Nitrate are natural constituents of all plants. Under normal conditions, plants take up nitrate through their roots and transport it to the leaves for use in photosynthesis. However, photosynthesis decreases under adverse environmental conditions (e.g., drought; leaf damage due to disease, hail, frost, insects, or herbicides; cool and cloudy weather; and other plant stressors). When photosynthesis is reduced, nitrate transportation to the leaves slows also. Potentially toxic nitrate concentrations can remain in the lower stalks and stems. In addition, heavy nitrogen fertilization provides large amounts of available nitrate for uptake. Plants with high stem-to-leaf ratios are more likely to be associated with nitrate intoxication. Nitrate remains high until photosynthesis resumes with new leaf growth. Resumption of normal photosynthetic rate provides the ability to utilize nitrites being translocated in the plant. However, hay, which has been cut and cured, remains a toxic hazard; nitrate concentrations do not decrease during drying. Ensiling high-nitrate forages crops may reduce nitrate concentrations by approximately 30-50%, however proper fermentation for at least 30 days by microbes is essential to convert nitrate nitrogen into microbial protein. Common crops in Kentucky that may accumulate nitrates include corn, wheat, sorghum-sudan hybrids, sudangrass, rye, pearl millet, soybeans, beets, Brassica spp. (rape, kale, turnips, swedes), and oats, although any heavily fertilized plant can be a culprit. Common nitrate-accumulating weeds include ragweed, pigweed, thistle, bindweed, dock, nightshades, jimsonweed, and johnsongrass. Note that these are not complete lists, but rather the weeds and forages that most often cause problems in Kentucky.

Nitrate/nitrite poisoning can occur in all animals, but cattle are considered most susceptible to nitrate. Rumen microorganisms rapidly convert non-toxic nitrate (NO$_3^-$) to the highly toxic nitrite (NO$_2^-$). Normally, dietary nitrate is reduced by rumen microbes from nitrate to nitrite to ammonia, which is eventually incorporated into microbial protein. Cattle consuming high-nitrate plants overwhelm the ability of the microbes to convert nitrates to true protein. Nitrite accumulates and is absorbed in large quantities, leading to poisoning. The classic forage situation in which plants develop an excessive amount of nitrate in the stalk is with drought-stressed Sorghum species and/or corn. These nitrate accumulators generally account for most of the forage-related cases of nitrate toxicity in livestock. The amount of nitrate that can be safely consumed in forages is approximately 45 grams of nitrate per 100 pounds of body weight. All potential sources of nitrate, including water and feed, must be considered. Surface water or water from shallow wells may contain nitrates, especially if there is run-off from fertilized land contaminating the water. Both water and forage should be analyzed to ensure that total nitrate does not exceed toxic levels. Nitrate poisoning in ruminants may also result from consumption of nitrate fertilizers. Salt-deprived cattle will seek out and ingest stored nitrate fertilizers and can consume toxic quantities very quickly.
**Cause of Poisoning**

Nitrates, when consumed more rapidly than they can be converted in the rumen to protein, enter the bloodstream as nitrite. The absorbed nitrites oxidize the hemoglobin of red blood cells, converting it to methemoglobin. Methemoglobin is incapable of transporting oxygen, and, as methemoglobin concentrations approach 80%, death occurs due to asphyxiation.

**Signs**

The first sign of nitrate poisoning may be the sudden death of one or more animals. Other signs include weakness; rapid, labored breathing; rapid, weak heartbeat; staggering; muscle tremors; and recumbency (inability to stand). Affected animals typically show signs of poisoning within one to four hours after consumption of a toxic dose of nitrates. Examination of the mucous membranes, especially the vaginal mucous membranes, may reveal a brownish discoloration that occurs well before other clinical signs. Chocolate-colored blood and a brownish cast to all tissues are hallmark signs of nitrate poisoning. Most deaths occur within 6-8 hours of onset of clinical signs, and largely depend on the quantity and rate of absorption of nitrite and the amount of stress or exercise to which the animal is subjected. The oxidation reaction is reversible, and methemoglobin will eventually reduce back to hemoglobin. Animals generally die or recover within 24 hours. Abortions can occur in pregnant animals at any stage of gestation due to the combined effects of decreased oxygen to the fetus and the limited ability of the fetus to metabolize nitrite. Abortions typically occur within a week of exposure, but they can be delayed in some cases.

