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Forage Nitrates and Prussic Acid

Sampling, Testing and Management Strategies

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Weather and cultural practices can impact forages in several manners. Poor growing conditions caused by drought, hail, cold, or excessively hot weather reduces a plant's ability to assimilate fertilizers or soil nitrate-N into amino acids and other nitrate-N containing compounds. Similarly, plants weakened by off target herbicide applications or recent cutting/harvesting may also cause accumulation of nitrates. These same derogatory plant growth factors can also cause some plants to accumulate prussic acid, a compound that is transformed into hydrogen cyanide in the rumen.

The impact of nitrates and prussic acid accumulation in forages, sampling and testing forages for these contaminants, and management strategies are highlighted in this fact sheet.

Nitrates in Forages

Nitrate accumulation

Ammonium nitrogen is the preferred nitrogen species for plant uptake due to energy considerations, however the nitrate form is the primary form taken up by plants. Even when ammonium and urea-based fertilizers are used, most of the nitrogen taken up by a plant is still in the nitrate form, as soil microorganisms quickly convert ammonium nitrogen to nitrate nitrogen (See TCE E-59). Nitrates are extremely soluble in water, and are subsequently taken up by the plant along with soil moisture. Under normal conditions, these nitrates are reduced to ammonium ions via the nitrate reductase metabolic pathway and then assimilated into amino acids and proteins for plant use. This energy-requiring process occurs in the roots for some grasses such as bermudagrass, while it occurs in the leaves, stems and stalks in other plants such as corn or sorghum. Under drought and elevated stress conditions, this pathway may slow or shut down, thus allowing the accumulation of nitrates in the plant. This accumulation will continue as long as this pathway function is impaired and the plant takes up nitrate-containing water.

Common causes of nitrate accumulation are listed below.

1. During high temperature and adequate moisture conditions, plants undergo a process known as photorespiration. Photorespiration results in the production of carbon dioxide, instead of the assimilation of carbon into energy building blocks (i.e., sugars, carbohydrates, etc.). As such, the reductase pathway slows or stops and nitrates will begin to accumulate and concentrate.

2. Minimum moisture for limited water uptake combined with presence of available fertilizer N (nitrate-N) may promote nitrate accumulation. Under these conditions, the reductase pathway slows or stops, but enough soil moisture continues to be present to allow plants to take up nitrate containing water. This causes the accumulation of nitrates. While some water is still being actively taken up, the plant has insufficient water to continue growing.

3. Nitrates may accumulate in plants following plant injury from herbicide applications. Many herbicide chemistries' modes of action interfere with one or more plant metabolic

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pathways, subsequently limiting the conversion and assimilation of nitrates. When surveying forages, pay close attention to weeds or other plants which may have been effected by recent herbicide treatments. Additionally, a forage producer should scan field edges for potential off target herbicide drift and any potential forage/plant damage.

Nitrate accumulating plants

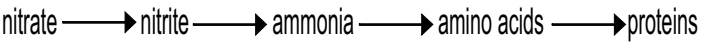
Nitrate accumulation to toxic levels is known to commonly occur in the following plants:

Forages	**Weeds**
alfalfa	Canada thistle
barley	dock species
corn	jimsonweed
flax	johnsongrass
millet	kochia
oats	lambsquarters
rape	nightshade species
rye	pigweed
soybean	Russian thistle
sorghum and sorghum hybrides	smartweed
sudangrass	sunflower species
sugarbeets	

While these plants are the most likely ones to accumulate nitrates, nitrates are present to some degree in all forages, including bermudagrasses. Nitrates are non-volatile and remain in non-ensiled forages/plants after cutting, curing and baling. Nitrates are soluble in plant tissues, and as such, can be leached from the plant during sustained rainfall. However, the significant reduction in protein and energy following weathering/leaching of hay bales strongly limits this process as a potential management tool. Furthermore, the movement of nitrates leached within a weathered bale may limit the use of the forage. Three to five days of active growth are needed to significantly reduce the nitrate levels.

Based upon findings of the Texas Veterinary Diagnostic Medical Laboratory (TVMDL), a forage containing 1% nitrate on a dry matter basis is the safe cut off point for healthy ruminants. Forages higher than 1% nitrate could be fed if nitrate-tainted forage is "ground" and mixed with nitrate free forage to reduce overall nitrate levels to <1% (dry matter basis). Feeding 0.5-1% nitrate containing forages/feeds to weakened cattle should only be done following consultation with your veterinarian. The 1% nitrate level is significantly higher than levels suggested by other southern universities. This research based value (TVMDL) assumes that healthy well-conditioned cattle are fed a high energy diet.

