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MONTANA STATE UNIVERSITY EXTENSION

MontGuide

# Forage Nitrate Analysis: What Method to Use?

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**Utilizing tests to determine nitrate accumulation in forage is an important tool for producers. This publication outlines the protocols and efficacy of the Nitrate QuikTest, Nitrate Strip Test, and Commercial Laboratory Analysis.**

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**ALL RANCHES RELY ON FORAGES SUCH AS** native range, introduced pasture, or hay to feed their livestock. Many livestock enterprises in Montana use a combination of several types of forages, including some that have the potential to contain toxic levels of nitrates ($NO_{3-}$). Nitrate toxicity associated with feeding forages reduces an animal's ability to transport oxygen in the blood, similar to how $NO_{3-}$ in well water can affect human infants, and can have major implications on livestock production. Animals subject to chronic $NO_{3-}$ toxicity due to sustained moderate levels of $NO_{3-}$ in feed exhibit reductions in appetite, reproduction, and productivity. Acute $NO_{3-}$ toxicity, which is characterized by animals consuming forages with toxic levels of $NO_{3-}$ in a short amount of time, can be fatal. Ruminants like cattle, sheep, and goats are more prone to $NO_{3-}$ toxicity than non-ruminants such as horses and pigs.

Annual cereal forages such as wheat, barley, and oats, are prone to accumulating $NO_{3-}$ that can harm livestock and ranch profits. Other species, including some grasses, sorghum, corn, millet, sweet clover, and alfalfa; and weeds such as kochia, lambsquarter, and pigweed, can also accumulate $NO_{3-}$. Beyond species, there are many factors that are implicated in toxicity, including: environment, water availability, forage maturity, herbicide, and fertilizer use, among others. This document will discuss various methods of testing forages for potential $NO_{3-}$ toxicity. For more information regarding $NO_{3-}$ toxicity in animals, please refer to the MSU Extension Montguide *Nitrate Toxicity of Montana Forages* (MT200205AG).

## Recommended Nitrate Levels

Nitrate levels in plants fluctuate depending on several factors including environmental conditions such as drought, hail, or frost; producer management techniques, including manure or fertilizer application; time of day at harvest; and forage species. Variation in plant $NO_{3-}$ levels has led to the development of field tests that can be used to measure $NO_{3-}$ levels in forage. These tests can be used prior to harvest to help producers reduce their chance of harvesting and processing forages that contain toxic levels of $NO_{3-}$, particularly important as levels do not decrease after harvest. Nitrate tests are also valuable for use post-harvest to adjust feed rations based on $NO_{3-}$ levels. Additionally, some of these tests can be used to test for $NO_{3-}$ in water. Water can have high $NO_{3-}$ content in some wells and ditches, which can be a significant $NO_{3-}$ source to livestock. It is important to account for both feed and water $NO_{3-}$ content when feeding forages to livestock. Recommended $NO_{3-}$ levels while feeding livestock are described in Table 1.

**TABLE 1.** Recommended Nitrate Levels for Feeding Livestock, from *Nitrate Toxicity of Montana Forages,* MT 200205AG.

| Nitrate (ppm) | <1500 | 1500-5000 | 5000-10,000 | >10,000 |
|---|---|---|---|---|
| Recommendation for feeding | Generally considered safe for all livestock. | Limit to 50% for calves, pregnant, or lactating animals. | Limit to 25-50% feed. Do not feed to pregnant animals. | Do not feed as is. Can cause animal mortalities. |

## Testing Methods

The ability to rapidly and accurately test $NO_{3-}$ levels in annual forages is important for producers in Montana. Currently, many producers test $NO_{3-}$ levels using the Nitrate QuikTest (Figure 1). The Nitrate QuikTest is a qualitative method developed in the 1960s that detects the presence of $NO_{3-}$ with a change in color of the testing solution, which consists of diphenylamine in 82% sulfuric acid. This test must be handled with caution and can only be accessed by trained and certified personnel, such as your local Extension Agent. The test is administered by first splitting the stem of a plant longitudinally and then dropping 1-2 drops of QuikTest solution onto the lower nodes, where plants tend to accumulate the highest levels of $NO_{3-}$. If the plant contains a detectable amount of $NO_{3-}$-, the solution will turn dark blue or black in color (Figure 2). While the test is simple to use, it does not provide a clear, quantitative measure of $NO_{3-}$ level, which is needed to determine whether a forage is safe to feed to livestock (Table 1).

