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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, especially young livestock, 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 and/or water 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, brassicas, millet, sweet clover, 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: 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 estimate NO_{3-}- levels in forage. These tests can be used prior to harvest to help producers reduce the risk of harvesting and feeding forages that contain toxic levels of NO_{3-}; this is particularly important as levels do not decrease after harvest. Nitrate tests are also valuable for use post-harvest to adjust feed rations based on NO3- levels. Additionally, some of these tests can be used to test for NO_{3-} in water. Water can have high NO3- content in some wells and ditches, which can be a significant NO3- 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 NO_{3-})	<1,500	1,500-5,000	5,000-10,000	>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 placing 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 purple 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 2 reports NO_{3-} as a percentage; however, each lab is different and may choose to report nitrate levels as parts per million (ppm) NO_{3-}, ppm nitrate as 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 2, 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 being considered. 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

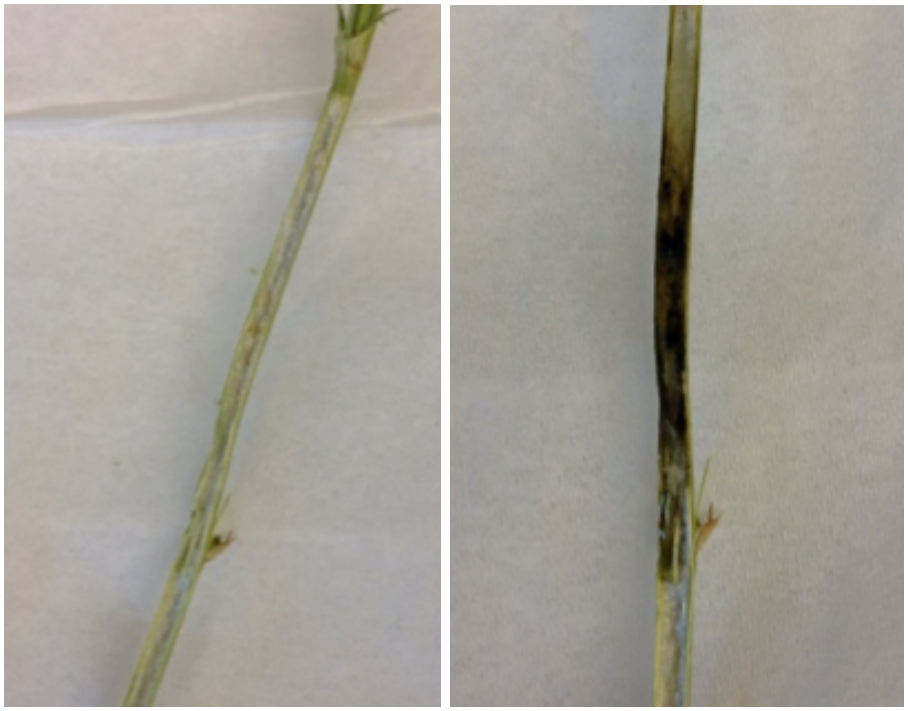


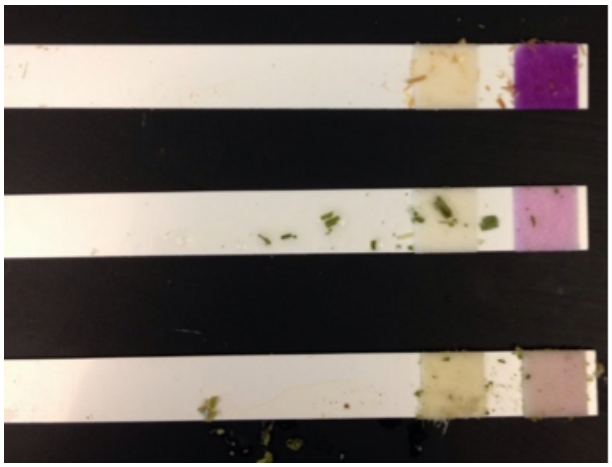
FIGURE 2. *Left:* Plant stem prior to QuikTest. *Right:* Plant stem after QuikTest. The dark blue color indicates the presence of nitrate in this plant.

