COMPARISON OF A QUALITATIVE FIELD TEST VERSUS LABORATORY ANALYSIS FOR NITRATE CONCENTRATION IN HYBRID SUDANGRASS HAYS.

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Story in Brief

A qualitative field test using 0.5% diphenylamine in concentrated sulfuric acid was compared to routine laboratory quantification of the nitrate (NO3) concentration in 1064 hybrid sudangrass hay samples. A visual scoring system was used to define the color changes of a single drop of the diphenylamine solution on the inner tissues of stems of the forage. The scores were 1 = clear, 2 = yellow, 3 = brown, 4 = light blue streaks, 5 = solid blue, and 6 = black. Diphenylamine has been reported to turn blue when it contacts nitrate in plant tissues. Color scores were assigned at 10 seconds and 20 seconds after the solution contacted the plant tissue. Two stems from each sample were tested with diphenylamine. After laboratory analysis, forage samples were grouped by assayed nitrate content into five classes: 0 - 2500 ppm; 2500 - 6000 ppm; 6000 - 10000 ppm; 10000 - 15000 ppm; and greater than 15000 ppm. The percentages turning blue of either stem sampled were 46%, 67%, 86%, 95%, and 100% for each of the classes, respectively. The correlation between the 20 second reading and the laboratory quantification was similar to the correlation of the 10 second reading and the laboratory quantification. The diphenylamine test is only a crude field test for the presence dangerous concentrations of nitrate. A higher percentage of false positives (60.6%) and a low percentage of false negatives (10.7%) indicates that the test is quite conservative.

(Key words: Qualitative Test, Nitrate, Hybrid Sudangrass.)

Introduction

Nitrate toxicity of livestock has plagued Oklahoma farmers and ranchers for years. Agricultural advisors including County Extension Agricultural

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Agents and veterinarians have used a field test to screen forage samples for dangerous concentrations of nitrates. The field test consists of placing a drop of diphenylamine in concentrated sulphuric on the inner tissues of the stems of plants. Plants that were exposed to a diphenylamine droplet and did not turn blue were assumed to be low in nitrate content. A blue or black color was assumed to represent a dangerous concentration of nitrate. Farmers or ranchers often were advised to send such samples of the forage to a commercial or university testing laboratory for more precise quantification of nitrate.

Little research has examined the error rate of the field test and to better determine the concentrations of nitrate that cause the diphenylamine to change colors. Also, time may elapse before the color change takes place. Therefore, nitrate content of numerous forage samples from three Oklahoma State University Agronomy Field Stations were examined by the diphenylamine test and then measured in the laboratory. This would allow for comparison of the diphenylamine field test with the laboratory analysis.

Materials and Methods

Forty different varieties of hybrid sudangrass being tested in 1990 for yield potential at three different agronomic experiment stations across Oklahoma were obtained. These stations are located in eastern Oklahoma at Haskell, central Oklahoma near Chickasha, and in western Oklahoma near Tipton. Two cuttings of the forages were made at the Tipton station. Each of the other stations supplied three cuttings. Each variety test was replicated four times at each location and one variety was subjected to five differing levels of nitrogen fertilization. Samples weighing approximately one pound were collected from each test plot. They were labeled and brought to Stillwater for analysis. A total of 1064 samples are included in this study.

Two stems from each sample were chosen randomly to be subjected to the diphenylamine test. A solution of 0.5% diphenylamine in concentrated sulfuric acid was stored in amber bottles with glass eye droppers. Each stem was disected longitudinally with a sharp surgical scapel. One drop of the test solution was placed in the soft, pithy, inner tissue of the stem. A color score was recorded at 10 seconds and again 20 seconds after the drop contacted with the stem. Scores were recorded as: 1 = clear;

2 = yellow;

3 = brown;

4 = light blue streaks;

5 = solid blue; and

6 = black.

After the samples had the two stems removed and tested, the remainder of the forage sample (including both stems and leaves) was analyzed for nitrate concentration using the salicylic acid method described by Cataldo et al.(1975). This procedure is used by the Oklahoma Animal Disease Diagnostic Laboratory to quantify nitrate concentrations in feedstuffs. The percentage dry matter was determined on each sample by using the mean of two duplicate estimates of dry matter. Nitrate concentration was calculated to parts per million (ppm) nitrate based upon a 100% dry matter forage. Nitrate concentration of two duplicate samples was within 1000 ppm or the samples were re-assayed until two duplicates were within 1000 ppm.

After laboratory analysis, the samples were categorized into one of five different groups based on nitrate concentrations. The first level included samples between 0 and 2500 ppm. Feeds with less than 2500 ppm are safe for all ruminants. The second level was 2500 to 6000 ppm. The third level of nitrate concentration consisted of samples between 6000 and 10000 ppm. Toxicologists at the Oklahoma Animal Disease Diagnostic Laboratory indicate that feeds above 6000 ppm need to fed with caution to pregnant livestock and may reduce milk production in lactating cows. At 10000 ppm, forage is potentially lethal, if animals are fed a larg. amount of the forage and are not acclimated to the nitrate. Samples in the fourth range, 10000 to 15000 ppm, can be utilized only as a fraction of the diet so as to dilute the concentration of nitrate in the total diet. Finally, the fifth category, above 15000 ppm, includes forages that must be fed only as a small part of the total diet or must be discarded because of their extremely dangerous concentration of nitrate. By comparing the various percentages of samples turning colors upon the presence of diphenylamine, the usefulness of the field test should be more apparent.

