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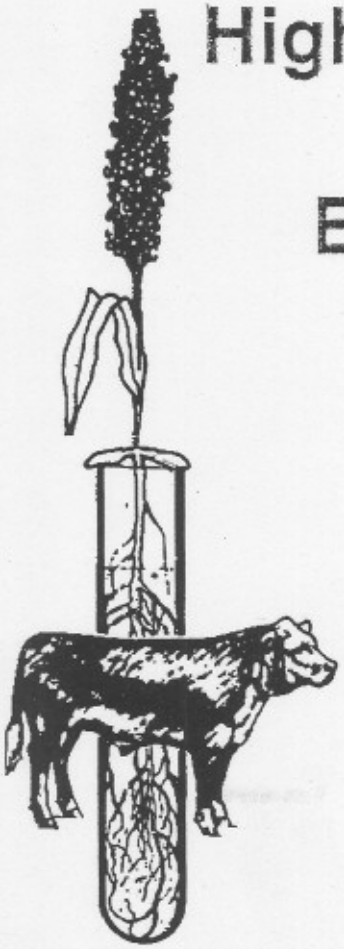
present

Management of High Nitrate Forages for Beef and Dairy Cattle



May 4, 1993
9:00 AM
Cherokee Strip
Conference Center
Enid, Oklahoma

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Plants & Growing Conditions Which Are Susceptible to High Nitrate Concentrations

Gary L. Kilgore
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Nitrate poisoning in cattle is reported to have occurred long before the use of commercial nitrogen fertilizer. Nebraska reported "cornstalk poisoning" in the late 1800's. A report of nitrate poisoning in Kansas was made in 1888. Many early day reports of nitrate poisoning was reported when cattle consumed immature oat hay, drought-damaged sorghum silage and grazing cornstalks.

Nitrate (NO_3) itself is not particularly toxic to animals. Most forages contain some nitrates. When feeds containing nitrates are consumed by ruminants, nitrates are changed in the rumen to ammonia that is, in turn, converted by bacteria in the rumen into microbial protein. Nitrite (NO_2) is one of the intermediate products in the breakdown of nitrate and is the actual cause of nitrate poisoning. If nitrate intake is faster than its conversion to ammonia, nitrites (NO_2) will begin to accumulate in the rumen. That nitrite is rapidly absorbed into the blood system where it oxidizes hemoglobin to methemoglobin. Red blood cells containing methemoglobin cannot transport oxygen and the animal dies from asphyxiation.

Table 1. Level of nitrate in forage (DM basis) and potential effect on animals.

PPM Nitrate	Effect on Animals
0 - 3,000	Virtually safe.
3,000 - 6,000	Moderately safe. Limit use for stressed animals. Don't feed dairy cows.
6,000 - 9,000	Potentially toxic. Should not be only source of feed.
9,000 and Higher	Dangerous to cattle.

Table 2. Conversion factors for expressing nitrate content of forage.

Potassium Nitrate x 0.61	= Nitrate (ppm)
Nitrate - Nitrogen x 4.42	= Nitrate (ppm)
% Nitrate x 10,000	= Nitrate (ppm)

Nitrate Accumulation in Plants

Practically all plants contain detectable amounts of nitrates. Excessive nitrate accumulation occurs when the uptake of nitrate exceeds the production of plant proteins.

Plant Factors

Plant Species. Crops such as forage sorghum, grain sorhum, sudangrass and hybrid pearl millet can accumulate high levels of nitrate. Weeds such as kochia, pigweed, lambsquarters and sunflower are routinely high in nitrate. Under certain environmental conditions, wheat, corn, oats, fescue, Johnsongrass and other plants can accumulate high levels of nitrate.

Stage of Growth. Nitrate content generally is highest in young plant growth and decreases with maturity. Sorghum and sudangrass plants can retain high levels even in mature plants. If plants are stressed at any stage they may accumulate nitrate.

Plant Parts. Nitrates usually accumulate in stalks. Highest nitrate levels occur in the lower one-third of the plant stalk. Concentrations of nitrate are low in leaves. Grain does not contain appreciable amounts of nitrate.

Environmental Factors

Drought. Nitrates accumulate in plants during time of dry weather because plant roots continually absorb nitrate but slow plant growth slows the conversion to amino acids. During severe drought, lack of moisture will prevent nitrate uptake from the soil. Following a rain, however, the roots rapidly absorb nitrate and accumulate high levels. After a drought-ending rain, it will require several days (4-8) before the nitrates will be metabolized to low levels, provided environmental conditions are good.

Sunlight. Nitrate reduction occurs in young leaves and requires light as an energy source. Shaded plants lack sufficient energy to convert nitrate to amino acids. Extended periods of cloudy weather increases nitrate content. High levels of nitrate can occur when wet, overcast days follow a period of drought.

Frost, Hail or Disease. These conditions will damage leaves and reduce photosynthetic activity. With less available energy, nitrate reduction is slowed and nitrates accumulate in the plant.

Temperature. Low temperatures (less than 55°F) retard photosynthesis of warm season plants and favor nitrate accumulation. Extremely high temperature also increases nitrate concentrations by reducing nitrate reductase enzyme activity.

