).

Nitrate poisoning occurs when animals eat forage material with high NO_3^- content. The most common causes of high NO_3^- content in forage tissue include: (i) high application of N fertilizers; (ii) drought conditions; (iii) damage to plant tissue (such as defoliation as a result of herbicide application); (iv) low light intensity, which reduce photosynthetic activity; and (v) presence of NO_3^- accumulating plant species, such as annual weeds. In this review paper, the processes of uptake and assimilation of N by plants, factors affecting NO_3^- accumulation in plants, and the treatment and prevention of nitrate poisoning in grazing animals are discussed.

Keywords: forage crops, methemoglobinaemea, methylene blue, N fertilizer, nitrate accumulation, nitrate poisoning, nitrate reductase

Introduction

Nitrogen (N) is taken up by plant roots almost exclusively in the form of NO_3^- and NH_4^+, but under most soil and climatic conditions uptake of NO_3^- dominates (Mengel & Kirkby 1982). Once absorbed by the plant roots, the NO_3^- is reduced by the NO_3^- reductase enzyme to NH_4^+, which is subsequently assimilated into organic compounds, such as protein (Raven & Smith 1976; Pate 1980). Under unfavourable growing conditions, when the rate of uptake exceeds the rate of NO_3^- reduction, accumulation of NO_3^- occurs (Maynard *et al.* 1976). High levels of NO_3^- in edible tissue may lead to toxicity problems in both human beings and farm animals (Wright & Davison 1964). Recently there has been increasing concern about NO_3^- accumulation in pasture and forage crops and the subsequent nitrate poisoning of animals (Hillman 2002).

A preliminary survey (Bolan 1998) indicated that a number of cases of pasture and forage poisoning ascribed to a high NO_3^- content have been reported in sheep and dairy cattle in New Zealand in the past 10 years (Table 1). For example, in 1999, 63 dairy cows died from nitrate poisoning in a Northland dairy farm after the ingestion of pasture with high levels of NO_3^-. Nitrate poisoning of cattle strikes without notice and in this particular case it was caused by cool and cloudy weather after a dry spell in the region. Ruminants are more susceptible to nitrate toxicity than simple stomached animals because of the action of rumen microbes (Walters & Walker 1978; Bruningfann & Kaneene 1993a & 1993b).

The toxicity symptoms include trembling, staggering gait, rapid respiration and prostration. Affected animals cease to eat and soon collapse and

Table 1 Recent cases of nitrate toxicity in grazing animals in New Zealand (1990 – 2001; Bolan 1998).

Animal	Pasture/forage	Toxicity
Dairy cows	Ryegrass	63 died; 10 treated
Dairy cows	Ryegrass	23 died; 10 aborted; 15 treated
Dairy cows	Brassica/Hay	13 died; 14 aborted; 5 treated
Dairy cows	Grass/hay	3 died; 5 treated
Bulls	Brassica/oats	2 died; 2 ataxic and treated
Sheep	Ryegrass	12 died
Sheep	Ryegrass	7 collapsed, went into coma with rapid respiration, and died
Sheep	Ryegrass	21 died suddenly
Dairy cows	Ryegrass	12 died; 7 treated
Dairy cows	Maize	3 died; 190 aborted (reason unknown); 6 treated
Dairy cows	Swedes/Brassica	5 died; 10 aborted and 40 had dead calves