Results and Discussion

Nearly one-third of the samples tested had greater than 6000 ppm nitrate. More than 10% of the samples had in excess of 10000 ppm nitrate (Table 1.). This means that producers must be cautious about feeding hybrid sudangrass hays and adequate on-farm and laboratory testing procedures are necessary to identify potentially dangerous forages.

The diphenylamine procedure of testing forages proved to be very conservative. Of those samples that eventually were estimated by laboratory analysis to have less than 2500 ppm, 45.7% had one or both stems giving a blue color. All of these are false positives, and would have raised concern about nitrates when in fact the nitrate concentration was of little concern. Samples with very high concentrations of nitrate gave very few false negatives. Only 5.1% of those samples between 10000 ppm and 15000 ppm and 0% of

Table 1. Nitrate content and percentage of 1064 samples giving positive (blue color) reaction when exposed to diphenylamine for 20 seconds, and least squares means of color scores.

	concentrations of nitrate (ppm)				
	0-2500	2500-6000	6000-10000	10000-15000	>15000
Number of samples	208	510	238	79	29
Percentage of 1064 samples	19.5	47.9	22.4	7.4	2.7
Percentage of samples one or two stems positive	45.7	66.7	86.1	94.9	100.0
Percentage of samples both stems positive	24.0	43.7	70.6	86.1	93.1
Mean color score					
Stem 1	3.0 ± 0.1^{a}	$3.6 \pm .06^{b}$	$4.5 \pm .09^{c}$	$4.9 \pm .16^{d}$	$5.3 \pm .27^{d}$
Stem 2	3.0 ± 0.1^{a}	$3.7 \pm .06^{b}$	$4.4 \pm .09^{c}$	$4.9 \pm .16^{d}$	$5.5 \pm .27^{d}$

[abcd] Means with differing superscripts are different ($P < .05$)

the samples greater than 15000 ppm were false negatives. All of the very dangerous samples had at least one of two stems that turned blue. However, the 5.1% of samples that contained between 10000 ppm and 15000 ppm and did not react to the diphenylamine raises concern. This data confirms that testing just one stem from a one pound sample of forage does not give as accurate an assessment of the nitrate content as does testing multiple stems.

The mean color score for each of the nitrate levels is presented in Table 1. Samples with more than 15000 ppm were not statistically different in the mean color score when compared to samples in the 10000 - 15000 ppm category. The other categories were statistically different in mean color scores. For both classifications less than 6000 ppm, mean color score was less than 4; thus indicating that the "average" stems in those groups did not turn blue. The mean color score for all groups with greater than 6000 ppm were greater than 4.0 which implied the "average" stem in those groups turned blue when exposed to diphenylamine. A large percentage (60.6%) of false positives (table 2) means that many "safe" samples are sent to a commercial or university laboratory for quantification. The small percentage (10.7%) of "potentially dangerous" samples that did not turn blue on either stem tested must be noted. The diphenylamine field test kit is not a perfect screening method to find forages with high nitrate concentration.

The correlations among the nitrate concentrations and the mean of the color scores for one stem or the mean of two stems at 10 or 20 seconds are presented in table 3. The correlation between the readings at 10 seconds and 20 seconds is quite strong (r = .954). The correlation between nitrate concentration and the 10 second reading was similar to the correlation between nitrate and the 20 second reading. Both correlations were low (r = .38, and .39, respectively. This means that only 15% of the variation in nitrate was accounted for by color change. The correlation between the mean score of stem 1 and stem 2 was moderate (r = .703) which indicates that small variation between plants grown in close proximity does exist.

The diphenylamine field screening kit for nitrate concentration of forages is only a crude estimater of the nitrate content. The producer and his advisor must remember that many false positives will occur and a few (potentially dangerous) false negatives will occur. Testing more stems will give a more accurate description of the potential for nitrate toxicity of a given hay source.

Table 2. Percentage of samples giving blue color in one or two stems in nitrate concentration categories of less than 6000ppm or greater than 6000ppm.

	Concentration of nitrate (ppm)	
	0-6000	>6000ppm
Both stems not turning blue	39.4%	10.7% (false negatives)
Blue detected in one or both stems	60.6% (false positives)	89.3%

Table 3. Simple correlations[a] for nitrate concentration and diplenylamine color scores.

	Mean of 10 sec. color scores of 2 stems	Mean of 20 sec. color scores of 2 stems	Stem 1 color score, mean of 10 & 20 sec.	Stem 2 color score, mean of 10 & 20 sec.
Nitrate concentration	.382	.392	.363	.360
Mean of 10 sec. color scores of 2 stems		.954	.915	.908
Means of 20 sec. color scores of 2 stems			.910	.915
Stem 1 color score, mean of 10 and 20 second				.703

[a] All correlations were greater than 0 ($P < .01$)

Variation in individual plants grown in identical situations exists so even the most accurate forage testing procedure is only an estimate of the entire cutting of hay. Suspicious forage samples should be sent to a qualified laboratory for more complete nitrate quantification.

Literature Cited

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