Management Factors

Fertilization. Nitrogen fertilization increases soil nitrate levels and so more nitrogen is available for plant uptake. Nitrogen cannot only come from added nitrogen fertilizer but also from animal manure and previous crop legumes like alfalfa or clovers. Also, plants growing in soils low in phosphorus, potassium or pH could accumulate nitrates because of poor plant metabolism.

Herbicides. Weeds sprayed by herbicides, but not killed, can have very high nitrate levels because of depressed enzyme activity and reduced leaf area.

Harvest Technique. Forages harvested as hay, nitrate concentrations remain virtually unchanged over time. Forages made into silage normally show a reduction in nitrate levels by 40 to 60 percent. If plants are fed as greenchop, the harvested forage should be fed soon after cutting. As the plants respire, nitrates are converted to nitrites which results in toxicity. If high nitrate forage is expected, cut plant 10-12 inches above the soil. Considerable amount of nitrate will be left behind in the lower stalk.

Nitrate Concentrations in Oklahoma Sorghum Forage Hay Crops

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The Story in Brief

Over a two year period (1990 and 1991) 2000 samples of sorghum forage hays were collected from three OSU Agronomy Research Stations. Forty varieties in 1990 and 34 varieities in 1991 were being evaluated for yield potential. Samples from the replicated plots were brought to Stillwater and analyzed forr nitrate content. Also samples from a nitrogen fertilizer rate study were analyzed so as to allow comparison of the nitrate accumulation in hays grown under different fertility regimes. A qualitative field test (diphenyalmine) was applied to each sample in 1990 and the results were compared to the laboratory chemical analyses to allow for the comparison of the field test to the laboratory nitrate quantification.

Introduction

Death loss from nitrate is an occasional problem in ruminants consuming certain annual forages, particularly sorghum hybrids. Nitrate accumulation usually results from plant stress such as drought and is accentuated by excessive soil nitrogen. Most nitrate accumulates in plant stems rather than leaves, and concentration tends to be highest in immature forage. A characteristic symptom of nitrate toxicity is a chocolate-brown color to the blood. Poisoning can be avoided with good management. Fertility programs consistent with plant needs and growing conditions minimize the problem. Potentially dangerous forage should be tested before feeding. Often hay containing excessive nitrate can be fed safely when diluted with other feed, particularly concentrates.

Annual forage crops like sorghums and small grains make valuable contributions to profitable beef production in Oklahoma. They are well adapted, very productive and provide high quality forage. Infrequently, some of these plants accumulate toxins that can result in costly livestock losses.

Nitrate is the primary nutrient form of nitrogen in most soils and is a normal constituent of plants. Normally nitrate is assimilated so rapidly following uptake from soil that its concentration in plant tissues is low. Occasionally, excessive levels occur in plants. The most notorious accumulators of nitrate in Oklahoma are the sorghums. Other annuals that less frequently accumulate nitrate are small grains (wheat, oats, rye and barley). Some perennial grasses (bermudagrass, fescue and johnsongrass) and certain weeds (pigweed, mustard, nightshade and lamb's quarters) also can contain dangerous levels.

Accumulation is usually triggered by some environmental stress, where plant growth is restricted but absorption of nitrate from soil continues. The most common stress of summer annuals is drought. Lack of moisture, together with excessive soil nitrogen for existing growing conditions, is a frequent cause of toxic levels of nitrate in sorghums. Other stress factors which favor buildup are reduced sunlight from cloudiness or shading, frost, certain herbicides including 2,4-D, acid soils, low growing temperatures, and deficiencies of essential nutrients like phosphorus and sulfur.

When more soil nitrogen is present than needed for maximum growth, some plants tend to accumulate nitrate even without environmental stress. This response is particularly true with hardy soil feeders like sorghums, noted for "luxury consumption" of certain nutrients.

When accumulation occurs, the concentration of nitrate in plant parts is greater in stems than leaves. Seeds seldom contain significant amounts. Rate of uptake diminishes with increasing maturity; thus mature plants usually contain less nitrate than immature ones. Differences in potential for accumulation exist among species and varieties.

The level of nitrate that causes toxicity in ruminants varies depending on rate of intake, diet, acclimation to nitrate and nutritional and reproductive status. As a rule, forage containing less than 6,000 ppm nitrate on a dry matter basis is safe for non-breeding cattle. Forage containing 6,000 to 10,000 ppm nitrate is considered potentially toxic when provided as the only feed. Forage containing over 10,000 ppm nitrate is considered dangerous but often can be fed safely after proper dilution with other feeds. Some diagnostic laboratories are even more conservative and suggest that 9000 ppm nitrate can be lethal.