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/kg 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 or distilled water and allow it to soak for about 30 minutes, usually at a ratio of 100 parts water to 1 part forage. Always refer to your protocol for correct amounts. 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 two quantitative methods for Nitrate detection, the Nitrate QuikTest and the Nitrate Strip Test, were compared to a Nitrate Laboratory Analysis in order to determine test accuracy. See Table 4 for a description of all three analyses. 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.

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	2-5 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.

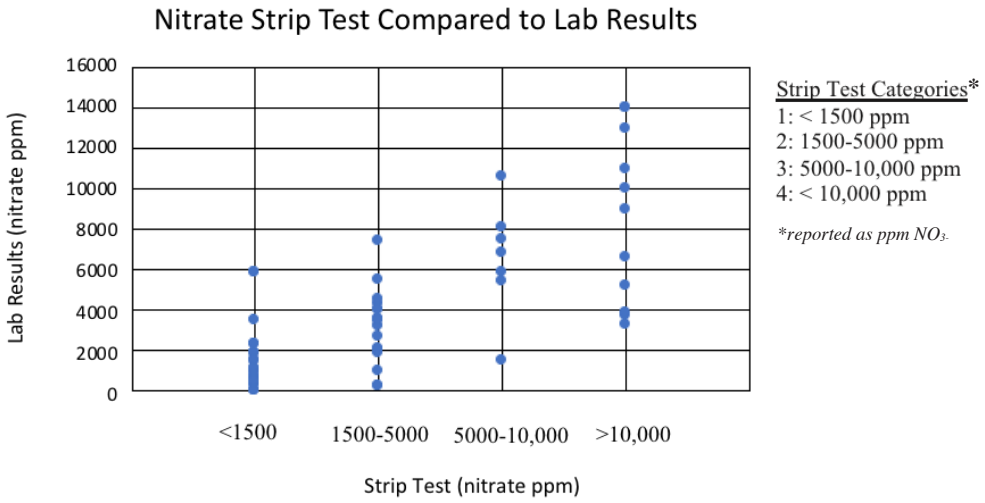


FIGURE 6. Compares results from the Nitrate QuikTest with commercial lab analysis for various forages grown in Montana. ***Yes*** using the QuikTest indicates a positive result, and the presence of nitrates, while ***No*** indicates a negative result, and lack of nitrates present.

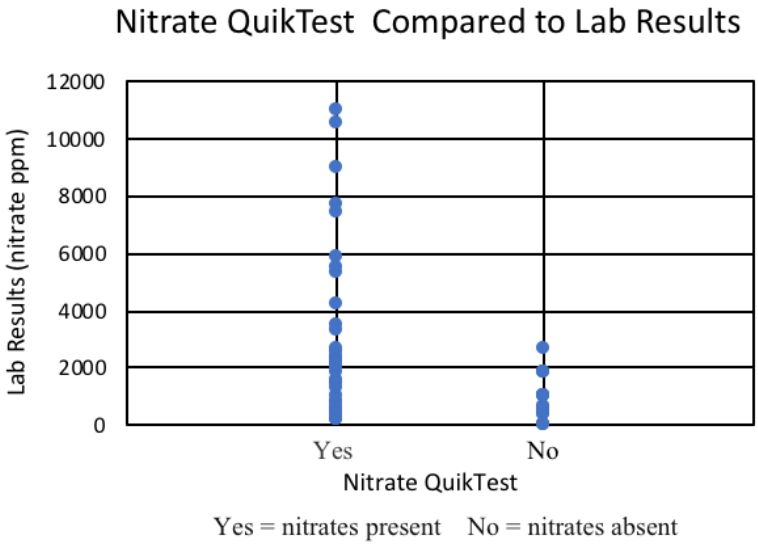


TABLE 5. A comparison of the accuracy of results of the Strip Test and the QuikTest compared to the laboratory analysis. A false positive means the individual test has higher levels of NO_{3-} than the laboratory analysis. A false negative means the individual test has lower NO_{3-} levels than the laboratory analysis.