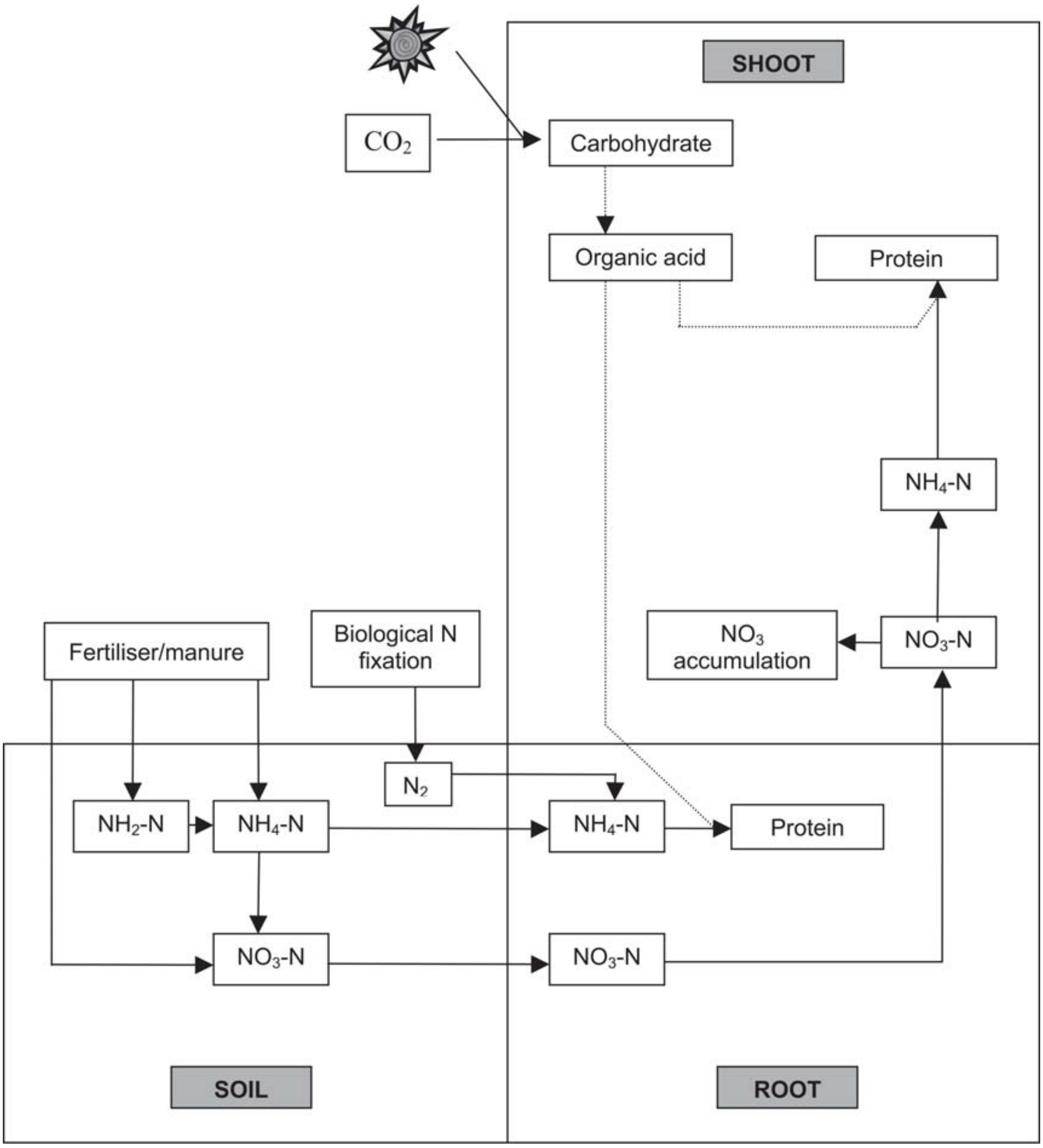
die without convulsions. Losses of weight and milk production and non-infectious abortion have been noted as sublethal effects in dairy cattle. The physiological reason for the poisoning is that the NO_3^- in the forage is converted to NO_2^- in the digestive tract. When NO_3^- is absorbed into blood it changes the haemoglobin into a form (methaemoglobin) that cannot transport oxygen and the animal is asphyxiated (Bruningfann & Kaneene 1993a & 1993b; Hill 1999).

This paper outlines the processes of uptake and assimilation of N by plants, factors affecting NO_3^- accumulation in plants, and the physiology and treatment of nitrate poisoning in grazing animals. The prevention of nitrate poisoning in grazing animals is discussed in relation to grazing management.

Uptake and assimilation of N

In legume plants, such as white clover, N is derived as ammonia during biological N fixation (Haynes 1986; Ledgard & Giller 1995). Non-legume plants, such as ryegrass, take up N almost exclusively in the form of NO_3^- and NH_4^+, but under most soil and

Figure 1 Uptake and assimilation of nitrogen.



climatic conditions the majority of N is taken up as NO_3^- because NH_4^+ ions undergo rapid nitrification, and also NH_4^+ ions are strongly retained onto the exchange sites (Clarkson & Hanson 1980; Russel 1973). When NH_4^+ ions are taken up they are converted directly to organic forms in the roots, thereby preventing NO_3^- accumulation. Whereas in the case of NO_3^-, once absorbed by the plant roots, the NO_3^- is reduced to NH_4^+ which is subsequently assimilated into organic compounds, such as protein (Beevers & Hageman 1969; Breteler 1973; Marschner 1995). Since NO_3^- reduction occurs mostly in the shoot, NO_3^- accumulation may occur in the leaves of those plants in which NO_3^- is translocated to the leaves at a faster rate than it is being reduced (Andrews 1986) (Figure 1). Protein synthesis is dependent on photosynthesis to convert NO_3^- into amino acids, thus accumulation of NO_3^- in plants is much greater in low light conditions such as a cloudy day (Crawford *et al.* 1961; George *et al.* 1971).