The most accurate method for quantitative $NO_{3-}$ detection is laboratory analysis. Samples that are sent to the lab are tested using wet chemistry methods that have been approved by the Association of Official Analytical Chemists. Table 2 (page 3) is a laboratory analysis of barley hay. The analysis in Table 3 reports $NO_{3-}$ as a percentage; however, each lab is different and may choose to report nitrate levels as $NO_{3-}$, nitrate nitrogen ($NO_{3-}N$), or as a percentage. Conversion factors can be found in the MontGuide *Nitrate Toxicity of Montana Forages* (MT200205AG).

Forages vary in moisture contents, so it is important to use dry weight/dry matter $NO_{3-}$ values when developing rations to feed to livestock. This allows comparisons to be made between forages of different moisture content and reflects actual amounts of feed ingredients excluding water content, which can dilute ingredient values. In Table 3, the $NO_{3-}$ content is 0.46% on a dry weight basis, which is equivalent to 4600 ppm $NO_{3-}$ (% $NO_{3-}$ × 10,000 = ppm). Based on the results of this lab analysis, consumption of this barley hay must be limited to below 50% of feed ration for calves and pregnant or lactating animals (Table 1).

Although laboratory analysis is the most reliable method available, it is also time consuming and costly, with a turn-around time from two days to several weeks. For this reason, other quantitative $NO_{3-}$ detection tests, such as the Nitrate Strip Test (Figure 3, page 3), are now commercially available. The Nitrate Strip Test consists of a reducing agent, an acidic buffer, and chemical compounds that interact and produce a red-violet dye. When a reaction occurs, $NO_{3-}$ levels can be measured semi-quantitatively by visually comparing the reaction zone of the test strip (Figure 4, page 3) to a color scale (Figure 3) representing different categories of $NO_{3-}$ levels.