**Treatment**

Animals showing signs of nitrate poisoning should be removed from the source, and a veterinarian should be contacted immediately. Severely affected animals are subject to sudden death, so stress associated with handling must be minimized. Administration of a 2% solution of methylene blue intravenously by the veterinarian will aid in converting methemoglobin back to hemoglobin, but withdrawal guidelines for food animals must be followed and are subject to change. Mineral oil or other emollients may be given to protect the lining of the digestive tract. Vinegar given orally via stomach tube will lower rumen pH and help prevent further nitrate reduction in the rumen.

**Prevention**

Several management strategies are available to reduce the risk of nitrate poisoning. These include:

1. Animals with reduced ruminal microbial activity or have ruminal digestive upset are at a greater risk. Newly purchased calves, water-deprived cattle, and sick cattle are examples of categories that may have a reduced ability to convert nitrite to microbial protein.

2. Nitrate fertilizer should be stored where cattle do not have access to it and accidental spills should be cleaned up promptly.

3. Animals should be provided with ample salt/mineral supplementation to prevent salt-deprived animals from seeking out nitrate fertilizers.

4. Avoid grazing warm season grasses fertilized with high amounts of nitrogen when growth ceases due to drought, cold damage, hail, or herbicide exposure. Warm-season grass stands that have received multiple sources of nitrogen (such as nitrogen fertilizer, manure, previous legume crops) can occasionally show elevated nitrate levels without environmental stress. When in doubt, test samples for nitrate before introducing cattle to the pasture.

5. Cool-season grasses and small grain pastures that have been heavily fertilized with nitrogen may be high in nitrates during early spring when cool, overcast days retard plant growth. Test before grazing.

6. Corn forage should be properly ensiled at least three weeks and tested for nitrates before feeding. Do not green chop forages suspected to be high in nitrates.

7. All suspected forages including silage and hay should be tested for nitrate levels. Instructions are provided in this publication: See “Sampling and Shipping of Forage Samples for Nitrate Testing.” A field test is also available to give a quick indication of whether the forage is potentially dangerous. If the test strip reacts, a forage sample should be sent to a laboratory for an accurate analysis of nitrate and a feeding recommendation. Consult your county Extension agent for information concerning sampling, sample preparation, field test, and location of a testing laboratory.

8. Delay harvest of high-nitrate forages until nitrate levels are safe. If not feasible to delay harvest, raise the cutter bar to 18” to avoid the base of plants.
If high nitrate forages must be utilized, the following suggestions will reduce the risk of poisoning:

1. Split forage feeding to twice a day. The diet should contain less than 5,000 ppm NO₃⁻ to avoid reproductive impacts. Non-pregnant animals could be fed slightly higher levels after being acclimated. Feeding low nitrate forage or hay before turning cattle on to high nitrate forages will also reduce the amount of nitrate consumed.

2. Forage with high nitrate levels can be mixed with forage known to be low in nitrate to reduce the risk from feeding.

3. Introduce the nitrate forage slowly. Give half the final amount of high-nitrate forage for the first two to three weeks. Cattle can increase their tolerance to dietary nitrates over time, as the rumen microbe population adapts to utilize the nitrates more efficiently. To aid in increasing this tolerance, the diet should be sufficient in vitamin A and trace minerals.

4. A gradual increase in the total energy content of the ration enhances metabolism in the rumen and helps cattle tolerate higher nitrate levels in their diet. Feeding three to four pounds of corn per head per day to mature cattle can help the rumen microbes convert nitrite to microbial protein faster. Low-energy diets increase an animal’s susceptibility to nitrite poisoning.

5. The best method to feed high-nitrate forage is grinding and mixing it in a total mixed ration (TMR). This can help to minimize sorting, or the boss cows eating more of a low-nitrate hay and leaving other cows to eat greater amounts of high-nitrate forage.