In healthy cattle, the nitrate consumed in normal forages is converted into protein in the rumen:



When animals consume forages too high in nitrates, the animals cannot complete the conversion of all nitrates to amino acids and proteins, and subsequently nitrite levels will build up. Nitrite is adsorbed directly into the bloodstream through the rumen wall. In the bloodstream, nitrite combines with hemoglobin to form methhemoglobin. Hemoglobin is the compound that carries oxygen in the blood, whereas, methhemoglobin does not carry oxygen. As such, the animal actually dies from asphyxiation, or lack of oxygen. The animal's blood turns to a chocolate brown color instead of the normal bright red. Monogastrics (i.e. horses, mules, swine, etc.) are less sensitive to nitrate toxicity. Since animal conditioning does influence the ability of an animal to assimilate or tolerate nitrates, consultation of your veterinarian is strongly suggested prior to feeding nitrate-containing forages.

Sampling for nitrates

Nitrate accumulation is often highest in the lower portions of the stem, and lowest in the leaves. The method of sampling forages for nitrate analysis is dependant on the method of harvest or grazing and forage species.

Limit grazing

Under grazing systems, two sampling scenarios exist depending on duration of grazing. Under limit grazing of traditional grasses, small grains or legumes, where only the upper 1/3-1/2 of the plant is consumed, a similar forage sample is obtained. When limit grazing corn, sorghum, sorghum-sudangrass and similar forages, sample only the plant leaves.

Rotational or single field grazing

When rotational grazing or single field grazing systems are used, a more conservative sampling approach is

warranted. The more conservative approach is needed, as livestock will likely consume the leaves and upper plant parts first, followed by the higher nitrate containing plant stems. Under these grazing scenarios, the lower 1/3-1/2 of traditional grasses, small grains and legumes is taken for sample analysis. Similarly, the lower 1/3 of corn stalks, sorghum, and sorghum-sudangrass stalks are taken for nitrate analysis.

A composite of sample for standing forage nitrate analysis should consist of plant parts from at least 10 to 15 plants from representative areas with the same fertility and moisture conditions. Do not mix plants from "good" and "bad" parts of the field. These should be composited individually and analyzed as multiple samples. All samples for nitrate analysis should be shipped to the laboratory in a clean paper sack. Avoid placing samples in plastic bags as the high moisture content of the forage will quickly result in molding and potentially skewing the nitrate analysis results.

Sampling existing baled forages for nitrates

If the forage has already been baled, take representative core samples from the bales using a bale probe. This sampling method is the only sampling method that will adequately evaluate the average nitrate levels in the bale. While this bale probe method is excellent for determining the average nitrate levels of a baled forage, it should not be used for sampling coarse stemmed forage such as corn, sorghum, sorghum-sudangrass or similar crop/hays, unless the bale will be ground prior to being fed. When bale sampling these coarse stemmed forages, split the bale open and selectively collect the lower stems of individual plants. This selective bale sampling method is needed to guard against high nitrate consumption by more timid feeders, following selective consumption of leaves and more palatable upper stems by the first animals at the bale.

Nitrate Testing

For forages grown under the conditions described above, proper management practices would include testing for nitrates. Testing for nitrates can be preformed both in the field and in the laboratory. Field methods include qualitative spot color methods, while some quantitative methods utilizing colorimeters and nitrate electrodes are available. Presently, field methods should be considered only as qualitative tools. The difficulty in obtaining uniform samples, varying moisture contents, and decreased accuracy and precision of these tests limits their use in mixing feeds to obtain sufficiently low overall nitrate levels.

One common nitrate quick test is the diphenylamine spot test. The Texas Cooperative Extension Soil, Water and Forage Testing Laboratory has historically manufactured these quick test kits for plant nitrate analysis. Recent difficulties in shipping these sulfuric acid containing kits is forcing the laboratory to investigate alternative quick test methods. The test is comprised of diphenylamine salt (0.1 grams) dissolved in sulfuric acid (30 ml-36N). A single drop of this acid reagent is placed directly on a freshly split plant stem. The immediate development of a dark blue color indicates the presence of nitrate (first 5 seconds). No immediate change in color indicates the absence of nitrate; however, a dark color (brown/black) will eventually develop if left in contact with the plant tissue for an extended time. The dark color is caused by acid caramelizing the plant sugars and carbohydrates.

The pictures below illustrate results of the nitrate spot test.