Nitrate Accumulation Data in Sorghum Forage Types from Oklahoma

During the summer of 1990, 17 varieties of sorghum x sudan, 12 varieties of sorgo x sudan, 5 varieties of sudan x sudan hybrids, and 6 varieties of pearl millets were being grown at 3 Oklahoma State University Agronomy Experiment Stations for yield evaluations. The second year of the study was conducted in 1991 with 18 varieties of sorghum x sudan, 9 varieties of sorgo x sudan, 2 varieties of sudan x sudan hybrids and 5 pearl millets. Six varieties were present both years. Field locations were: Eastern Oklahoma Agronomy Experiment Station at Haskell, Oklahoma in Muskogee County; South-Central Oklahoma Agronomy Experiment Station in Grady County near Chickasha; and the Southwestern Oklahoma Station near Tipton in Tillman County. Four replicated plots were randomly assigned to each variety at each experiment station. Each plot was approximately 15 feet by 9 feet in size. Fertilization of the plots consisted of phosphorus and potassium according to the soil test. Nitrogen was applied in split applications of 50 pounds of actual nitrogen per acre at planting and 50 pounds of actual nitrogen top-dressed after each cutting of forage. Planting was done in late May and harvesting was initiated as plants reached the pre-boot to boot stage of seed head development. Harvesting was done by hand and plants were cut approximately 2 to 4 inches above the ground. Sun-cured hay samples (approximately 1 pound) consisting of leaves and stems were obtained from each plot. Samples were labeled and brought to Stillwater for nitrate concentration analysis and percentage dry matter determination. Duplicate nitrate analyses were made on each sample and were within 1000 ppm nitrate or the nitrate procedure was repeated. The average of the two readings was then considered the nitrate content after being adjusted to 100% dry matter.

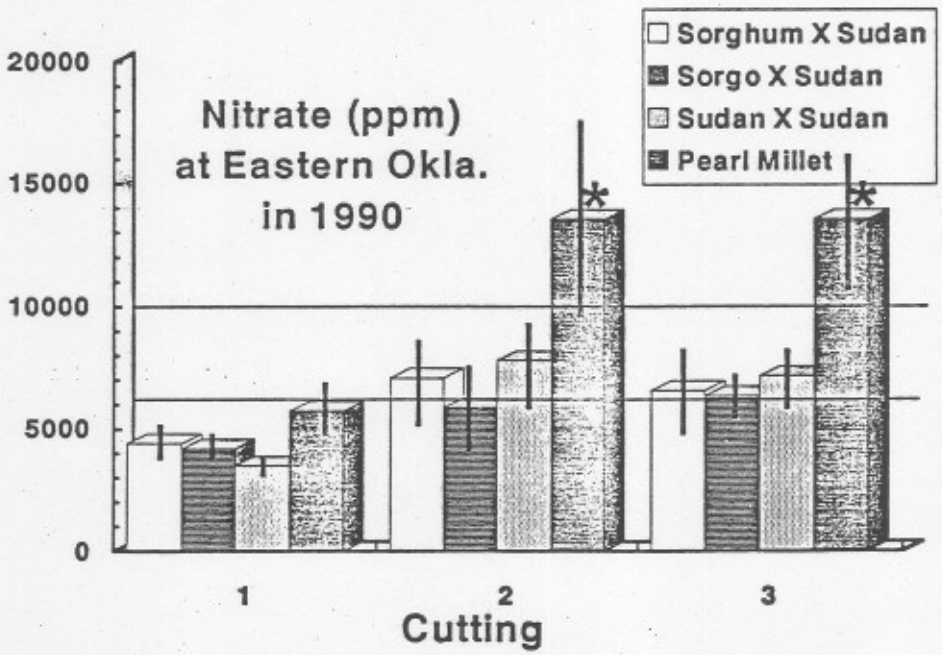
Two or three cuttings were made at each location each year. In the second year, very heavy rains at the Eastern Station forced the re-planting of the plots in late June rather than the late May plantings at the other locations. The very hot dry months of July and August then produced heat and drought stress on the plants at that location. The very high concentrations of nitrate in the first cutting of 1991 at the Eastern Station apparently were the result of those weather conditions. Data from the hay samples of the first cutting at the South-Central station in 1991 were not available.