6. Offer excessive amounts of hay, so cattle will eat leafy portions and leave the high-nitrate stems behind.

7. There is no assurance that the forage samples submitted for testing are representative, and some bales may test even higher than reported. Thus, err on the side of caution, especially when feeding pregnant cattle.

8. There are propionibacterium products available in bolus or powder form that are reported to reduce nitrate and nitrite levels in the rumen by approximately 40 percent. These products must be established in the rumen for at least 10 days before allowing cattle to consume high-nitrate feedstuffs.

### Table 1. Nitrate levels and feeding options for cattle.

<table>
<thead>
<tr>
<th>Total Dietary Nitrate (NO₃⁻) in dry matter</th>
<th>Feeding Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5,000 ppm (0.5%)</td>
<td>Generally safe for cattle. Be cautious with pregnant and young animals when nitrate concentrations approach 5,000 ppm and dilute with other feeds.</td>
</tr>
<tr>
<td>&gt;5,000 but &lt;10,000 ppm (&gt;0.5% but &lt;1%)</td>
<td>Dilute with other feeds and introduce slowly. Consider options to reduce nitrate in fresh forage (ensiling, delayed harvest, other). Limit to a maximum of 50% of the total dry matter in pregnant animals.</td>
</tr>
<tr>
<td>&gt;10,000 ppm (1%)</td>
<td>Very dangerous; can cause acute nitrate poisoning and death in cattle. Do not feed.</td>
</tr>
</tbody>
</table>

### Testing for Nitrates

All sources of dietary nitrate, including feeds, forages, supplements, and water should be taken into consideration when determining total dietary nitrate concentration. The guidelines for interpretation of forage nitrate concentrations are provided in Table 1. Representative sampling is crucial for proper interpretation of results. Nitrite, a breakdown product of nitrate that can be found in forages, is directly toxic, as opposed to nitrate, which requires conversion in the rumen. Much lower levels of nitrite can cause poisoning and death, and all species are susceptible.

Horses are much less sensitive to nitrate than are cattle or other ruminants, and can tolerate much higher concentrations of nitrate, but exact threshold values have not been established. Horses are extremely sensitive to nitrite, so any preformed nitrite in forages can pose a significant risk. Please consult with a veterinary clinical toxicologist for interpretation of nitrate/nitrite concentrations in horse feeds. Sheep and goats are less susceptible than cattle to nitrate toxicity and camelids are rarely affected.

Many commercial laboratories conduct nitrate testing. However, be aware that nitrate levels can be reported a variety of ways, and the method of expression can differ between laboratories. Nitrate can be reported as nitrate (NO₃⁻), nitrate-nitrogen (NO₃⁻N), or potassium nitrate (KNO₃). These numbers are NOT equivalent, as they represent different chemical structures. Make sure the feeding guidelines used for a particular result match the type of analysis performed. To convert between the different methods of reporting, use the conversions in Table 2.

Forage nitrate results can also be reported using a variety of units. The most common units of measurement are parts per million (ppm) or percentage (%). Results are usually reported on a dry matter basis. To convert from ppm to %, move the decimal point four places to the left (eg, 5,000 ppm = 0.50%).

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Laboratory Contact Information:

Two veterinary diagnostic laboratories in Kentucky perform nitrate testing on forages, the University of Kentucky Veterinary Diagnostic Laboratory and the Murray State University Breathitt Veterinary Center. Both are accredited by American Association of Veterinary Laboratory Diagnosticians.

University of Kentucky Veterinary Diagnostic Laboratory (UKVDL)
1490 Bull Lea Rd
Lexington, KY 40511
Phone: (859) 257-8283

Murray State University Breathitt Veterinary Center
715 North Drive
Hopkinsville, KY 42241-2000
Phone: (270) 886-3959

Sampling and Shipping of Forage Samples for Nitrate Testing

Contact the laboratory you intend to use or your local county agent for specific instructions, but some general guidelines are listed below.

