University of Missouri Extension

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Qualitative Nitrate Detection for Toxicity Potential

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This test is designed for survey use and for advising clients of the need for quantitative nitrate analysis when the level of nitrate detected could possibly cause nitrate/nitrite poisoning. The test can be used on water supplies, hay, silage, fodder, pasture samples and mixed ground feeds.

Preparing the reagent

The reagent is prepared by placing diphenylamine (0.5 grams) in a graduated cylinder or flask containing 20 milliliters of distilled water. While stirring, **slowly** add 88 milliliters of concentrated sulfuric acid (H_2SO_4). This will dissolve the diphenylamine and generate heat.

Allow the reagent to cool, then place it in a clean dropper bottle. Since the reagent contains sulfuric acid, it must be handled with caution. Burning of skin, charring of clothing, etc., can best be stopped by washing with water or, even better, with baking soda (sodium bicarbonate).

If, over time, the reagent becomes highly discolored, brown or blue, discard it. The brown color is caused by foreign matter and blue is probably due to touching the sample with the dropper tip. Don't allow the dropper tip to contact the samples.

Diphenylamine crystals in 0.5 grams per packet are available upon request from the MU Department of Biochemistry. Sulfuric acid solutions are not mailable. Distilled water and sulfuric acid are available at area soil testing laboratories.

Testing solid substances

To acquaint yourself with the test, split a normal appealing corn stalk and test each node from the ground up with a few drops of reagent. The concentration should be highest at the lower end and become less toward the top of the stalk. If ample nitrogen has been available, a dark blue spot would be expected to develop almost instantaneously at the first node. Each node toward the tassel should show a light blue spot or take longer to become dark blue.

When an ear has formed and proper nitrogen fertility is present, the node above the ear should be very light blue and a brown color may begin to appear. The brown coloration indicates carbohydrates and may occur anywhere in the plant if nitrates are absent. Only a blue, blue-black color is a measure of nitrate concentration.

All gradations of blue color from light blue to blue-black are possible. A rating system of 1+, 2+, 3+ may be useful to correlate with quantitative data on samples tested.

For testing silage, hay and pasture samples, two procedures may be used. The simplest is to drop the diphenylamine reagent on the cut cross section of the stem or pith and observe the color. The second method is to squeeze out liquid from silage or pasture samples onto a white spot plate and add a few drops of the reagent. With dry silage and hay, enough water should be added to moisten the sample throughout and then express the liquid and test as described above.

Testing liquids

Pour a few milliliters of water into a clean test tube and carefully pour 1 to 3 milliliters of the diphenylamine reagent down the side of the slanted test tube. If a blue color does not appear at the interface of the water and sulfuric acid within 30 seconds, hold the test tube between the thumb and index finger and tap gently to start a swirling action. Approximately 1/2 of the reagent layer should be left. Samples that do not show blue coloration at the interface within 30 seconds are quite low in nitrate (less than 20 ppm). Expressed liquids from silage, hay and pasture samples can also be handled this way, but they take more time than the spot plate.

Interpreting the test

The moisture content of material to be tested markedly affects the interpretation. Wet silage may show less blueness than dryer silage but still have equal nitrate content.

Silage

For silages, since the nitrate content is higher in the stem or stalk at time of harvest, sort out several stems or pieces of pith for testing. Drop reagent on the material and observe.

If the silage is moist enough, express a few drops on a spot plate to test. A light blue color indicates some nitrate present. A rapidly developing dark blue color that appears nearly black indicates the silage should be analyzed quantitatively for nitrate. To further test, take the leafy portion of the silage and express a drop of liquid from it. Add reagent. If it rapidly develops a dark blue-black color, feed the silage cautiously until a quantitative test is made.

Green chop

For green chop, a few drops of liquid usually can be expressed and tested. Rapidly developing dark blue and black color indicates a level of nitrate that requires a quantitative analysis. For corn that has a small to large normal ear, split the stalk and test by nodes. If only a weak test is observed above the ear node, there will be a dilution of at least half of the nitrate in the base nodes nitrate when chopped. No problems should be anticipated in direct feeding or ensiling.