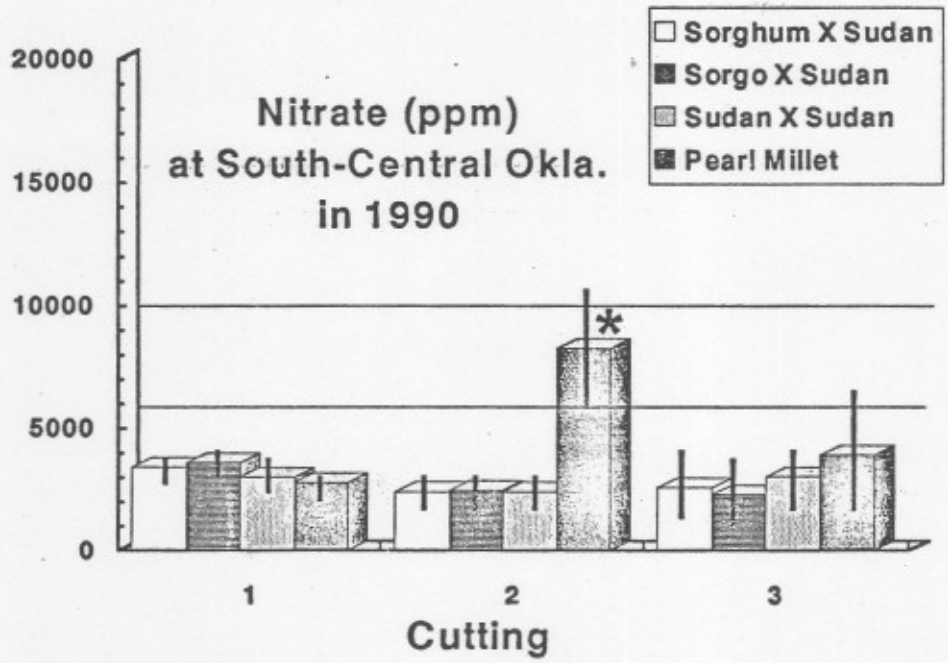
Results

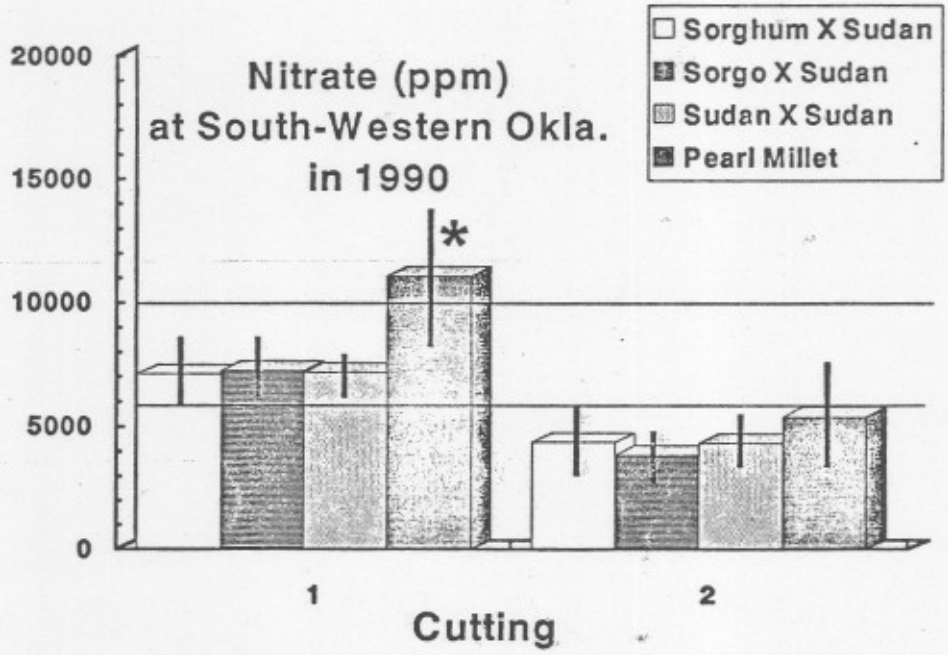
The statistical analysis of this large data set was performed by the analysis of variance procedure with differences due to variety within forage type, cutting within location and year, field station location, and year as the sources of variation studied. Remember the four forage types included in the data were 1) Sorghum x Sudan; 2) Sorgo x Sudan; 3) Sudan x Sudan; and 4) Pearl Millet. **Varieties within forage type were not different from each other,** therefore we could quickly conclude that different varieties within any of the four types accumulated nitrates similarly. However, **there were differences between the four forage types** that were worth considering.

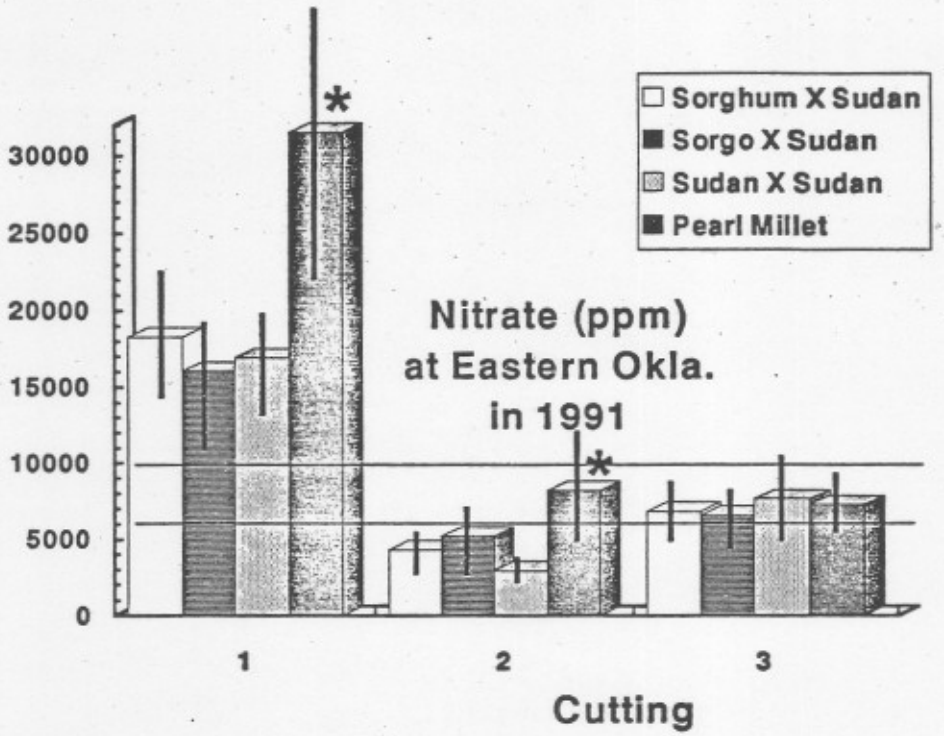
A statistically significant four way interaction meant that we should look at each cutting at each location each year to study the differences in nitrate accumulation among the four forage types.