The diphenylamine field test only indicates the presence or absence of nitrate. As it does not determine the actual nitrate concentration and as with any field test method, it should only be used as a screening tool. Any positive result from the spot test should be followed up with a laboratory analysis for quantification. The field quick tests can provide a

rapid estimate of what stem height will provide a nitrate-free forage. Additionally, most field test methods only work for moist plants that have thick enough stems (corn, sorghum, and similar) to split and apply the test reagent. Dried plant tissue, hay, silage, fine-stemmed grasses and other similar tissues should undergo laboratory testing.

Interpretation different laboratory's reports

Nitrate results may be expressed as actual nitrate ($\%NO_3$) or nitrate-N ($\%NO_3$-N) values. The Texas Cooperative Extension Soil, Water and Forage Testing Laboratory reports the results for forage nitrate analyses as actual nitrate. The industry standard is to report forage nitrates as percent nitrate, which differs from plant nitrate analysis, which is expressed as ppm nitrate-N. To convert nitrate-N levels to actual nitrate, multiply by 4.42. Results may also be reported in parts per million (ppm). To convert ppm to percent, divide by 10,000. Confusion by the testing laboratory could result in a toxic nitrate level being considered safe for feeding if results are expressed as nitrate-N.

Nitrate management considerations

The management of nitrate containing forages is dependant on forage type and harvest method. All suggested scenarios should be done in tandem with proper sampling and analysis methods outlined earlier in this factsheet.

Managing standing forages

1. The grazing of forages containing elevated and potentially toxic levels of nitrates maybe preformed by limit grazing the upper 1/3-1/2 of the grass, legume or leaves of coarse stemmed forages (corn, sorghum and etc.). Prior to this type of grazing scenario, a manager should carefully evaluate nitrate levels in these plant parts. Additionally, this type of grazing management requires close monitoring of remaining forage stocks and ability to remove the livestock off these forages when the nitrate-safe portion of the forage is consumed.

2. Close and timely monitoring of nitrate levels in the lower 1/3-1/2 of the plant or coarse stems is required if livestock are to be released into a field with questionable or potentially toxic nitrate levels. Generally, forage nitrate levels will drop significantly 3 to 5 days following sufficient rainfall.

3. A third management practice is to cut the forage for hay, field cure and bale. This practice will not lower the nitrate levels; however, if these bales can be ground and mixed with "nitrate-free" forages, a higher overall protein and energy value will be maintained.

4. The forage could be harvested and ensiled. During the ensiling process, nitrates are converted to volatile nitrous oxides. These volatile nitrous oxides, also referred to as "silo gases" are highly toxic and extreme care should be used prior to entering silo pits and bunkers where nitrate tainted forages have been ensiled. A common safety practice is to remove tarps from a portion of the silo 1-2 days prior to removing silage from that part of the pit.

Managing existing nitrate containing hay (baled)

1. Fewer options are available for managing baled forages. The safest method is the grinding of the entire hay bale and thoroughly mixing the nitrate tainted hay with other nitrate-free hay. Sufficient quantities of good forage should be ground and mixed to reduce the nitrate level to less than 1%. This practice is best performed in concert with similar forage types, thus providing a more uniform particle size distribution. The optimum size is dictated by stem size; however, in general, the smaller the better. A handful of ground and mixed feed should contain portions of all plant parts from all forages used in the mix. The uniform size will help limit selective feeding by livestock. This grinding and mixing method should not be used for forages containing greater than 2.5% nitrate.

2. If nitrate levels are greater than 2.5% or no method of grinding and mixing the forage exists, the baled forage should not be used for feeding or bedding of livestock.

Prussic Acid in Forages

The production of prussic acid by certain forages and non-forage plants can pose a significant risk to certain grazing and barnyard livestock. The following list of plants have been reported to accumulate prussic acid.

Accumulating plants

Forage or plant	Prussic acid potential
pearl/foxtail millet	very low
sudangrass/sudangrass hybrids	low or moderate
sorghum-sudangrass hybrids	moderate to high
forage sorghum	moderate to high
shattercane	high
johnsongrass	high to very high
grain sorghum	high to very high
sorghum almum	high to very high
arrowgrass	low
velvetgrass	low
white clover	low
birdsfoot trefoil	low
chokecherry*	low
pincherry*	low
wild black cherry*	low
apricot*	low
peach*	low
apple*	low
elderberry*	low

**Prussic acid maybe found in the leaves and seeds of these tree species, however low potential of poisoning is generally considered due to limited livestock intake. Avoid housing livestock in areas where these trees exist and limited other traditional forages are present.*

