6 Nitrate and Nitrite

The nitrate (NO_3^-) and nitrite (NO_2^-) ions are intermediates in the biological nitrification cycle and the primary source of nitrogen (N) for plants in the soil. Plants accumulate NO_3^- from soil to synthesize protein via a multistep process; excessive NO_3^- accumulation as a result of this process may cause poisoning of grazing animals.[312] Nitrate or NO_2^- may contaminate water as a result of contact with natural minerals (e.g. niter), agricultural runoff (fertilizer, manure) or industrial processes.[312-314] Nitrate is the more stable of the two N species and therefore more common in surface waters.[315] Nitrite (NO_2^-) usually results from biological reduction of NO_3^-, but it may also be an industrial contaminant or exist in ground waters where pH and redox potential prevent oxidation to NO_3^-.[315] Both ions are extremely water-soluble and therefore water-mobile.

Essentiality

Although N is an essential macro element for mammals, NO_2 and NO_3 are not essential *per se.*

Metabolism

While the NO_3^- ion is readily absorbed in the upper gastrointestinal (GI) tract and possesses intrinsic toxicologic properties such as vasodilation, the condition referred to as "nitrate poisoning" actually depends upon reduction of NO_3^- to NO_2^- in the upper GI tract.[312,316-318] This process occurs in human infants[319,320], and it is the basis of current human drinking water standards, but nitrate toxicity is primarily a problem in ruminant species, as the rumen microflora are well-suited to catalyze NO_3^- reduction. Radiotracer studies indicate that NO_2^-, formed from NO_3^- in the rumen, may either be further reduced and incorporated via NH_3 into amino acids or reduced via nitric oxide to N and expelled.[321] Unfortunately, neither of the latter pathways (i.e. amino acids or N) is as fast as the initial reduction to NO_2^-, and dangerous NO_2^- concentrations accumulate in the GI tract when assimilatory pathways are overloaded.[321-323] Under the proper dietary conditions (mainly adequate carbohydrates) the assimilatory pathways can adapt to high NO_3^- concentrations, and very little NO_3^- or NO_2^- escape the rumen.[314,322,324-327] Sustained exposure to moderately high NO_3 diets results in induction of the assimilatory pathways, and this ability may be acquired by transfer of GI flora from one ruminant to another.[322,323] Once absorbed into the bloodstream, the NO_3^- ion is rapidly distributed throughout the body water and excreted via urine and saliva, whereas the NO_2^- ion is oxidized to NO_3^- via a coupled reaction with hemoglobin and eliminated as NO_3^-.[316,318,328,329]

Other differences between ruminants and monogastrics in the metabolism of the NO_2^- and NO_3^- ions, while not directly tied to the ruminal metabolism of NO_3^-, probably reflect evolutionary pressure of the constant background of NO_2^- ruminants receive from ruminal metabolism. The blood $t_{1/2}$ of NO_2^- is similar in monogastrics and ruminants, but the elimination of NO_3^- from blood is much slower in monogastrics[316] and the normal background metHb concentration is higher in monogastrics.[330] In ruminants only a small portion of an oral dose of NO_3 is eliminated in the urine, whereas in monogastrics most ingested NO_3 is eliminated via urine.[314]

Some sources suggest the NO_3^- ion is more bioavailable in water than feedstuffs.[312-314] The experiments this conclusion was drawn from, however, were based upon aqueous NO_3^- administered directly into the stomach vs. contaminated feedstuffs offered ad libitum.[314] The most important determinant of NO_3 toxicity seems to be how rapidly a given dose of NO_3^- is administered, rather than whether it is in feed or water.[314,331,332] For example, the single oral median lethal dose ($SOLD_{50}$) of $NaNO_3$ in cows was reported to be 328 mg/kg BW when given at once, but when the same dose was spread over 24 hours, the LD_{50} increased to 707-991 mg/kg.[331]

