

Nitrate Toxicity

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Plants and drinking water both can contain concentrations of nitrate that prove toxic to animals. Bacterial species found normally in the digestive tract (rumen of ruminants; cecum and colon of horses; stomach and intestine of infants less than 6 months of age and young nonruminants) reduce nitrate (which is non-toxic) to nitrite (which is toxic) and ammonia. When nitrate is reduced to nitrite faster than nitrite is removed, nitrite is absorbed from the digestive tract. Reacting with nitrite, hemoglobin is converted to methemoglobin, a compound that cannot transport oxygen. Tissue oxygen depletion results in rapid, labored, or noisy breathing, rapid pulse rate,

and, in severe cases, death. Some animals recover spontaneously, but surviving cows in their first trimester of pregnancy often abort within two weeks due to death of the fetus.

Origin of Nitrate. All growing plants absorb nitrate from soil and transport it to leaves where nitrate is reduced stepwise to nitrite, hydroxylamine, and finally ammonia. Ammonia becomes a component of amino acids and protein of the plant. With various types of plant stress - water shortage, cool and

uptake of nitrate from soil continues causing nitrate to accumulate, particularly the lower stems. Most cultivated plants including corn, wheat, oats, rye, barley, millet, sorghum (sorghum-sudan hybrids, particularly) as well as weeds accumulate nitrate. When nitrogen (nitrate or animal waste) fertilization rate exceeds that needed for plant growth, nitrate accumulation is enhanced. Nitrate concentrations within plants in a field vary due to local soil nitrate and moisture conditions. Forages with less than 1,000 ppm nitrate-N are considered safe for all cattle and levels below 1,500 ppm nitrate-N are safe for non-pregnant cattle. Forages above 1,500 ppm should be diluted to 1,500 ppm before feeding, but forage containing over 4,000 ppm nitrate-N is not readily diluted. Recorded nitrate-N include over 12,000 ppm for bales of oats hay, 19,000 ppm in growing weeds, and 11,000 ppm in sorghum-sudan pastures! Forages rich in nitrate may burn explosively. Nitrate from surface or well water adds to the nitrate load of animals. Primarily from leached fertilizer, nitrate under 10 ppm nitrate-N in water is considered safe for humans; levels below 40 ppm nitrate-N (some research says 100 ppm) are considered safe for ruminants.

Nitrate measurement. A diphenylamine spot test is used to detect concentrations of nitrate-N above 1,000 ppm. Forage rich in nitrate becomes deep blue or blue-black when contacted with a drop of 0.5% diphenylamine in 80% sulfuric acid. Quantification, during feed analysis or at a diagnostic lab, involves colorimetry or use of a nitrate-sensing electrode. Measurements can prove confusing with results being variously expressed as ppm of nitrate-N, ppm nitrate (being 4.4 times that for nitrate-N), or ppm potassium nitrate (being 7.0 times that for nitrate-N). Some laboratories also report percentages rather than ppm (1% equals 10,000 ppm).

Toxicity prevention. Methods to prevent nitrate toxicity include: 1) reducing nitrate intake, 2) preventing conversion of nitrate to nitrite, 3) increasing nitrite reduction to non-toxic compounds, and 4) increasing an

Plant management. By splitting soil N application across the growing season and avoiding excess application, plant nitrate can be reduced. Acid and phosphorus deficient soils produce plants with more nitrate. After a drought-ending rain, grazing or crop harvest should be delayed for 3 to 14 days to allow plant nitrate levels to subside. In contrast, following frost or hail damage, immediate harvest is recommended because live roots continue to amass nitrate but the damaged upper plant is unable to use nitrate. More mature forage has less nitrate than lush green forage; forage grazed or harvested in the afternoon, when nighttime accumulations of nitrate have been depleted, will have lower nitrate levels. Some forages (sorghum-sudan hybrids) known to accumulate nitrate may be avoided. At harvest, the forage harvesting equipment can be adjusted to leave the higher nitrate lower stems in the field. Nitrate remains stable in forage stored dry, but 30 to 80% of forage nitrate is lost during silage fermentation. During the first few days of fermentation, nitrate is released as a toxic, yellowish-brown gas - nitrogen dioxide. On contact with water, this gas forms nitric acid. Reacting with lung tissue, it can cause permanent damage that may prove lethal.