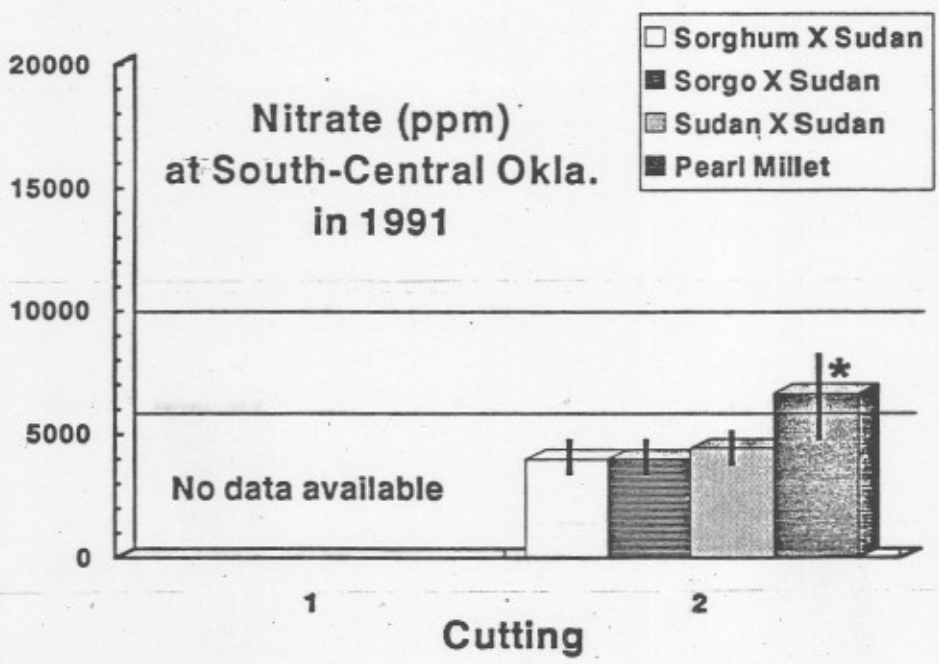
The following graphs illustrate the average concentration of nitrates in parts per million (100% dry matter) for each forage type. The black vertical lines in the middle of each column represents the standard deviation of each group of samples. The standard deviation would include about 2/3 of the samples represented by that average. This gives us a look at the variation in the samples as well as the overall average. Super imposed on each graph are horizontal lines representing 6000 ppm or 10000 ppm. Those are the biologically important concentrations of nitrate that may affect the productivity or the survivability of the animal. The asterisk above certain bars indicates that the mean of those nitrate concentrations is statistically greater than the other forage types in that cutting. The final graph presents the raw means of nitrate concentrations across all locations, cuttings and years. Remember that the interaction previously discussed tells us that the individual cuttings may or may not fit this pattern. The accompanying OSU Current Reports list the varieties, yield data, and rainfall totals by month at each of the experiment stations.