Toxicity

Although the most common source of NO_3 poisoning in livestock is contaminated feedstuffs, NO_3^--contaminated drinking water *has* poisoned people and animals.[320,333-336] The mechanism of poisoning involves GI microbial reduction of the NO_3^- ion to NO_2^-, which is absorbed into the bloodstream where it oxidizes the ferrous iron atoms (Fe^{+2}) in hemoglobin (Hb) to the ferric (Fe^{+3}) state, resulting in methemoglobin (metHb), which can-

not transport oxygen from the lungs to the rest of the body. The exact mechanism by which Hb is oxidized is the subject of some controversy but is currently thought to be a multi-step autocatalytic process involving several free radicals.[328] The end product of this reaction is NO_3^-, which is eliminated via urine and saliva. As might be expected, the clinical signs of acute NO_3 poisoning (cyanosis, hyperpnea, muscle tremor, weakness, collapse, and death) reflect the effects of anoxia on critical organs such as the brain and heart. Pregnant animals that survive episodes of acute NO_3 poisoning during the latter part of pregnancy may abort within one to two weeks.[333,337-340] Poisoning during earlier pregnancy does not usually result in abortion.[313,331,333,341]

As might be expected from the toxic mechanism, monogastric animals (with the exception of human infants) seem to be relatively resistant to the effects of NO_3 compared to ruminants. Textbooks suggest NO_3 is approximately 10-fold more toxic in ruminants than monogastric animals.[317] Burwash et al.[342] fed six mares high NO_3 (1.7-1.85%) oat hay for 13 days. Although the serum NO_3^- concentration increased significantly, there was no change in metHb concentration, no effects on blood chemistry parameters, and no clinical signs of poisoning. They concluded it is safe to feed horses diets containing 2% NO_3 and "likely much higher" concentrations. Mice exposed to drinking water containing 1,000 mg NO_3^-/L for 18 months excreted more ammonium ion than controls early in the study and may have died slightly sooner (17.5 months vs. 18 months) but did not show any signs of poisoning.[343] Seerley et al.[344] fed water containing 1,465, 2,900, or 4,400 mg NO_3^-/L as $NaNO_3$ to weanling pigs for 84 days with no effect upon rate of gain, water consumption, or clinical signs of toxicity. The difference in toxicity between animal species is not nearly as pronounced for NO_2.[317] Although rare, monogastric animals have been acutely poisoned by the NO_2^- ion from water[335] and feedstuffs.[345]

Wright and Davison[346] reviewed the literature and concluded the LD_{50} of NO_3 in ruminants was between 700-985 mg NO_3/kg BW when fed as dry feed. Experimentally, sheep have been acutely poisoned by NO_3 doses as low as 300 mg/kg BW[323,325,329,347]; however, doses as large as 800 mg/kg BW have been fed without measurable effects.[323,326,327,340,347,348] Field reports have incriminated feedstuffs containing as little as 2% ppm dietary NO_3 (which would provide about 500 mg NO_3/kg BW) as causing acute lethality in sheep .[349,350] Experimentally, 3.4% dietary NO_3, fed as pigweed or oat hay killed two of five ewes. The lethal dosages, calculated from consumption data, were 660 and 730 mg NO_3/kg BW, respectively. Campagnolo et al.[351] reported the accidental poisoning of several animals, including sheep, by water containing 6,000 mg NO_3^-/L at a county fair; however, the water contained several other substances that might also have been toxic.

Cattle have been experimentally poisoned by 520 mg/kg BW or more NO_3 incorporated into feedstuffs[341,346,352,353], and as little as 200 mg/kg BW may be toxic if given by gavage.[354] Several investigators[324,354,355] consistently produced sublethal toxicity with 200-300 mg NO_3/kg BW in order to test various protective strategies. Other investigators[322,356,357] failed to demonstrate toxicity at dietary concentrations as high as 0.9% (approximately 225 mg NO_3/kg BW), although one[322] reported that sustained exposure enhanced the ability of the rumen microflora to degrade NO_3^- and NO_2^-. Calves were experimentally poisoned by drinking water containing 2,500 mg NO_3^-/L (250 mg/kg BW), but none were affected by 2,000 or less.[338] Older texts and reviews variously describe the minimum toxic dose in cattle as 169-500 mg NO_3/kg BW.[313,317,358]