Factors affecting nitrate accumulation

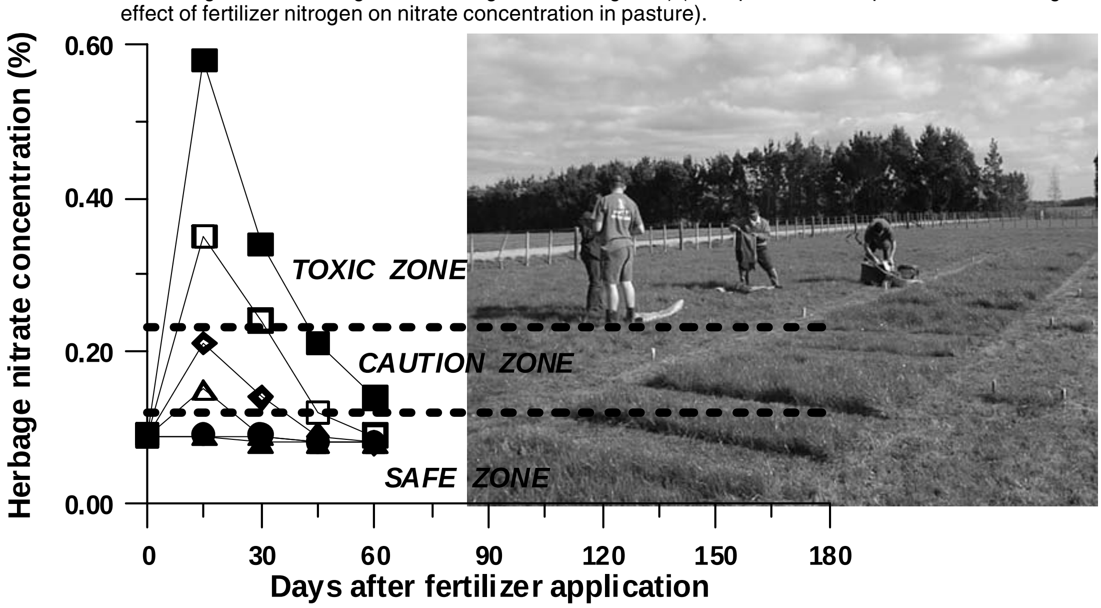
The rate of NO_3^- accumulation in higher plants is dependent on supply of N, plant species, and the production and distribution of dry matter (Whitehead 1995).

Nutrient input

Nitrogen input is considered to be one of the important factors controlling NO_3^- accumulation in pasture (Goh & Haynes 1986; Whitehead 1995). In New Zealand, traditionally a small amount of N fertilizer is added to legume-based pastures mainly during the winter/spring period for the following reasons (Haynes & Williams 1984). Firstly, any marginal increase in pasture production during this period is likely to have a high return in terms of milk production because of low pasture growth rate and high feed requirements by the lactating cows. Secondly, during the winter/spring period the rate of biological N fixation by legume is not adequate to meet the demand of the pasture. Thirdly, the rate of breakdown (mineralisation) of organic matter is very slow, especially during the winter period, resulting in low levels of mineral N for plant uptake and hence pasture plants respond to N fertilizer application.

There has been a steady increase in the use of N fertilizers in grazed pasture, especially in the intensive dairy sector (Bumb 1995; Jarvis *et al.* 1995). For example, the sale of N fertilizers in New Zealand increased from approximately 45 000 tonnes in 1990/91 to 160 000 in 2000/01 (Bolan *et al.* 2003). Various reasons have been attributed to the increase in the use of N fertilizers in dairy pasture: (i) extra feed can be produced throughout the year which can be used to increase the stocking rate, achieve early calving, extend the lactation later into autumn and to make more high quality silage to feed later in the lactation; (ii) feed obtained from the N fertilizer application can be used to replace the more expensive feed supplements; and (iii) the productivity and the profitability of the farm can be increased by fertilizer

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).



N application.

In legume-based pastures, application of N fertilizers is likely to increase NO_3^- concentration in the herbage (Whitehead 1995). Two reasons have been attributed to fertilizer-induced NO_3^- accumulation in pasture (Wilman 1965). Firstly, the application of N fertilizers decreases the rate of biological N fixation and hence the legume plants become dependent on soil N, mostly the NO_3^--N. Secondly, under poor growing conditions with sufficient N supply the N uptake increases more than the dry matter yield resulting in NO_3^- accumulation.

A field experiment was conducted at Massey University No 4 Dairy unit in June 2002 to examine the effect of urea fertilizer application on NO_3^- accumulation in legume-based pasture. Six rates of N fertilizer (0, 50, 100, 200, 400 and 600 kgN/ha) were applied to a plot size of 9 m^2. The two highest rates (400 and 600 kgN/ha) were applied in 2 split doses with 5 days interval in order to avoid pasture burning resulting from excessive urea application. Although it is unlikely that more than 50 kgN/ha is applied at one single dose to grazed pasture, the objective of the experiment was to examine the effect of excessive application of N fertilizer on NO_3^- accumulation in pasture. Four replications were used for each fertilizer treatment. Pasture samples were taken at regular intervals and analysed for total N and 2% acetic acid extractable NO_3^- N. The results indicated that the herbage NO_3^- concentration increased with increasing level of N application, but the concentration decreased with increasing time after fertilizer application (Figure 2). The NO_3^- concentration in pasture reached the toxic level (> 0.23 %) immediately after fertilizer application, for only levels 200 kgN/ha and above. This indicated that at the optimum level of N fertilizer application (50 – 100 kgN/ha) nitrate toxicity may not be a serious issue.