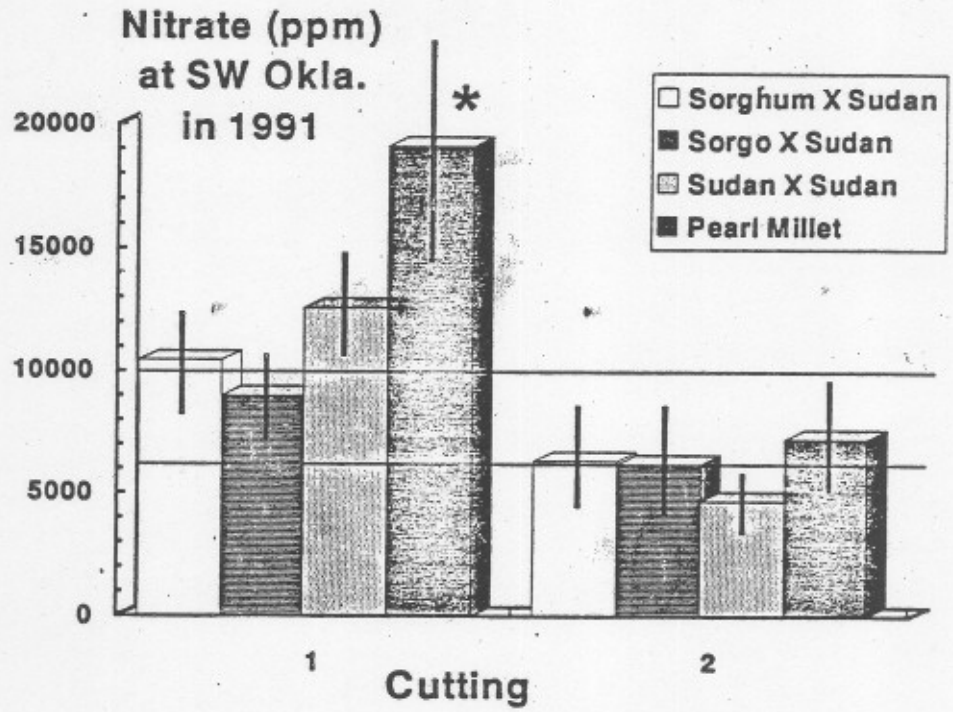


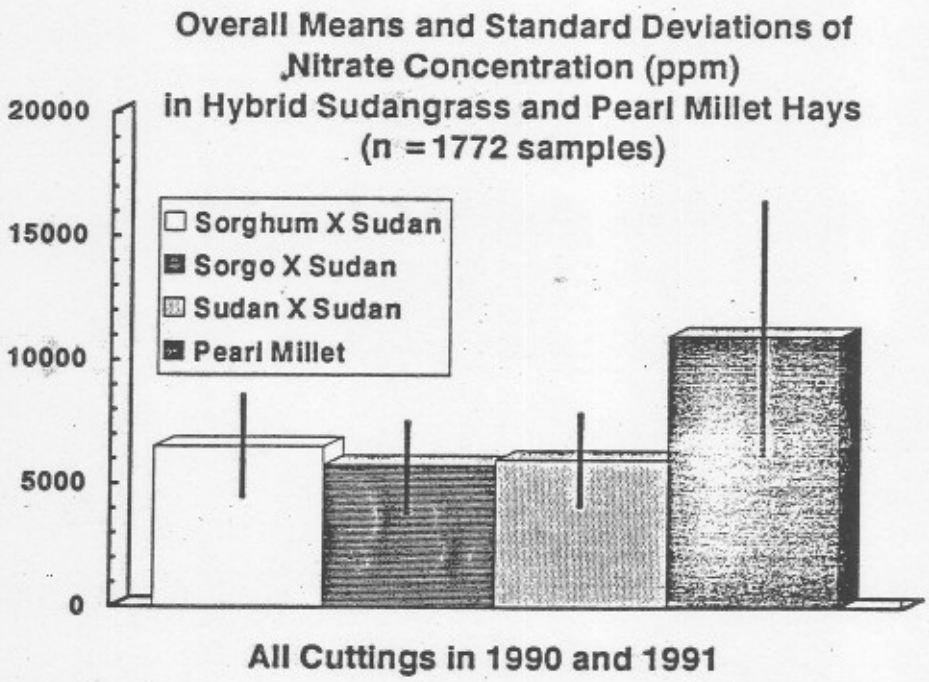

Nitrate (ppm)
at South-Central Okla.
in 1990
Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
20000
15000
10000
5000
0
*
1
2
3
Cutting


Nitrate (ppm)
at South-Western Okla.
in 1990
Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
20000
15000
10000
5000
0
*
1
2
Cutting


Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
Nitrate (ppm)
at Eastern Okla.
in 1991
30000
25000
20000
15000
10000
5000
0
*
*
1
2
3
Cutting


Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
Nitrate (ppm)
at South-Central Okla.
in 1991
20000
15000
10000
5000
0
No data available
*
1
2
Cutting


Nitrate (ppm)
at SW Okla.
in 1991
*
20000
15000
10000
5000
0
Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
1
2
Cutting


Overall Means and Standard Deviations of
Nitrate Concentration (ppm)
in Hybrid Sudangrass and Pearl Millet Hays
(n = 1772 samples)
20000
15000
10000
5000
0
Sorghum X Sudan
Sorgo X Sudan
Sudan X Sudan
Pearl Millet
All Cuttings in 1990 and 1991

Of the fourteen cuttings represented in this data, pearl millets had numerically the greatest concentrations of nitrate in 12 of the cuttings. Furthermore, pearl millets had statistically greater concentrations of nitrate in 8 of the 12 cuttings. The other hybrid crosses (sorghum x sudan, sorgo x sudan, and sudan x sudan) were very similar for nitrate accumulation. All of the forage types accumulated dangerous concentrations of nitrate when severely stressed (i.e. Eastern Oklahoma, 1991). In those cuttings where all of the forage types had an increased nitrate concentrations, pearl millets were even more prone to accumulate nitrate. However, when a suitable growing period took place and all of the forage types had low concentrations of nitrate, then the difference between pearl millet and the other hybrids was diminished, if present at all.

Previous research conducted in Oklahoma in 1982 produced similar results. Lemon and McMurphy studied the nitrate content of 3 varieities of pearl millet versus a sudan x sudan hybrid and a sorghum x sudan hybrid. The plants were grown in central Oklahoma at Perkins or at Lahoma. The plots at Perkins all had very low concentrations of nitrate and there was no difference between the millets and the other hybrid grasses. However, drought stressed the plants at the Lahoma site and all of the varieties contained dangerous concentrations of nitrate. Pearl millet varieties accumulated 2 to 3 times as much nitrate under the stress as did the other varieities.

As producers interpret this data, they must remember that the samples tested in the current study were hay samples cut quite close to the ground. Entire stems and leaves were ground together to make up the samples tested for nitrate content. No selective grazing would be occuring with this method.

NITROGEN FERTILIZATION RATE STUDY

Varying levels of nitrogen fertilization for hybrid sudangrass were studied at two Oklahoma State University Agronomy Research Stations; one located at the Eastern Research Station near Haskell and another in the South-Central Research Station near Chickasha. The Eastern Research Station plots were planted on a Taloka silt loam soil. Whereas the South Central Research Station is located on a Reinach silt loam. The nitrogen fertilization rate study was conducted in two successive years. Three cuttings of hay were taken at each location in the first year. Three cuttings were obtained from the Eastern Research Station in year two; whereas, only two cuttings were available from the South Central Research Station in year two. Five fertilization treatments were replicated four times at each of the locations each year. Therefore a total of 220 samples were obtained. A randomized complete block design was used at each location. At time of planting both soil and moisture conditions were good at both locations for germination and emergence. The five levels of nitrogen fertilization studied were:

Treatment 1: No nitrogen fertilization applied
Treatment 2: Two or three split applications of 50 pounds of actual nitrogen per acre (50 pounds at planting time and 50 pounds per acre after each cutting)
Treatment 3: One application of 100 pounds of actual nitrogen per acre at planting
Treatment 4: One application of 150 pounds of actual nitrogen per acre at planting
Treatment 5: One application of 200 pounds of actual nitrogen per acre at planting.