There are numerous anecdotal reports of acute NO_3 poisoning in cattle associated with contaminated feedstuffs. O'Hara and Fraser[359] summarized 10 episodes of acute NO_3 poisoning in New Zealand in which mortality varied from less than 1% to almost 50%. Forage concentrations associated with these cases ranged from 0.3-5.3% NO_3 (mean = 3.3%) with variations of 1-2% NO_3 between samples from the same premise. In one extensively investigated case, 23 of 50 calves turned into a ryegrass pasture containing 6.6-8.9% KNO_3 (4-5.3% NO_3) died within a 12-hour period.[359] In another instance, calves died if left on a ryegrass pasture containing 3.6% NO_3 for more than one hour.[360] A dose of NO_3, later calculated to be 170 mg NO_3/kg BW, from contaminated hay killed seven of 200 heifers. The herdsman tried to dilute the toxic hay in half and killed seven more.[361] The authors speculated that concurrent overfeeding of monensin enhanced ruminal reduction of NO_3^- to NO_2^-, thus potentiating the toxicity of the hay. Harris and Rhodes[362], summarizing the experience of farmers during a severe drought in Victoria, Australia, reported several hundred animals were killed by plants containing "over 1.5% NO_3." Three cows fed hay containing 1% NO_3 died within 30 minutes.[353] Eleven cows aborted, and 73 of 153 died when fed sudax hay containing 1.1-3.1% NO_3.[363] McKenzie summarized several cases with acute

mortalities of 16-44% on button grass (2.4-7.2% NO_3) grown in N-rich soil in Queensland, Australia.[349] Animals grazing the same grass in adjacent paddocks without the extra N were unaffected.

Although not as common as poisoning from feedstuffs, contaminated water has resulted in acute poisoning, including abortions and death. Seven of 12 cows died shortly after drinking water containing 2,790 mg NO_3^-/L.[336] Several authors reported lethality as a result of fertilizer-contaminated water (1,000-6,000 mg NO_3^-/L).[333,351,364] Contaminated liquid whey, fed in addition to water and containing 2,200-2,800 mg NO_3^-/L, killed 17 of 360 cattle. Whey containing only 400-800 mg NO_3^-/L did not kill any animals, but it did result in 26 of 140 cows aborting.[333] Yong et al.[334] reported that water, contaminated with 4,800 and 7,000 mg NO_3^-/L as a result of blasting water holes, killed 16 of 100 and four of 90 cows in two separate incidents in Saskatchewan, Canada.

It is known the NO_2^- ion may react with secondary amines (common in many foodstuffs) under conditions typical of the adult human stomach (pH 1-4) to form nitrosamines.[365-367] Many nitrosamines are potent animal mutagens and carcinogens. Bacterial reduction of NO_3^- to NO_2^- does not occur under the acid conditions necessary for nitrosamine production, or *vice versa*, but it is theoretically possible NO_2^- excreted in saliva or ingested in water might cause cancer in people. In practice, however, salivary secretion contributes much less NO_2^- than other sources such as vegetables, and attempts to link nitrosation, mutagenesis, and/or cancer with drinking water NO_3 consumption have been negative or only equivocal.[365-371] This, together with the fact most herbivores have GI conditions that are even less prone to nitrosamine formation than humans, suggests cancer is not a likely sequella of NO_3 exposure in our species of interest. Elevated NO_2 *is* a potential acutely toxic hazard, however. Four of four sows were killed by drinking 1,940 mg NO_2/L water.[335] Nitrite is reported to be 2.5 times more toxic than NO_3 in ruminants and 10 times more in monogastrics[317], and the minimum toxic dose is reportedly between 20-90 mg NO_2/kg BW in pigs and 90-170 mg NO_2/kg in cattle and sheep.[313,358]