As long as the crop is green and has not been severely drought damaged (whitish-green to gray leaves), normal ensiling will further reduce the nitrate. There will be some reduction of nitrate between the time of cutting and feeding. Overnight storage on wagons is not recommended because nitrite could build up. Chop and feed as needed. There is one exception: where cyanide (prussic acid) is a problem in some sorghums, overnight storage on wagons or trucks would result in loss of cyanide.

Dried fodders and hays

Test stems and stalks. If dark blue-black color rapidly develops, check the leaves. If leaves are also very positive, make a quantitative analysis before feeding the forage or feed it cautiously.

Pastures

Test stems of plants. If stems test positive, then test leaves. If both are positive, excessive nitrates are present and quantitative analysis is needed along with cautious feeding. Sudan, Sudex, etc., are more likely to be a problem than cool season grasses. You may occasionally find that grasses in old feedlots or well fertilized pastures are a problem

Mixed ground feed

Grains would be free of nitrates as measured by this reagent. Contamination, accidental addition of nitrate salts to rations, and the use of beet pulp or molasses with high nitrate content would be the most likely cause for a positive test. Moisten the feed to paste consistency on a white spot plate and drop reagent on the edge of the paste. Observe the color of the reagent. Only a blue color is positive because the high sugar-starch content of the feed will react with the sulfuric acid to form a charred mass that is black and impart a brown color to the excess reagent.

Water

Water should be tested by using a test tube and layering of the reagent. A small light blue ring at the interface indicates between 20 and 30 ppm of nitrate (NO_3^-). A dark blue ring indicates at least 50 ppm. If the entire reagent layer becomes dark blue, the nitrate level is in excess of 100 ppm nitrate. U.S. Public Health recommended safe limit is 45 ppm nitrate. Pond water should be negative by this test. Often a green chlorophyll color will be seen if there is algae in the water.

Whenever nitrate is detected in pond water, there is a source of contamination (be careful not to test suspended soil particles), and that source should be determined. If the source is removed. the organisms in the pond will soon use up the nitrate.

Water testing warning

Nitrite at a few parts per million causes the reagent to become a violet-blackish color, usually seen as violet to greenish above the dark black ring. If reagents are available, make a test for nitrite. Pond water and water from livestock tanks or other non-sterile holding basins (cisterns) often contain water with nitrite present if nitrate contamination is also present.

Sampling procedures

Sampling should be done carefully. The analysis can be no better than the sample.

Water
Collect water in a clean, sterile bottle (2 ounce minimum). When collecting from a water system, allow enough flow to replace all the water in the lines and dilute out the pressure tank. Then take the sample.

Ponds may be sampled from the drain pipe. In unimproved ponds, sample so as to avoid mud contamination.

Dry forages
Whenever possible, send in a core sample from 15 to 20 bales. (Use Penn State Sampler or equivalent). For loose hay or without bale sampler, open 10 to 15 bales and take a grab sample of approximately one handful from each. Cut the forage into 2- to 4-inch pieces, being careful to preserve all leaf material. Pack tightly in a container.

Silage and fresh grass
Pack the sample (approximately 5 pounds) in a plastic bag. This sample should be taken from a composite sample of at least four areas in the silo or pasture.

Samples should be taken or sent immediately to the laboratory. If transit time is more than a few hours, freeze the samples of water, silage, fresh grass or green chop to minimize bacterial action. Otherwise, test results will be lower than in the water or feed offered to livestock.

Chemical preservatives such as mineral acids, chloroform, toluene, etc., should not be added unless they have been cleared by the laboratory doing the analysis.

Related MU Extension publications

- G9800, Nitrate Problems in Livestock Feed and Water
  http://extension.missouri.edu/p/G9800
- G9802, Terminology of Reporting Nitrate Concentration
  http://extension.missouri.edu/p/G9802
- G9808, Nitrate and Water
  http://extension.missouri.edu/p/G9808
- WQ103, Nitrate in Drinking Water
  http://extension.missouri.edu/p/WQ103

Order publications online at http://extension.missouri.edu/explore/shop/ or call toll-free 800-292-0969.

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