**FIGURE 1.** Nitrate QuikTest

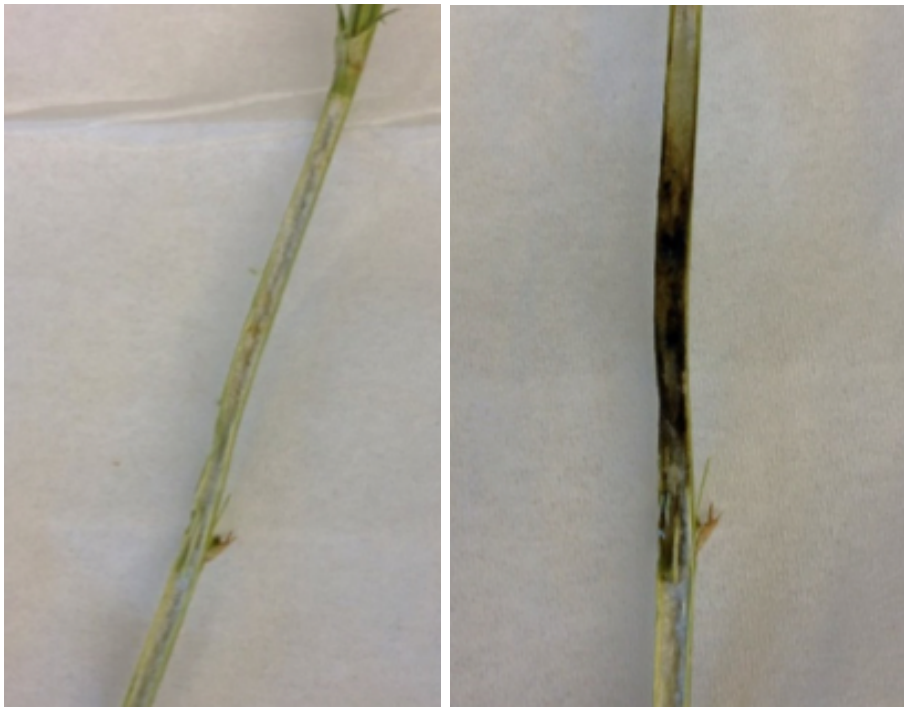


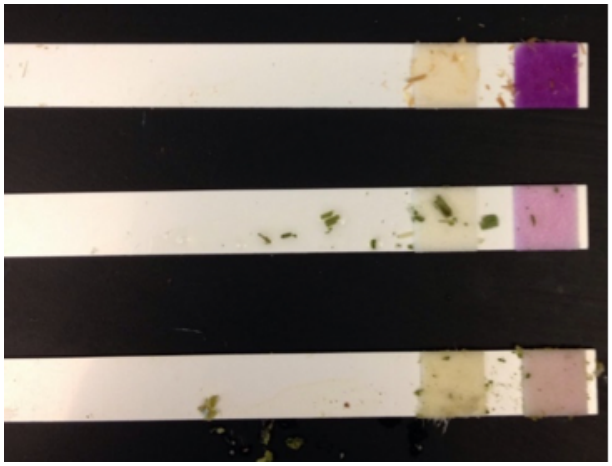
**FIGURE 2.** *Left:* Plant stem prior to QuikTest. *Right:* Plant stem after QuikTest. The black color indicates the presence of nitrate in this plant.

**TABLE 2.** Laboratory analysis of barley hay in Montana

| Analysis | Level Found As Received | Level Found Dry Weight | Units | Reporting Limit | Method |
|---|---|---|---|---|---|
| Sample ID: **HBH BARLEY HAY** | Lab Number: **12569727** | | | | |
| Moisture | 14.19 | /////// | % | 0.01 | AOAC 930.15 * |
| Dry matter | 85.81 | /////// | % | 0.010 | Calculation * |
| Protein (crude) | 9.95 | 11.6 | % | 0.20 | AOAC 990.03 * |
| Fiber (acid detergent) | 32.5 | 37.9 | % | 0.5 | ANKOM Tech. Method * |
| Fiber (neutral detergent) | 52.7 | 61.4 | % | 1.0 | ANKOM Tech. Method * |
| Total digestible nutrients | 50.9 | 59.3 | % | 0.1 | Calculation * |
| Net energy (lactation) | 0.52 | 0.61 | Mcal/lbs | 0.01 | Calculation * |
| Net energy (maint.) | 0.50 | 0.58 | Mcal/lbs | 0.01 | Calculation * |
| Net energy (gain) | 0.30 | 0.35 | Mcal/lbs | 0.01 | Calculation * |
| Relative Feed Value | | 90 | | 0.0 | Calculation * |
| Nitrate (NO3) | 0.40 | 0.46 | % | 0.02 | EPA 353.2 * |

**FIGURE 3.** Nitrate Strip Test


**FIGURE 4.** Strips that have been used to test plants for nitrates



| Species | Nitrate (mg/L $NO_{3-}$) | Feeding Recommendation |
|---|---|---|
| Barley | 50,000 | DO NOT feed to any livestock |
| Corn | 10,000 | DO NOT feed to pregnant livestock |
| Alfalfa | 1,000 | Safe to feed to livestock |

**TABLE 3**. Nitrate Strip Test Reading and Corresponding Forage Nitrate Content

| Test Strip Reading (mg/L $NO_{3-}$) | Forage Nitrate (mg/L $NO_{3-}$) |
|---|---|
| 0 | <1,000 |
| 10 | 1,000 |
| 25 | 2,500 |
| 50 | 5,000 |
| 100 | 10,000 |
| 250 | 25,000 |
| 500 | 50,000 |

Instructions for the Nitrate Strip Test vary depending on the brand selected for use. Generally, forages must be dried and ground prior to testing. These two steps can be done at home using a microwave to dry the forage and either a blender or a coffee grinder to grind the forage. After collecting a fresh forage sample, cut the sample into 1-2 inch lengths using scissors. Spread the cut samples in a single layer onto a microwave dish and microwave on high setting using 30-second intervals or until dry. Be careful not to heat the samples rapidly, or for extended periods of time, to avoid charring. Place the dried sample into a coffee grinder or blender and grind until the particle size of the dried forage resembles that of salt or sugar. Then, combine the ground sample with low $NO_{3-}$ tap water and allow it to soak for about 30 minutes. After 30 minutes, dip the Nitrate Strip Test strip into the forage-water mixture for 2 seconds, remove, and allow to react for 1 minute. After 1 minute, the strip can be compared to the color scale provided on the testing kit to determine the semi-quantitative $NO_{3-}$ concentration in the forage being tested (Figures 3 and 4; Table 3).