In addition to excess N, an imbalance of other soil nutrients can also affect forage NO_3^- levels. Plants growing in soils deficient in phosphorus, potassium, and some trace elements, such as molybdenum and manganese have high NO_3^- concentrations (Buwalda & Warmenhaven 1999).

Plant species

In legume-based pastures, NO_3^- accumulation is less likely to occur in legume plants which are actively fixing N in their root nodules as ammonia (Ledgard & Giller 1995). In grasses, however, high concentration of NO_3^- is found in the year of sowing and it decreases with the age of the crop (Jarvis *et al*. 1995). While in some plant species (e.g. Italian ryegrass, *Lolium multiflorum*), accumulation of NO_3^- is mainly caused by a high NO_3^- uptake, in others (e.g. oats, *Avena sativa*) NO_3^- accumulation is mainly caused by a slow rate of NO_3^- reduction (Harada *et al*. 2000; Hillman 2002; Harada *et al*. 2003). In actively growing young plants, most of the N is assimilated into protein resulting in high organic N, whereas in old and dead leaves only a small amount of N is assimilated into organic N, thereby resulting in high NO_3^- accumulation (Marschner 1995). Differences in NO_3^- accumulation have been found between species and varieties (Hillman 2002). Nitrate accumulating species include oats, maize (*Zea mays*), rye (*Secale cereale*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) and non-accumulators include timothy (*Phleum pratense*), browntop (*Agrostis capillaris*) and cocksfoot (*Dactylis glomerata*). In general, forage crops which produce considerable amounts of leafy materials, such as ryegrass convert NO_3^- N to organic N, whereas grain crops such as oat converts less NO_3^- to organic N, thereby resulting in NO_3^- accumulation. Most weed species tend to accumulate NO_3^- due mainly to their slow growth rate (Geurink *et al*. 1979; Cash *et al*. 2002).

Growth stage

Generally the NO_3^- concentration is high in new growth and decreases with maturity (Maynard *et al*. 1976). However in mature leaves NO_3^- reduction is limited leading to the accumulation of NO_3^-, especially soon after N fertilizer application. If plants are stressed at any stage of growth, they can accumulate NO_3^- (Viet & Hageman 1971). Nitrates normally accumulate in stems and conductive tissues. Concentrations tend to be low in leaves because of high NO_3^- reductase enzyme levels (Hageman & Flesher 1960). Grain does not contain appreciable amounts of NO_3^-.

Drought

It has often been observed that NO_3^- concentration in herbage is high after a short period of drought, for two reasons (Wright & Davison 1964; Denium & Sibma 1980). Firstly, during the drought period the NO_3^- concentration builds up in the soil and most of the N is taken up in this form. Secondly, the moisture stress during the drought period causes dry matter yield depression, thereby resulting in less reduction of NO_3^- to organic N. It has been observed that drought during the heading and ripening period increases the NO_3^- concentration in oats (Wright & Davison 1964). In New Zealand the accumulation of NO_3^- in pasture has been noticed under cool and cloudy weather conditions, especially after a dry spell (Goh & Haynes 1986). During a long dry spell the NO_3^- levels build up in the soil and when it rains the plants tend to take up excessive amounts of the NO_3^-.

When a plant experiences moisture shortage there is a general disturbance of assimilatory process. The rate of reduction of NO_3^- is slowed, in part by a drop in NO_3^- reductase enzyme. Nitrate accumulates in plants during periods of moderate drought because the roots continue to absorb NO_3^-, but high daytime temperature is likely to inhibit its conversion to amino acids. During a severe drought lack of moisture prevents NO_3^- absorption by plant roots. Following rain, roots rapidly absorb NO_3^- and accumulate high levels (Whitehead 1995).

Sunlight

Diurnal variations in NO_3^- concentration in higher plants have often been observed (Deinum & Sibma 1980; Steingrover *et al.* 1986). Sunlight affects NO_3^- accumulation through its direct effect on NO_3^- reduction and indirect effect on drymatter yield. Nitrate reduction occurs in young leaves and requires light as an energy source. Shaded plants lack sufficient energy to convert NO_3^- to amino acids (Nowakowski & Cunningham 1966). Extended period of cloudy weather has been shown to increase NO_3^- content in plants. Forages harvested or grazed after several days of cloudy weather have been found to contain higher NO_3^- levels than after sunny weather. In North America, crops grown in summer fallow were found to contain high level of NO_3^- (Wright & Davison 1964). The synthesis of organic compounds would be limited because of low light intensity at that period. However, the uptake of N is generally maintained at a high level because of relatively high soil temperature and good supply of water.