		Strip Test	QuikTest
		Sample Number (Percent of Total Samples)	
Correct		54 (71%)	45 (71%)
Incorrect	*False +*	10 (13%)	15 (23%)
	False -	12 (16%)	4 (6%)
Total		76	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 forage classes in 14 counties.

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. Nitrate results were then placed into four different categories: Category 1: <1500, Category 2: 1500-5000, Category 3: 5000-10,000, and Category 4: >10,000 ppm NO_{3-} (Figure 5, page 5). This study found that the Strip Test was a reasonably reliable method for quantitative NO_{3-} detection, with an accurate result compared to the commercial analysis 71% of the time (Table 5, page 5). Of the 29% 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. When evaluating the incorrect results, about 73% of them were only off in estimation by one category, but 6 of the 22 incorrect samples were off by at least 2 categories. It is also important to note, that when the strip test yielded a false negative, the results were still always below 5,000 ppm, meaning they would have still been considered "safe" for non-pregnant animals. Caution should be used when feeding pregnant animals, but for open livestock, this test could be a reliable option.

Based on these results, it is recommended that producers are cautious when using the Strip Test to detect quantitative NO_{3-} values because it was only accurate 71% 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, and was found to be similar in accuracy compared to the Strip Test. In this study, the QuikTest reported correct results 71% of the time compared to commercial quantitative analysis (Table 5). Of the total samples, 23% of the samples were false positives, while only 6% were false negatives. This is in accordance with reports from producers who have stated that they commonly have to delay harvest due to QuikTest results, but receive "safe" results after submitting for commercial analysis.

It should be pointed out that of the 4 false negatives, 3 of those samples had a nitrate level around 1900 ppm, which while incorrect and reported as a false negative, is quite close to the recommended threshold safe level for feeding. It is also worth noting that even with the false negative samples reported by the QuikTest, all of those samples were still below 5,000 ppm, and would have been safe to feed to non-pregnant livestock, similar to the Strip Test.

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. This research indicates that both of the tests are similar in accuracy, correctly estimating nitrate levels about 70% of the time when compared to a commercial analysis. Of the inaccuracies, the Strip Test was almost equal in reporting false positives versus false negatives, while the QuikTest reported false positives much more commonly. These can both provide a reasonable estimate of assessing nitrate risk, but they cannot be relied upon to give accurate results 100% of the time. There is a benefit to using the Strip Test in that it is a semi-quantitative estimate, and many of the

samples that were marked as incorrect still actually detected levels of nitrate, they just did not indicate the appropriate category, or the interpretation of the color change was incorrect. The benefit of the QuikTest is that it much more commonly provided false positives compared to false negatives, decreasing risk of feeding unsafe feedstuffs, however both tests still have potential for inaccurate interpretation.

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 Extension agent, Extension Forage Specialist, Certified Crop Adviser, 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 Extension agent to access this test or to become certified to use this test.

Nitrate Strip Test

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

**The means reported in this publication are least squares means. Least squares means are means that have been adjusted for other terms in a model (county, year, treatment).*

Terminology for this study

False negative: results that indicated lower levels of nitrates using the qualitative test compared to quantitative testing. For the QuikTest, this means there were no color changes, but were positive quantitative results. For the Strip Test, this means that the test estimated the nitrates were in a lower category than the quantitative test.

For example, the Strip Test may indicate that a sample was category 2 (1500-5000ppm NO_{3-}), but if the quantitative estimated it was >5000ppm NO_{3-}, it was marked negative.

False positive: results that indicated higher levels of nitrates using the qualitative tests compared to the quantitative analysis. For the QuikTest, this means that there was a color change but no elevated levels. (<5000ppm NO_{3-}) on the quantitative test. For the strip test, this means the test rated the risk as higher than it was.

For example, the Strip Test may indicate that a sample was in a category 3 (5,000-10,000ppm NO_{3-}), but it may have actually only been in category 1 or 2 (<5,000ppm NO_{3-}) based on the quantitative test.

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