Regardless of the testing method used to determine $NO_{3-}$ in forage, it is important to obtain a representative sample for analysis. Samples are considered adequate for analysis when they represent the variations within a pasture or hay stack as possible. The best way to achieve an adequate sample is to walk in a zig-zag pattern and select clippings randomly throughout the pasture, or to sample several bales throughout the stack. The number of samples should increase with increasing pasture size or number of bales to achieve a representative sample for the entire pasture. Collected samples should be mixed and either dried, or frozen, prior to shipping to the lab. For more information on collecting a representative forage feed sample for analysis refer to the MontGuide *Collecting a Forage or Feed Sample for Analysis* (MT201610AG).

## Montana Research

In order to provide more accurate and expedited results to producers, the three most commonly used $NO_{3-}$ detection tests: Nitrate QuikTest, Nitrate Strip Test, and Nitrate Laboratory Analysis were analyzed. Table 4 describes each test, reports whether the test is quantitative or qualitative, and describes the turnaround time, price, and training necessary for each test. Laboratory analysis is considered to be the "gold standard" for testing because it is the most accurate method for $NO_{3-}$ determination. Lab analysis directly measures the $NO_{3-}$ concentration contained within the sample via wet chemistry methods. For this reason, both the Strip Test and QuikTest are compared to lab analysis to determine accuracy.

**Terminology for this study**

False negative: results that came back negative using the QuikTest or the Strip Test, but were positive on the commercial lab results (>1,500 ppm $NO_{3-}$)

False positive: results that came back as positive using the QuikTest or Strip Ttest, but were negative on the commercial lab results.

**TABLE 4.** Comparison of nitrate detection tests available for use by producers.

| Test | Description | Quantitative or Qualitative | Turnaround time | Price | Training |
|---|---|---|---|---|---|
| QuikTest | Rapidly detects the presence of $NO_{3-}$ in a forage sample on site | Qualitative | Immediate | Free through your local MSU Extension agent | Requires personnel training and certification for use |
| Strip Test | Rapidly analyzes a semi-quantitative amount of $NO_{3-}$ in a forage sample on site | Semi-quantitative | Immediate | Free through some local MSU Extension agents or $8-$80 online, depending on package size | Does not require training or certification |
| Lab Analysis | Detects a quantitative amount of $NO_{3-}$ in a forage sample | Quantitative | 2-3 business days | $16-$21/sample | Samples are sent to trained personnel |

**FIGURE 5.** Compares the results of samples analyzed using the Strip Test and commercial lab analysis for various forages grown in Montana.