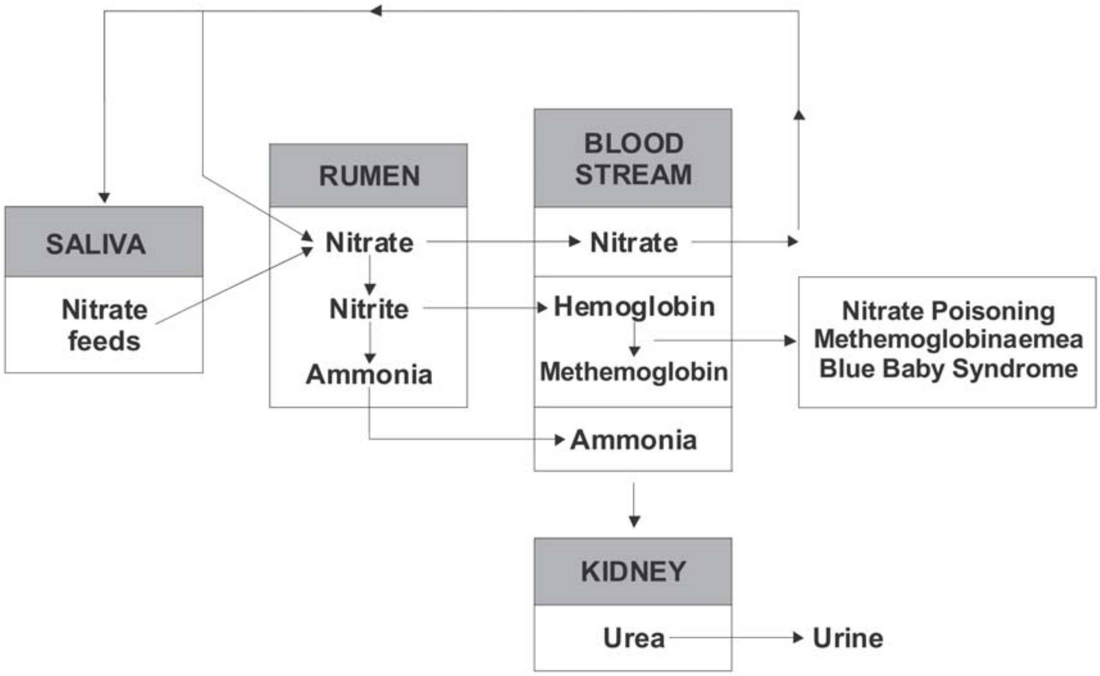
Plant stress factors

Plant stress factors, such as hail, light frost, or plant disease can damage plant leaf area and reduce photosynthetic activity. These stress factors can increase NO_3^- hazard in animals (Wright & Davison 1964). For example, hail usually reduces leaf area, leaving a higher proportion of stems for animal consumption, where NO_3^- concentration is high. Also, the stress of reduced leaf area reduces NO_3^- conversion. Similarly, herbicides, such as 2,4-D can temporarily increase NO_3^- content of forage and weeds; however, since many weeds accumulate NO_3^- the overall hazard can be reduced if weeds are killed. Herbicide-damaged weeds are likely to accumulate NO_3^- because of decreased leaf area and reduced NO_3^- reductase enzyme activity (Kim *et al.* 1997; Nazaryuk *et al.* 2002).

Nitrate poisoning in grazing animals

Nitrate in forage consumed by ruminant animals is reduced to NO_2^- and then to ammonia. The ammonia is then converted to protein by microbes in the rumen. Excess ammonia is absorbed by the blood and passed

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



in the urine as urea. Ruminant animals with high NO_3^- levels in their diets accumulate NO_2^-. Nitrite is absorbed into the blood and combines with hemoglobin to form methemoglobin, a substance that is incapable of transporting oxygen (Figure 3). The blood can no longer transport oxygen to the body tissue and the animal's heart rate increases and muscle tremors develop. This condition is known as nitrate poisoning (methemoglobinaemea or blue baby syndrome in human beings). Clinical signs begin to appear when methaemoglobin reaches 30-40% within the blood and moves on to convulsions followed by death at 80-90% (Kvasnicka & Krystal 1996; Hill 1999).

A number of cases of acute symptoms and deaths in cattle following the ingestion of oat hay or straw have been reported in many countries and referred to as oat-hay poisoning (Wright & Davison 1964; Nolan & Stephen 2001). In these countries, corn stalk as well as oat hay, corn silage, sorghum (*Sorghum vulgare*) silage and alfalfa (*Medicago sativa*) hay have been found responsible for nitrate poisoning. The physical symptoms noted include trembling, staggering gait, rapid respiration and prostration. Affected animals cease to eat and soon collapse and die without convulsions. Cyanosis of the tongue and sclera are observed and the blood turns reddish brown due to the presence of methaemoglobin (Bruningfann & Kaneene 1993a & 1993b; Elbahri *et al.* 1997).