Chronic NO_3 poisoning is another area of controversy. Mice exposed to 1,000 mg NO_3^-/L in drinking water for 18 months (life time) died prematurely starting at 17.5 months. The result was of only marginal statistical significance, and no possible mechanism for the result was proposed.[343] Mice exposed to 100 mg NO_3^-/L showed no effects in any parameter measured (liver function, kidney function, serum protein, etc.). Seerley et al.[344] fed breeding gilts $NaNO_3$ in water to provide 1,320 mg NO_3^-/L for 105 days with no effect. Similar results were reported for weanling pigs.[372,373] Fan et al.[374] reviewed the veterinary literature on chronic NO_3 toxicity and concluded it "failed to provide evidence for teratogenic effects attributable to NO_3 or NO_2 ingestion." A retrospective epidemiologic study of pregnant women in the Mt. Gambler region of Australia indicated a "statistically significant increase in risk of bearing a malformed child" in women who drank water with more than 66 mg NO_3^-/L, but it did not take into account other factors associated with the water wells. Bruning-Fann et al.[375] surveyed water from 712 swine operations in the United States and found no differences in litter size or piglet mortality attributable to well-water containing NO_3^- (1-443 mg/L).

After a widespread drought in the American Midwest in the mid-1950's, several authors summarized the experience of multiple field investigations.[376-378] Purportedly, feed concentrations greater than 0.5% NO_3 or water supplies containing more than 500 mg NO_3^-/L were hazardous to cattle fed "poor quality" rations. Case[377] was first to propose that NO_3 interfered with vitamin A metabolism. The results of many controlled experiments since then have rendered this theory "questionable".[312] Sheep fed 2.5% $NaNO_3$ (approximately 1.75% NO_3) diets for 135 days had slightly lower liver vitamin A concentrations than controls, and gains were depressed. A second replicate of the same experiment did not exhibit decreased vitamin A nor was there an increase in metHb or signs of toxicity in either group.[348] Fourteen yearling steers were divided into seven groups and treated with various combinations of NO_3^- in drinking water, NO_2^- in drinking water, *E. coli*, and a "thyroid depressant." Creative use of statistics demonstrated depressed carotene utilization, but there were no other effects.[379] On the other hand, heifers fed various amounts of NO_3 up to 0.9% NO_3 in diets containing 20% or 40% concentrate did not exhibit any difference from controls in carotene conversion or hepatic retinol concentrations.[356] Feedlot cattle fed 0.81% dietary NO_3 as $NaNO_3$ exhibited poor gains as a result of decreased feed consumption. Gains were not improved by supplementing with 12,000 IU vitamin A.[357] Emerick[380] reviewed the literature and concluded that chronic effects involving vitamin A, thyroid function, and other hypothetical chronic mechanisms only occurred at doses that were nearly toxic due to metHb formation.

Winter and Hokanson[381] fed varying amounts of NO_3 (330-690 mg/kg BW) to dairy heifers as part of their ration to maintain metHb levels at either 25-30% or 50% for the last six months of pregnancy. One animal aborted, possibly as a result of NO_3 intoxication and two died of acute poisoning after a diet change, but no chronic effects could be ascertained. Crowley et al.[332] in what, to date, has been the most rigorous experimental attempt to produce chronic NO_3 poisoning in dairy cattle, fed high NO_3^- water (384 mg NO_3^-/L) for 35 months with no effects on conception rate, twinning, stillbirths, abortions, retained placenta, or a variety of production parameters. The only statistically significant effect was a slightly lower first-service conception rate in the NO_3 group. The authors concluded that, in a herd fed a balanced ration, "water containing up to 400 ppm NO_3 should not cause any serious problems."[332] Ensley[382] attempted an epidemiologic approach to the question of high NO_3 water for dairy cattle. In a survey of 128 Iowa dairies with water concentrations from 1-300 mg NO_3^-/L, he found water NO_3 concentrations were positively correlated with services per conception, which agrees with the results of Crowley et al., but several other potentially confounding factors such as the size of the farm and other contaminants in the water were also positively correlated with NO_3 concentrations.