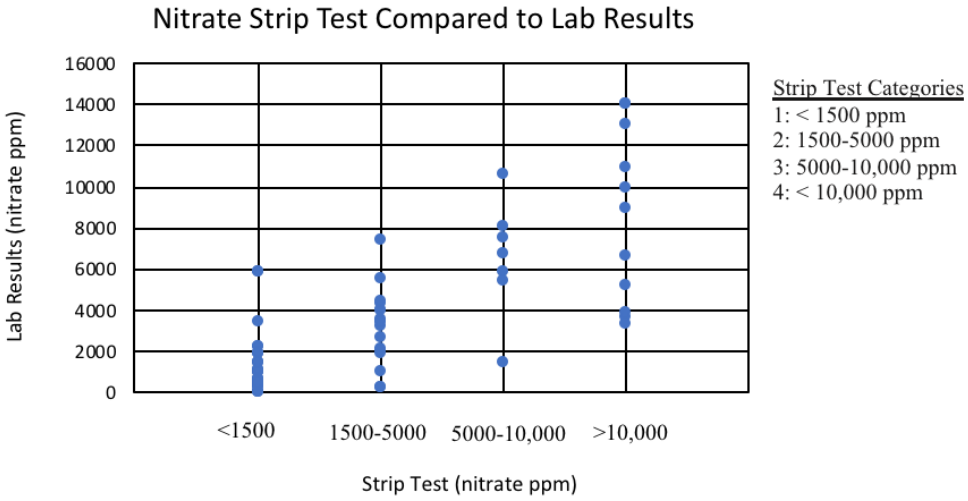



**FIGURE 6.** Compares results from the Nitrate QuikTest with commercial lab analysis for various forages grown in Montana. ***Yes*** using the QuikTest indicates a positive result, and the presence of nitrates, while ***No*** indicates a negative result, and lack of nitrates present.

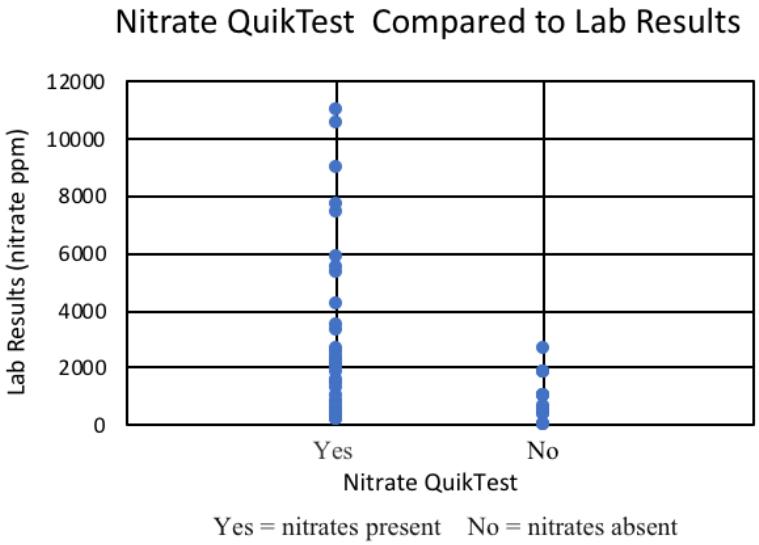



**TABLE 5.** A comparison of the accuracy of results of the Strip Test and the QuikTest compared to the laboratory analysis. A false positive is reported as the individual test estimating the sample to have higher levels of $NO_3$. compared to the laboratory analysis. A false negative is reported as the individual test having lower $NO_3$. levels compared to the laboratory analysis.

| | | Strip Test | QuikTest |
|---|---|---|---|
| | | Sample Number (Percent of Total Samples) | |
| **Correct** | | 53 (73%) | 44 (69%) |
| **Incorrect** | *False +* | 9 (12%) | 0 |
| | *False -* | 11 (15%) | 20 (31%) |
| **Total** | | 73 | 64 |

A two-year study in Montana, conducted by Meccage et al., evaluated $NO_{3-}$ levels using both the QuikTest and Strip Test, and compared the results to lab analyses. The purpose of the study was to determine the validity of using the Strip Test and QuikTest to detect $NO_{3-}$ in a production setting, and to evaluate the accuracy of the Strip Test in detecting quantitative $NO_{3-}$ levels. Data for the study was compiled using samples taken by Montana State University Extension agents across the state. The data consisted of $NO_{3-}$ detection through the use of the Nitrate QuikTest, Nitrate Strip Test, and commercial laboratory analysis for 16 classes of forages in 14 counties. Each forage sample was tested using all three $NO_{3-}$ detection tests so comparisons could be made.

## Effectiveness of Individual Tests

### *Strip Test*

The Strip Test offers producers the opportunity to obtain a semi-quantitative measure of $NO_{3-}$ accumulation in forage. Meccage et al. found that the Strip Test was a reasonably reliable method for quantitative $NO_{3-}$ detection, with an accurate result compared to the commercial analysis 73% of the time (Table 5, page 5). Of the 27% inaccurate estimates, 45% overestimated $NO_{3-}$ levels compared to the commercial lab analysis and the remaining 55% underestimated $NO_{3-}$ levels compared to the commercial lab analysis. While this can still potentially lead to the feeding of forages with toxic levels of $NO_{3-}$, the Strip Test was found to be more accurate than the Quiktest.