Cattle appear to be more susceptible than sheep and horses. Losses of weight and milk production and non-infectious abortion have been noted as sublethal effects in dairy cattle. It is thought that NO_2^- may interfere with progesterone synthesis, which is the primary hormone involved in supporting pregnancy (Yaremcio 2001).

Uncertainty exists about the level of NO_3^- ingestion that is considered the minimal lethal dose. Studies have indicated that 7.6 - 9.0 g NO_3^- N per 100 kg body weight is lethal to animals. Assuming that the average daily pasture intake by dairy cattle is 4% of the body weight, pasture with a NO_3^- content of more than 0.3% is likely to be toxic to animals with a live body weight of 300 kg. It is important to remember that the pasture NO_3^- levels above which toxicity occurs depend on the rate of pasture ingestion by the animal (Carrigan & Gardner 1982; Murphy & Power 1995; Fjell *et al.* 2002).

Treatment for nitrate poisoning

The first and foremost step to be taken in treating a suspected case of nitrate poisoning is to immediately remove the animal from high NO_3^- feed source and offer plenty of water. A high carbohydrate, low NO_3^- feed should be offered as this will decrease rumen NO_3^- concentration and also decrease the rumen pH, which slows down the reduction of NO_3^- to NO_2^- in the rumen (Kvasnicka & Krystal 1996).

The treatment recommended is methylene blue, which proves to be an effective antidote for NO_3^- toxicity (Burrows 1984; Vandijk *et al.* 1983; Mondal & Pandey 2000). Acutely affected animals should be treated intravenously. A 1 to 4% aqueous solution given at the rate of 2 g methylene blue per 300 kg body weight is adequate, although methylene blue in 5% glucose or 1.8% sodium sulphate has been suggested. Methylene blue is thought to act as an electron acceptor for the methemoglobin reductase enzyme in blood, thereby speeding the reconversion of methemoglobin to haemoglobin.

Preventing nitrate accumulation in forages and nitrate poisoning in grazing animals

Essentially farmers should concentrate more on prevention than treatment, as once an animal begins to show nitrate toxicity symptoms there is likely to be only a short period of time to save the animal, as the health of the animal deteriorates quickly. Nitrate poisoning in cattle can be minimised by proper grazing management practices that include (Geurink *et al.* 1982; Koch 2002):

- Precautionary measures should be taken if excess NO_3^- is accumulated in the forage. Forages with > 0.3 % NO_3^- should be considered potentially toxic and should be mixed with feeds that are low in NO_3^- but high in carbohydrate and protein.
- Hungry cattle should not be allowed to graze new pasture in cloudy weather conditions, especially after a dry period. Under this condition, the pasture samples should be tested for NO_3^- levels and animal should be given pasture only as part of the ration.
- Nitrogen fertilizer should not be applied in high rates of single does during very dry weather. This will predispose the pasture or forage crop to elevated soil NO_3^- levels. Lush re-growth of heavily N fertilized grasses generally contain high NO_3^- levels and should not be grazed.
- Ensiling the forage crop, rather than harvesting as hay, will reduce NO_3^- by 40-60% during the ensiling process. However, forages with extremely high NO_3^- levels at harvest may still be dangerous after ensiling and should be analysed before feeding.
- Young animals should be weaned gradually onto diets with high NO_3^- content. Over time the rumen microbes are able to assimilate high levels of NO_3^-, thereby preventing a flush of NO_2^- build up within the rumen.
- Break-feed high NO_3^- content pasture, alternating with a high carbohydrate feed source. This will

prevent rumen NO_3^- reaching toxic levels and the low pH created by high carbohydrate concentration of the diet will slow down the reduction of NO_3^- to NO_2^- within the rumen.

- Keep stock in good health as healthy livestock are less susceptible to toxicity than stock in poor health condition. For example, stock suffering from parasite infection are shown to display high susceptibility.
- Care must be taken when grazing plants with tissue damage (eg. after herbicide application). Any damage to plant tissue will result directly in a decrease in the plant's photosynthetic potential which will indirectly affect the NO_3^- levels within the plant.
- Stocking rate should not be too high because overgrazing forces cattle to eat the stems, which contain the highest NO_3^- levels.

ACKNOWLEDGEMENT

We would like to thank the C. Alma Baker Trust (NZ) for funding this project.

REFERENCES

Andrews, M. 1986. Minireview: The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant, Cell and Environment 9*:511-519

Beevers, L.; Hageman, R.H. 1969. Nitrate reduction in higher plants. *Annual Review of Plant Physiology 20*: 495-522.

Bolan, N.S. 1998. Nitrate accumulation in pastures. *New Zealand Dairy Exporter 73*: 134-135.

Bolan, N.S.; Saggar, S.; Luo, J.; Bhandral, R.; Singh, J. 2003. Gaseous emission of nitrogen from grazed pastures: environmental implications. Advances in Agronomy (in press).