Animal management. To prevent toxicity from high nitrate forages, cattle should be fed hay prior to grazing to reduce intake of forage and gradually adapted to the forage by increasing grazing time over several days. Grazing pressure should be kept low so that cattle do not consume stems and weeds, and fresh water should be provided. High nitrate forage should be diluted with low nitrate forage or grain. Providing small, intermittent meals and totally mixed rations helps to avoid temporal nitrate and nitrite peaks within the rumen. Grain, always low in nitrate, dilutes nitrate from other sources, provides energy for bacterial nitrite reduction, and may reduce ruminal pH below that ideal for nitrate reduction (pH = 6.5) to a pH closer to that ideal (pH = 5.6) for nitrite reduction.

Mineral and vitamin supplementation. Several minerals are involved with nitrate reduction. Nitrate reductases, enzymes that convert nitrate to nitrite, contain molybdenum. When tungsten replaces molybdenum in this enzyme, it no longer reduces nitrate. Daily intakes of 3 to 10 mg tungsten per kg body weight from sodium tungstate will reduce or halt nitrite formation in the rumen. Although less than 1% of dosed tungsten appears in milk and soil contamination appears to have minimal effect on either soil microbes or plants, environmental effects of tungsten remain of concern. Copper and sulfur, involved with nitrite detoxification enzymes and capable of forming insoluble complexes with molybdenum, must be supplied in adequate quantities to avoid nitrite accumulation. Vitamin A depletion, noted for cattle fed high nitrate silage, reflects carotene destruction by nitrite under acidic (silage) conditions; similar conditions do not exist in the digestive tract, so no extra vitamin A is needed when nitrate intake is high.

Microbial inoculation. Because it inhibits clostridia, nitrite is added to cured meats to prevent botulism. However, enterobacteria, including *Escherichia* and *Salmonella*, when grown without oxygen, obtain energy for growth by reducing nitrate to nitrite. Reduction of nitrite to amino acids anaerobically requires energy. Microbial reduction of nitrite to amino acids in the rumen is stimulated by nitrate intake, and this capacity is transferred among ruminants. Though never field-tested, increased intake of nitrate or, preferably, nitrite, might be used to pre-condition animals to handle high nitrate feeds or water. Isolated strains of propionibacteria, a bacterial species used in cheese production, when inoculated through the feed or as an oral paste, become permanent residents in the ruminal ecosystem,

actively reducing nitrite and preventing nitrate toxicosis. Such cultures are available commercially (Bova-Pro; about \$6 per head).

Diagnosis and treatment. Acute and chronic toxicoses differ. With either, chocolate coloration of blood provides a strong visual diagnostic clue. Diagnosis of chronic toxicity involves measurement of nitrate in ocular fluid or serum or methemoglobin concentration of blood. Under chronic conditions, cattle gradually adapt to high intakes of nitrate and can remain productive with up to 50% of hemoglobin as methemoglobin (compared to 3% normally). Treatment for toxicity consists of converting methemoglobin back to hemoglobin via intravenous infusion of a 2 to 4% methylene blue solution at a rate of 1 ml per 5 kg live body weight. When weather conditions make nitrate toxicity probably, veterinarians should carry and livestock producers should maintain a supply of methylene blue for immediate treatment of acutely affected animals. Although infused ascorbate has been used to treat methemoglobinemia, potential beneficial effects of dietary antioxidant supplements (e.g., vitamins C or E) have not been examined.

Future prospects. Selection for increased plant yields and growth rates are likely to increase the potential for nitrate accumulation in forage. However, soil nitrate may be reduced through 1) legislative limits on nitrate fertilization, 2) modified fertilization practices, e.g., split applications, water-sensitive slow release fertilizers, and foliar fertilizers, 3) greater legume use, 4) development of nitrogen-fixing soil microbes or grasses, and 5) reduced nitrate application based on long-range prediction of drought. Plants might be selected or developed that regulate nitrate uptake based on soil moisture or that accumulate less nitrate. Plant stress, sensed through satellite infra-red technology combined with regional plant nitrate assays could be used to issue local nitrate alerts, similar to weather warnings, for forage harvesters. Hay, silage, and ruminants can be inoculated with nitrite-metabolizing microbes. Water purification systems using electrochemistry, anion exchange, reverse osmosis, and fixed enzyme columns can remove nitrate from drinking water. Finally, improved nutrient status of animals, e.g., less anemia due to internal and external parasites and enhanced nitrate utilization by microbes in the digestive tract, should help reduce the prevalence of nitrate toxicity.