The hybrid sudangrass variety utilized at both locations and both years was a "Sudan X Sudan" hybrid named Monarch V*. Planting date in year 1 was in late May for both locations. In year 2, the original planting in both Eastern Oklahoma and South Central Oklahoma was late May. However that planting was washed away at the Eastern Research Station and replanting took place in late June. Each location was cut prior to seed head exertion and plots in treatment 2 were top dressed to provide the additional nitrogen. The hays were harvested with the cutter bar height at 4 inches above the ground. Hay was harvested from each of the 15 feet by 9 feet plots, and an approximate 1 pound sample consisting of stems and leaves was labeled and taken to Stillwater for dry matter and nitrate concentration determination. Nitrate concentrations were determined by the salicyclic acid method described by Cataldo in 1975. Nitrate concentration for each sample was expressed as

*Cal/West Seed Co.

parts per million and adjusted to a 100% dry matter basis.

Data were analyzed by utilizing analysis of variance to test for main effects of treatment, year, location, and cutting. All possible two-way interactions of main effects were examined and then removed from the regression model if non-significant. Least squares treatment means were compared by examining "protected least significant differences".

Results

The mean nitrate concentration across all treatments for each location and cutting from both years is presented in Table 1.

Table 1. Least squares means of nitrate concentrations (ppm) across all treatments for each research station and cutting in 1990 and 1991.

	Location	
	Eastern	South Central
Year 1		
Cutting 1	5144	3685
Cutting 2	5301	4978
Cutting 3	5547	5330
Year 2		
Cutting 1	21382	4210
Cutting 2	1427	6204
Cutting 3	8954	N/A

Year, location, cutting, and fertilizer treatment were all significant sources of variation for nitrate content of the samples. ($P < .05$) There were no significant two-way interactions among treatments and the other main effects. However, significant ($P < .05$) interactions between year and location, year and cutting, and location and cutting were present. These interactions could be attributed to weather stresses that occurred in each of the two years, but at different times and to differing degrees of severity at the two locations. Table 2 illustrates the monthly rainfall for the two locations each year.

Drought stressed sudangrass plants have been shown to be nitrate accumulators. The lack of rainfall at the Eastern Oklahoma Research Station in the summer months of the second year of the study resulted in greater nitrate concentrations in the first cutting of the second year.

Table 2. Monthly rainfall totals for Eastern Oklahoma Research Station and South Central Oklahoma Research Station in 1990 (year 1) and 1991 (year 2).

	Eastern Research Station									
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
Year 1	4.07	3.09	5.44	7.64	7.38	0.86	2.65	1.42	8.30	1.77
Year 2	0.81	0.06	1.10	2.36	6.56	3.51	0.97	0.51	5.15	4.59

	South Central Research Station									
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
Year 1	1.92	5.00	6.42	5.21	5.59	1.92	2.47	3.48	2.76	1.90
Year 2	1.49	0.05	1.51	3.28	6.72	3.80	3.41	3.75	9.88	3.47

Because there were no significant interactions of treatment with year, location, or cutting, least squares means of the treatments across both years, both locations, and all cuttings are presented in Table 3.

This data clearly illustrates that nitrogen fertilization affects nitrate accumulation in hybrid sudangrass hays. Treatment 2 (50 pounds nitrogen applied in two or three split applications) versus treatment 3 (100 lb of nitrogen applied at planting) or treatment 4 (150 lb of nitrogen applied at planting) revealed no signifcant difference in nitrate accumulation. Therefore the practice of splitting nitrogen applications in order to reduce nitrate toxicity appears to be questionable. Applying 200 lb of nitrogen per acre resulted in increased concentrations of nitrate compared to all treatments except where 150 lb were applied.

Table 3. Least squares means of nitrate concentrations (ppm) of hybrid sudangrass grown under different nitrogen fertilization schemes.

Treatment				
0 lb. N	50 lb. 2-3 X	100 lb	150 lb	200 lb
3631^{a}	6282^{b}	6098^{b}	7083^{bc}	8432^{c}

^{a,b,c} Means with different superscripts are different ($P < .05$)

Nitrogen fertilization increased nitrate content of hybrid sudangrass hays grown under differing rainfall amounts and in different locations of the state. As large as these increases due to fertilization were, they were still not as large as the differences noted between cuttings. The differences due to cuttings were unquestionably related to weather differences wherein hot, dry weather caused plant stress that resulted in even greater changes in nitrate accumulation. Splitting the application of nitrogen did not show a significant decrease in nitrate accumulation in the sudangrass hays tested in this study.

Comparing the Diphenylamine Field Kit to Laboratory Analysis of Nitrate

Two stems from each sample in the samples collected in 1990 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. One-half gram of diphenylamine is added to 20

ml of water, then brought to volume of 100 ml with sulfuric acid. Each stem was dissected longitudinally with a sharp surgical scalpel. 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. This chemical 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. By comparing the various percentages of samples turning colors upon the presence of diphenylamine, the usefulness of the field test should be more apparent.