Breteler, H. 1973. A comparison between ammonium and nitrate nutrition of young sugar-beet plants grown in nutrient solutions of constant acidity. 1. Production of dry matter, ionic balance and chemical composition. *Netherlands Journal of Agricultural Sciences 21*:227-424.

Bruningfann, C.S.; Kaneene, J.B. 1993a. The effects of Nitrate, nitrite and N-nitroso compounds on animal health. *Veterinary and Human Toxicology 35*: 237-253

Bruningfann, C.S.; Kaneene, J.B. 1993b. The effects of Nitrate, nitrite and N-nitroso compounds on human health – a review. *Veterinary and Human Toxicology 35*: 521-538.

Bumb, B.L. 1995. World nitrogen supply and demand: An overview. pp. 1-40. *In*: Nitrogen fertilization in the environment. Ed. Bacon, P.E. Marcel Dekker, New York.

Burrows, G.E. 1984. Methylene-blue – effects and disposition in sheep. *Journal of Veterinary Pharmacology and Therapeutics 7*: 225-231.

Buwalda, F.; Warmenhoven, M. 1999. Growth-limiting phosphate nutrition suppresses nitrate accumulation in greenhouse lettuce. *Journal of Experimental Botany 50*: 813-821.

Carrigan, M.J.; Gardner, I.A. 1982. Nitrate poisoning in cattle fed Sudax (Sorghum SP hybrid) hay. *Australian Veterinary Journal 59*: 155-157.

Cash, D.; Funston, R.; King, M.; Wichman, D. 2002. Nitrate toxicity of Montana forages. http://www.montana.edu/wwwpb/pubs/mt200205.pdf.

Clarkson, D.T.; Hanson, J.B. 1980. The mineral nutrition of higher plants. *Annual Review of Plant Physiology 31*: 239-298.

Crawford, R.F.; Kennedy, W.K.; Johnson, W.C. 1961. Some factors affecting nitrate accumulation in forages. *Agronomy Journal 53*: 159-162.

Denium, B.; Sibma, B. 1980. Nitrate content of herbage in relation to nitrogen fertilization and management. pp. 95-102. *In*: Proceedings of the International Symposium of European Grassland Federation on Role of Nitrogen in Intensive Grassland Production.

ElBahri, L.; Belguith, J.; Blouin, A. 1997. Toxicology of nitrates and nitrites in livestock. *Compendium on Continuing Education for the Practicing Veterinarian 19*: 643-649.

Fjell, D.; Blasi, D.; Towne, G. 2002. Nitrate and prussic acid toxicity in forage http://www.oznet.ksu.edu/library/crpsl2/MF1018.PDF. Cooperative Extension Service Kansas State University.

Gashaw, L.; Mugwira, L.M. 1981. Ammonium-N and Nitrate-N effects on the growth and mineral compositions of triticale, wheat and rye. *Agronomy Journal 73*: 47-51.

George, J.R.; Rhykerd, C.L.; Noller, C.H. 1971. Effect of light intensity, temperature, nitrogen and stage of growth on nitrate accumulation and dry matter production of a sorghum-sudan grass hybrid. *Agronomy Journal 63*: 413-415.

Geurink, J.H.; Malestein, A.; Kemp, A.; Korzeniowski, A.; Vantklooster, A.T. 1982. Nitrate poisoning in cattle. 7. Prevention. *Netherlands Journal of Agricultural Science 30*: 105-113.

Geurink, J.H.; Malestein, A.; Kemp, A.; Vantklooster, A.T. 1979. Nitrate poisoning in cattle. 3. Relationship between nitrate intake with hay or fresh roughage and the speed of intake on the formation of methemoglobin. *Netherlands Journal of Agricultural Science 27*: 268-276.

Goh, K.M.; Haynes, R.J. 1986. Nitrogen and agronomic practice. pp.379-468. *In*: Mineral Nitrogen in the Plant-Soil System. Ed. Haynes, R.J.