Impact of prussic acid on livestock

The plants listed above produce cyanogenic glucosides (prussic acid) during their growing stages. Glucosides are sugar compounds that break down into glucose sugars through the process of hydrolysis in the rumen. In cyanogenic glucoside forming plants, hydrolysis frees the cyanide from the sugar and forms hydrocyanic acid. Hydrocyanic acid (HCN) is commonly known as cyanide. The HCN combines with hemoglobin to form cyanoglobin, which does not carry oxygen. Cyanide poisoned livestock exhibit similar respiratory stress as those described under the nitrate poisoning section of this factsheet. A blood sample can quickly discriminate between nitrate and prussic acid poisoning. Blood from prussic acid poisoned livestock will appear cherry red, unlike the chocolate brown coloring resulting from nitrate poisoning. Horses, hogs and other non-ruminant animals are less affected by prussic acid. The strongly acidic (hydrochloric acid) stomach of non-ruminants converts the prussic acid to less toxic formic acid and ammonium chloride.

Treatment for prussic acid poisoning maybe effective if administered immediately upon the first poisoning symptoms. Two common treatment methods include an intravenous injection (125-250ml) of 1.2% sodium nitrate or 7.4% sodium thiosulfate. The veterinarian should insure the symptoms are not related to nitrate poisoning prior to administering the sodium nitrate treatment.

Fate of prussic acid in plants

Actively growing, healthy tissues, from this class of plants, contain low levels of cyanogenic glucosides, as these compounds are broken down, thus eliminating toxic accumulations. Unlike nitrate, prussic acid may be present for a time then dissipate from plants properly cured for hay.

Common causes/occurrences of prussic acid accumulation

Common management or growing conditions which may lead to prussic acid accumulation are listed below.

1. Poor growing conditions resulting in reduced stem development.

2. Slow and stunted growth of new plant tissue due to recent hay harvest or grazing activities.

3. Over application of nitrogen fertilizers or plants grown on soil with other soil fertility or nutrient imbalances.

4. Newly developing tissue generated following prolonged drought.

5. Damaged tissues following herbicide injury, frost, hail or other plant injuries.

Sampling for prussic acid

In cyanogenic glucoside forming plants, the prussic acid primarily accumulates in the leaf tissue. Prussic acid concentrations are highest in new growth tissue. Generally, these levels are many times higher in the leaves than in the plant stems. Since livestock typically select leaves prior to stems, samples taken for prussic acid analysis should be largely comprised of leaves. This is especially true for sampling fields where cattle will be allowed to graze. Under limited grazing conditions, stem nitrate accumulations will likely not be ingested by the cattle.

Unlike nitrates, prussic acid may volatize from cut/harvested forages. The rate of volatization is weather, and hay bale dependant. The amount of volatization can be limited when sampling and shipping forages to a laboratory by placing the samples in resealable plastic bags or pint glass jars. When filling the bag or jar, allow 25-50% head space.

When sampling standing forages for prussic acid content, selectively remove the newest upper leaves from 10-12 plants in different areas of the field, that are the most probable locations for prussic acid problems (review common causes/occurrences list). Place leaves in sealable container and ship under refrigeration (on ice, but not frozen) immediately to testing laboratory. The sampling of baled forages requires the use of a bale probe. Avoid grab samples from the outside portion of the bales, as losses of prussic acid due to volatization is highly probable. A single bale core should be placed in a sealed pint jar. More detailed instructions on sampling and shipping samples for prussic acid analysis can be obtained by contacting the TVDML at (979) 845-3414.

A testing laboratory can evaluate the prussic acid concentration either qualitatively or quantitatively. While references exist suggesting 250 ppm HCN is safe, the extreme variability in prussic acid sampling and shipping and rapid field changes limit the value of a quantitative result. A simple qualitative analysis indicating the presence or absence of prussic acid will generally suffice. If a forage is determined to contain prussic acid, the producer is urged not to feed or graze the forage until levels decline to sub-detectable concentrations.

Management Considerations

The management of prussic acid containing forages is dependant on the forage type and harvest method.

1. Standing forages testing positive for prussic acid which will be grazed should be sampled every 3-4 days. Frequent sampling intervals will help lesson losses of forage nutritive value due to increasing plant maturity.

2. Standing forages can be green chopped and ensiled. Prussic acid is enzymatically converted to free cyanide which will readily escape upon silage removal. Extreme caution is required when entering silage pits where prussic acid containing forages were ensiled. Additional benefits of green chop/silage systems is the overall dilution effect of prussic acid by elimination of free choice selection of leaves or plant stems.