There were four different categories of $NO_{3-}$ accumulation listed by the manufacturer for the Strip Test used in this study: <1500, 1500-5000, 5000-10,000, and >10,000 ppm $NO_{3-}$ (Figure 5, page 5). Based on these results, it is recommended that producers are still cautious when using the Strip Test to detect quantitative $NO_{3-}$ values because it was only accurate 73% of the time. To minimize the chance of achieving inaccurate results, it is especially important to obtain a representative sample, and samples that test anywhere near 1500 ppm for $NO_{3-}$ need to be sent to a commercial laboratory for analysis prior to being fed to livestock.

### *QuikTest*

The Nitrate QuikTest offers producers the opportunity to obtain a qualitative measure of $NO_{3-}$ accumulation in forage, however, this method was found to be fairly inaccurate. When Meccage et al. compared QuikTest results to lab analysis, they found that the two were not positively correlated (Figure 6, page 5). In this study, the QuikTest commonly reported false negative results, however they did not find any false positive results (Table 4). The QuikTest had the same results as the commercial analysis 69% of the time (Table 4). Of the 31% inaccurate samples, 87% of those were false negatives. False negative/positive results can cause unnecessary delays in harvest and can be dangerous for producers, as the QuikTest does not provide quantitative values. Additionally, false negatives can lead to feeding of potentially dangerous feedstuffs.

## Summary

Nitrates in plants fed as processed feed, and as forage, are a concern for livestock producers. Quick, accurate methods of quantitative $NO_{3-}$ detection are crucial to minimize delays in harvest and for turning animals onto $NO_{3-}$ containing forages. The Nitrate QuikTest and Nitrate Strip Test are two cost-effective methods in which producers can quickly and easily test for the presence of $NO_{3-}$. The Nitrate QuikTest is qualitative and is only useful in detecting the presence of $NO_{3-}$ in plants. The Nitrate Strip Test is semi-quantitative and is limited to categorizing plants into a general range of $NO_{3-}$ content. Both of these tests are useful for preliminary $NO_{3-}$ detection; however, research indicates that these tests can give misleading and inaccurate results. Research in Montana indicates that the Nitrate QuikTest is only 38% accurate in detecting the presence of $NO_{3-}$ , and the test tends to detect false negatives more often than it detects false positives, which is dangerous for livestock. The same research indicates that the Nitrate Strip Test is a reasonably reliable method for quantitative $NO_{3-}$ detection, correctly identifying the nitrate levels almost 80% of the time, however, this test did lead to more false negatives which underestimate $NO_{3-}$ levels in plants. Caution must be exhibited when utilizing these two tests to detect $NO_{3-}$ in forages that are meant to be consumed by livestock.

Laboratory analysis is still the most reliable method for quantitative $NO_{3-}$ detection, and although sending samples to the lab is the most time consuming, it is the only way to obtain the actual numerical $NO_{3-}$ content of a plant. The most important aspect of sampling for $NO_{3-}$, regardless of the detection test being used, is obtaining a representative sample. Failure to use a representative sample for analysis will increase the chance of inaccurate results and can be dangerous for livestock.

## Resources

### *Commercial Laboratory Analysis*

Information regarding commercial laboratories can be found by contacting a local MSU Extension agent, Extension Forage Specialist, Certified Crop Advisor, or through individual laboratory websites. A complete list of certified laboratories can be found on the MSU Extension Forages Website. Generally, lab protocol will require producers to acquire a representative sample to submit for analysis.

### *Nitrate QuikTest*

The Nitrate QuikTest is only available for use by trained and certified personnel. Contact your local MSU Extension agent to access this test or to become certified to use this test.

### *Nitrate Strip Test*

The Nitrate Strip Test has no restrictions and is available for use by any personnel. Contact your local MSU Extension agent to access this test.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