Other attempts to produce chronic NO_3 poisoning in ruminants have been unsuccessful. Sinclair and Jones[327] dosed ewes with 15 g KNO_3 (similar to 1.5% in forage) for two months and then sprayed the same dose of NO_3 on the daily hay ration for another seven weeks. Ewes were fed diets containing 0.2-2.6% NO_3 as $NaNO_3$ or from natural sources for 12 weeks with no effects on health or pregnancy.[340] Despite elevated serum NO_3^- concentrations, there were no effects on metHb, body condition, or reproduction in the treated group. Whethers fed $NaNO_3$ in drinking water to provide 1,465, 2,900, or 4,400 mg NO_3^-/L for 84 days did not differ from controls in gain and water consumption, and only modest increases in metHb concentrations were seen at the highest dose.[344] Feeder lambs fed 3.2% dietary KNO_3 (1.9% NO_3) until slaughter differed from controls only in "carcass quality[326]." Emerick[380] reviewed the literature in 1974 and concluded that feeds containing less than 0.44% NO_3 and water with less than 440 mg NO_3^-/L were "well within a safe range for all classes of livestock."

Summary

There is no question NO_3 contamination of drinking water can result in acute death and/or abortion in ruminant livestock. Cattle are usually reported to be more susceptible than sheep, with monogastrics such as horses and swine being relatively resistant. Surprisingly, we were able to find only one report of NO_3 poisoning (from feedstuffs) in wild ruminants[383], but, given the physiological similarities with domestic animals, it is reasonable to assume deer, antelope, and elk are also susceptible.

The chronic toxicity of very low doses of NO_3 is controversial. Despite repeated attempts (and failures) to reproduce vitamin A deficiency, hypothyroidism, or other chronic forms of NO_3 toxicity, experimentally it does not seem that dietary concentrations significantly less than those required for acute intoxication cause measurable ill-effects in domestic ruminants. While there is no question NO_3 can produce abortions in ruminants, the dose required appears to be very near that required for acute toxicity. The most scientifically rigorous examination of chronic NO_3 toxicity to date[332] concluded that water concentrations less than 400 mg/L (the concentration tested) should not pose any hazard to a well-managed herd.

The lowest toxic dose of NO_3 in cattle in the experimental studies we reviewed is somewhat less than 200 mg NO_3^-/kg BW, although there were several experiments that failed to produce any effect at considerably higher (as much as 800 mg/kg BW) doses. Clinical (i.e. anecdotal) reports, in particular those of Yeruham et al.[384] and Slenning et al.[361], push the minimum toxic dose down to near 100 mg NO_3/kg BW. There are some uncertainties associated with these two reports. Yeruham did not specify the amount of toxic whey consumed (we assumed 20% BW when figuring a dose as it occurred in a hot climate), and there was a two-fold variation in analytical results between samples. Slenning et al. suggested other factors, notably overfeeding an ionophore, might have potentiated the toxicity of NO_3. The next lowest concentration reported to be acutely toxic was 1% NO_3 in *Chenopodium* hay, which would provide approximately 250 mg NO_3/kg BW in cattle under the assumptions outlined in the Introduction. Nitrate in water is additive with NO_3 in feedstuffs, with a given dose in water being somewhat more potent than in feed because it is consumed more rapidly.

Assuming negligible forage NO_3 concentrations, a water NO_3 concentration of 500 mg NO_3^-/L (measured as NO_3^- ion) would provide 100 mg/kg BW, which would provide a two-to-three fold margin below the 200-250 mg/kg BW dose above. If forage concentrations are higher (not a rare occurrence in the Great Plains) the permissible water concentration should be adjusted downward.

The NO_2^- ion is commonly described as approximately 2.5-fold more toxic than the NO_3^- ion in ruminants (10-fold more toxic in monogastrics), which implies a safe threshold of about 200 mg/L. We were, however, unable to find sufficient experimental studies or well-documented field investigations upon which to base any conclusion about maximum safe concentrations. This is probably due to the fact NO_3^- is the more stable form of the two in surface waters and feedstuffs, and NO_2^- is only rarely present in negligible concentrations. Garner describes the minimum lethal dose of NO_2 in swine (the most sensitive species) as 40 mg/kg BW[358], which translates to 200 mg NO_2^-/L in drinking water under very conservative assumptions. Intravenous administration (the most potent route of exposure for most toxicants) of 12 mg NO_2^-/kg BW to cattle and 17.6 mg NO_2^-/kg BW to sheep did not produce any reported toxic effects.

Obviously, this is an area that needs further research, but we believe, based upon the existing knowledge base, 100 mg NO_2^-/L (as the nitrite ion) should not cause poisoning in livestock.