3. Standing forages can also be cut, field cured and baled. As with all baled forages, proper sampling using a bale probe is required. Bales should be repeatedly sampled until prussic acid is no longer detected. Additional safety can be achieved if whole bales are ground, thus diluting elevated new leaf concentrations with stems.

Forage analyses at Texas A&M University

Two Texas A&M University laboratories routinely analysis producer forage samples. The Texas Cooperative Extension Soil, Water, and Forage Testing Laboratory (SWFTL) provides routine analysis of plant nitrates, protein, ADF, and mineral analysis. Simply analyzing a forage for nitrate concentrations does not provide any insight to forage nutritive value and the need of energy or protein supplementation. The laboratory does not provide prussic acid analysis. The SWFTL website is http:soiltesting.tamu.edu. For further information about the Soil, Water and Forage Testing Laboratory call 979-845-4816.

The other laboratory mentioned throughout this factsheet is the Texas Veterinary Medical Diagnostic Laboratory, which specializes in toxicological analysis of feeds, forages and animals. The TVMDL can be reached at 979-845-3414.

Additional References and Sources of Information

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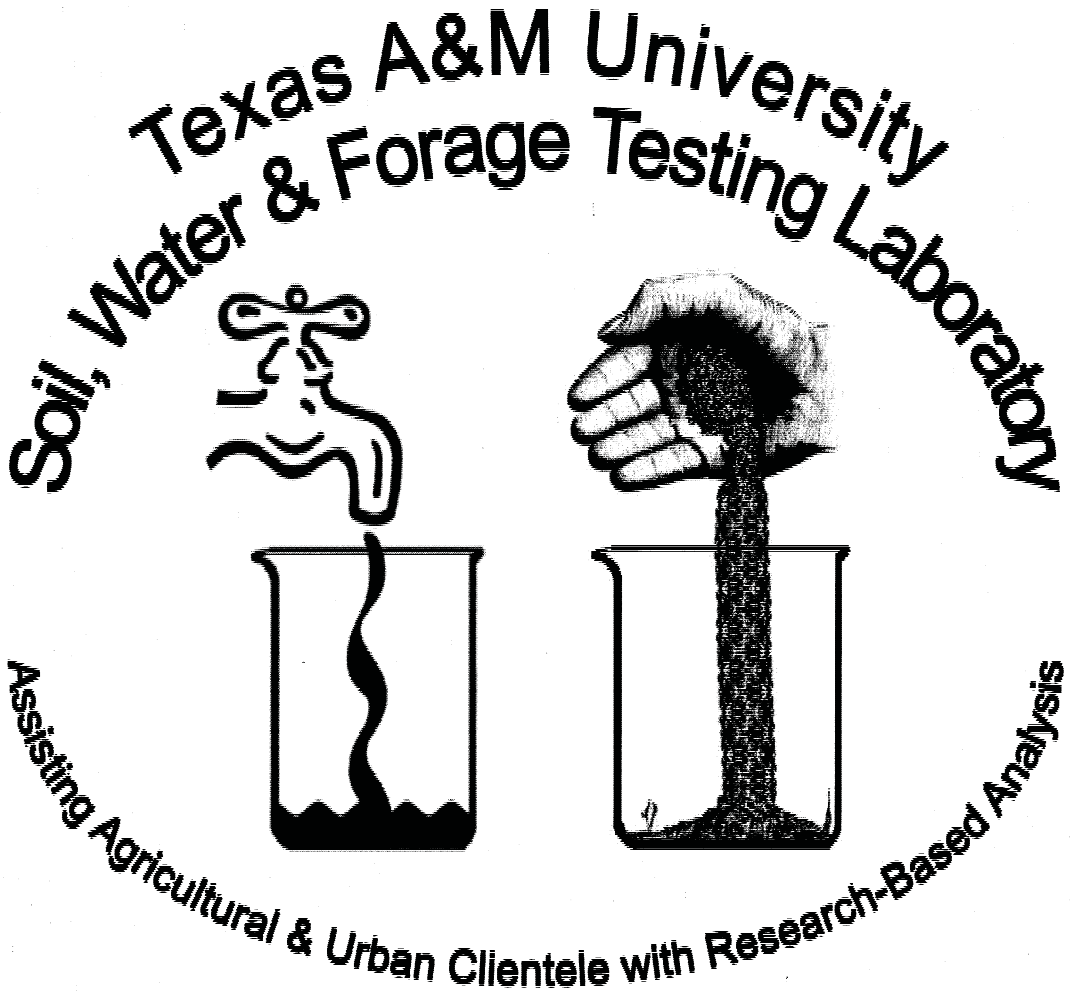

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