Sample Collection Guidelines for Nitrate Testing

Proper sample collection is crucial for proper interpretation of results. The sample should represent what the animals will be eating, so collect the entire part of the plant that will be fed. Collect a number of smaller samples to form a large representative composite sample. If different regions of a field were treated differently, then separate composite samples should be submitted for each different region. Different cuttings, batches, or fields should be sampled separately, and submitted as separate samples.

Preferably at least a pound of total composite sample should be submitted. More sample is better than too little, so when in doubt, collect more. Be sure to mark each bag legibly with forage/sample type and identification information.

Note: Nitrate concentrations tend to be higher at the base of the plants, and higher in the stalks than the leaves. Grains, seeds, and leaves do not accumulate significant nitrate levels. Plants with high stem-to-leaf ratios are the most likely to cause nitrate intoxication.

Dry forage (hay, bedding). Use a hay probe to take core samples. Randomly select 10 or more bales that are representative of a cutting/batch. Take one or more core samples per bale, and mix all the cores to make one large composite sample.

Silage, balage, haylage. Use a hay probe to take core samples if possible. Randomly select 10 or more bales that are representative of a cutting/batch. Take one or more core samples per bale, and mix all the cores to make one large composite sample. Reseal the hole created in the wrap with tape after sampling. For bagged silage, select at least 10 areas to sample that are representative. If core sampling is not possible, unload some silage material and collect large handfuls from 10 or more different locations. Mix to form a large composite sample.

Corn stalks. Cut the stalks at the anticipated harvest level and submit the entire part of the stalk that will be fed. Collect stalks from several areas of the field; five or more stalks are recommended. Stalks can be cut or folded prior to shipping. Alternatively, if the corn stalks are going to be chopped, you can collect representative samples from the fresh chop. Or, if shipping volume is an issue, only the bottom halves of the stalks could be submitted, but remember that the result will be higher than the actual overall average nitrate concentration for the entire plant.

Pasture grasses. Collect handfuls of forage from 10-20 different areas in the field. Cut the grass at the anticipated harvest or grazing height and submit the whole part of the plant that will be ingested. Mix thoroughly to make one large composite sample.

Note: Grains do not accumulate nitrate, so nitrate testing is not typically performed on grains.

<table>
<thead>
<tr>
<th>Method of expression</th>
<th>Chemical designation</th>
<th>To convert to NO₃, multiply by</th>
<th>To convert to NO₃-N, multiply by</th>
<th>To convert to KNO₃, multiply by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>NO₃</td>
<td>1.00</td>
<td>0.23</td>
<td>1.63</td>
</tr>
<tr>
<td>Nitrate-nitrogen</td>
<td>NO₃-N</td>
<td>4.40</td>
<td>1.00</td>
<td>7.20</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>0.61</td>
<td>0.14</td>
<td>1.00</td>
</tr>
</tbody>
</table>
A real-world example of nitrate toxicosis was described in the June 2020 issue of the California Animal Health and Food Safety (CAHFS) newsletter. Six deaths occurred out of 50 dry dairy cows on pasture that were supplemented with oat hay. A new bale of oat hay was offered in the morning, and “four hours later three cows were dead, and three others showed signs of paresis [weakness/partial paralysis], urine dribbling, and staggering before falling, developing agonal breathing and dying. Field necropsies revealed brown blood. Eye fluid from four cows had toxic levels of nitrate (130-170ppm, toxic >25ppm). The hay also had toxic levels of nitrate (11,000-13,000ppm, toxic >10,000ppm).”

Sample Storage and Shipment Guidelines

Moist samples (such as fresh green grasses, silage) must be placed in plastic bags and immediately put in a cooler on ice or ice packs. These samples should be kept chilled or frozen until shipment and should be shipped with ice packs. Dry samples such as relatively dry corn stalks and hay should be placed in paper bags and kept at room temperature until shipped; ice packs are not needed for shipment of dryer samples. Regardless, samples should be shipped as soon as possible after collection. Samples should be shipped overnight or delivered directly to the laboratory.

Note: Storage of moist plant samples in plastic bags at room temperature will result in bacterial growth and reduction of nitrate to nitrite, resulting in inaccurate nitrate results.