Academic Press, New York.
Hageman, R.H.; Flesher, D. 1960. Nitrate reductase activity in corn seedling as affected by light and nitrate content of nutrient media. *Plant Physiology 35*: 700-708.
Harada, H.; Yoshimura, Y.; Sunaga, Y.; Hatanaka, T.; Sugita, S. 2003. Breeding of Italian ryegrass (*Lolium multiflorum* Lam.) for a low nitrate concentration by seedling test. *Euphytica 129*: 201-209.
Harada, H.; Yoshimura, Y.; Sunaga, Y.; Hatanaka, T. 2000. Variations in nitrogen uptake and nitrate-nitrogen concentration among sorghum groups. *Soil Science and Plant Nutrition 46*: 97-104.
Haynes, R.J. 1986. Origin, distribution and cycling of nitrogen in terrestrial ecosystem. pp.1-51. *In*: Mineral Nitrogen in the Plant-Soil System. Ed. Haynes, R.J. Academic Press, New York.
Haynes, R.J.; Williams, P.H. 1984. Nitrogen cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy* 49: 120-199.
Hill, M.J. 1999. Nitrate toxicity: myth or reality? Invited commentary: *British Journal of Nutrition 81*: 343-344.
Hillman, R.B. 2002. Nitrate poisoning. *http:// web.vet.cornell.edu /CVM/HANDOUTS/ plants/ nitrates.html*
Jarvis, S.C.; Schofield, D.; Pain, B. 1995. Nitrogen cycling in grazing systems. pp. 381-420. *In*: Nitrogen Fertilization in the Environment. Ed. Bacon, P.E. Marcel Dekker, New York.
Kim, S.; Han, S.; VandenBorn, W.H. 1997. Effect of chlorsulfuron on assimilate transport: Ultrastructural implications. *Weed Science 45*: 470-473.
Koch, D. 2002. Managing forages to minimize nitrate poisoning. *http://www. uwyo.edu/ ces/psas/SMRR/ nitrate.html*
Kvasnicka, B.; Krystal, L.J. 1996. Nitrate poisoning in livestock. *http:// www.forages.css. orst.edu/Topics/ Pastures/Species/Grasses/Animal_issues/ Nitrate.html*
Ledgard, S.F.; Giller, K.E. 1995. Atmospheric N_2 fixation as an alternative N source. pp. 443-486. *In*: Nitrogen Fertilization in the Environment. Ed. Bacon, P.E. Marcel Dekker, New York.
Marschner, H. 1995. Mineral Nutrition of Higher Plants. 2nd Ed. Academic Press, London.
Maynard, D.N.; Baker, A.V.; Minotti, P.L.; Peck, N.H. 1976. Nitrate accumulation in vegetables. *Advances in Agronomy 28*: 71-118.
Mengel, K.; Kirkby, E.A. 1982. Principles of Plant Nutrition. International Potash Institute, Bern. 655pp.
Mondal, D. B.; Pandey, N. M. 2000. Dosage regimen of methylene blue and its comparative therapeutic efficacy with tolonium chloride and ascorbic acid in induced acute nitrate toxicity in goats. *Indian Journal of Animal Science 70*: 572-575.
Murphy, S.A.; Power, E.P. 1995. Poisoning of dairy-cows by a high nitrate concentration in Italian ryegrass. *Irish Veterinary Journal 48*: 395-397.
Nazaryuk, V.M.; Klenova, M.I.; Kalimullina, F.R. 2002. Ecoagrochemical approaches to the problem of nitrate pollution in agroecosystems. *Russian Journal of Ecology 33*: 392-397.
Nolan, R.H.; Stephen, K.B. 2001. Nitrate toxicity. *http:// www.ibc.iastate. edu/content/ newsrel 2001/ 701/nitrate_toxicity.htm.*
Nowakowski, T.Z.; Cunnigham, R.K. 1966. Nitrogen fractions and soluble carbohydrates in Italian ryegrass. 2. Effects of light intensity, form and level of nitrogen. *Journal of the Science of Food and Agriculture 17*: 145-150.
Pate, J.S. 1980. Transport and partitioning of nitrogenous solutes. *Annual Review of Plant Physiology 31*:313-340.
Raven, J.A.; Smith, F.A. 1976. Nitrogen assimilation and transport in vascular land plants in relation to intercellular pH regulation. *New Phytology 76*:415-431.
Russell, E.W. 1973. Soil Conditions and Plant Growth. 10th Ed. Longman, London. 848 pp.
Steingrover, E.; Ratering, P.; Siesling, J. 1986. Daily changes in uptake, reduction and storage of nitrate in spinach grown at low light-intensity. *Physiologia Plantarum 66*: 550-556.
Vandijk, S.; Lobsteyn, A.J.H.; Wensing, T.; Breukink, H.J. 1983. Treatment of nitrate intoxication in a cow. *Veterinary Record 112*: 272-274.
Viets, F.G.; Hageman, R.H. 1971. Factors affecting the accumulation of nitrate in soil, water and plants. *United States Department of Agriculture. Report 413.*
Walters, C.L.; Walker, R. 1978. Consequences of accumulation of nitrate in plants. pp. 637-648. *In*: Nitrogen Assimilation of Plants. Eds. Hewitt, E.J.; Cutting, C.V. Academic Press, New York.
Whitehead, D.C. 1995. Influence of fertilizer nitrogen on the composition and nutritional quality of grassland herbage. pp. 644-684. *In*: Grassland Nitrogen. CAB International, Wallingford, UK.
Wilman, D. 1965. The effect of nitrogenous fertilizer on the rate of growth of Italian ryegrass. *Journal of British Grassland Society 20*: 248-254.
Wright, M.J.; Davison, K.L. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. *Advances in Agronomy 16*: 197-247.
Yaremcio, B. 2001. Nitrate poisoning and feeding nitrate feeds to livestock. *http://www. agric. gov.ab.ca/agdex/400/0006001.html*