Means of the color scores were compared by using t-tests. Simple correlations were calculated to examine relationships between color score and laboratory determination of nitrate concentration at 10 and 20 seconds in the first and second stem of each sample tested. The percentages of samples "turning blue" was the percentage of samples in each class that had scores of 4.0 or greater.

Results

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. Therefore 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 (table 4). All of these are false positives, and would have raised concern about nitrates when in fact the nitrate concentration was of little concern. Some of these false positives may result from the interference of other ions as previously mentioned. Others result from the relatively large leaf-to-stem ratio. The stems contain considerable nitrate, while the leaves contain very little. The nitrate concentration of the entire sample is diluted to a moderate level by predominance of leaves in the sample.

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 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 450 g- 500 g sample of forage does not give as accurate an assessment of the nitrate content as does testing multiple stems.

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.

Table 4. Percentage of 1064 samples giving positive (blue color) reaction when exposed to diphenylamine and least square means and standard errors of color scores.

	concentrations of nitrates (ppm)				
	0-2500	2500-6000	6000-10,000	10,000-15,000	>15,000
Percentage of samples one or two turned blue	45.7	66.7	86.1	94.9	100.0
Percentage of samples both turned blue	24.0	43.7	70.6	86.1	93.1
Mean color score					
Stem1	3.0+0.1	3.6+.06	4.5+.09	4.9+.16[a]	5.3+.27[a]
Stem 2	3.0+0.1	3.7+.06	4.4+.09	4.9+.16[a]	5.5+.27[a]

[a]Means with common superscripts are not different (P>.05)

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 means that many "safe" samples are sent to a commercial or university laboratory for quantification. False positive reactions may occur with bromides, iodates, chlorates, molybdates, iron, antimony, and perioxides. These ions are usually not present in plant tissues in sufficient concentrations to produce a positive reaction so these are little concern when testing hay. However, soil with high iron content (common in Oklahoma) could produce a false positive. 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 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. Approximately 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 estimator 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. Variation in individual plants grown in identical situations exists, therefore 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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EFFECTS OF NITRATES IN PREGNANT BEEF AND DAIRY COWS

J.P. Sonderman and K.G. Odde

INTRODUCTION

Nitrate toxicity in cattle from roughages was reported in the literature as early as the 1890's in Kansas (Mayo, 1895). Nitrate (NO_3) was first identified as a toxic compound by Bradley et al. (1940). Subsequent research confirmed that high levels of dietary nitrates were potentially toxic.

Over the last several years, there has been an increase in the number of abortions attributed to nitrate toxicity, primarily "chronic" nitrate toxicity. We began to investigate how nitrate intake influences the conceptus and possibly causes abortion. In this paper, we will review nitrate metabolism, present the results of some case studies from field investigations, and also present the results from some of our controlled studies.

NITRATE METABOLISM IN RUMINANTS

Nitrates (NO_3) are non-toxic (Archer, 1982). They become toxic when they are reduced to nitrite (NO_2) or another intermediate hydroxylamine (NH_2OH), before being reduced to ammonia in the rumen (Wang et al., 1961). This is portrayed in Figure 1.

Ruminants and nonruminants with significant microfloral fermentation in the lower gastrointestinal tract (i.e., horses) are more susceptible to nitrate poisonings than nonruminants, with less microfloral fermentation in the lower tract. Within a few hours after entering the rumen, over 25% of nitrate is converted to nitrite by bacterial nitrate reductase. This membrane-bound enzyme requires molybdenum for activity (Korzeniowski et al., 1980) and has an optimal pH of 6.5-6.6 (Tillman et al., 1965). Nitrite is reduced by

nitrite reductase, which requires copper, iron and magnesium, at an optimal pH of 5.6-5.8 (Tillman et al., 1965). Nitrite reductase is inhibited by nitrate. It is further converted to hydroxylamine (requires copper, iron and manganese) and eventually ammonia (requires magnesium and manganese) (Owens and Dubeski, 1989).

Nitrite accumulation in the rumen occurs when nitrate ingestion and reduction to nitrite exceeds that of nitrite reduction to ammonia, due to differences in levels of reductive enzymes (Allison, 1978). Nitrate toxicity occurs when nitrite oxidizes the ferrous iron of hemoglobin to ferric iron producing methemoglobin, which cannot transport oxygen to body tissues (Figure 2). Hydroxylamine, formed from nitrite in the blood, is also capable of converting hemoglobin to methemoglobin (Winter, 1962). Clinical signs of nitrate toxicosis may appear when methemoglobin concentrations reach 40 (Deeb and Sloan, 1975) to 50 % (Geurink et al., 1982). NADPH reductase can reconvert small amounts of methemoglobin to hemoglobin (Vertregt, 1977), but this reconversion can be exceeded. When methemoglobin levels reach 70-90%, death may result. However, death may occur at lower levels in susceptible or stressed animals (Winter, 1962).

Considerable quantities of nitrate can be absorbed into the bloodstream, but once absorbed, nitrate cannot be reduced to nitrite (Wang et al., 1961; Winter, 1962). Within a few hours after dosing, significant amounts of nitrate (25%) are excreted in the urine (Wang et al., 1961).

Milk Production

Effects of nitrates on milk production have been variable. Wright and Davison (1964) reported that nitrate did not lower milk production until feed intake was reduced or

acute toxicity occurred. In other studies (Davison et al., 1963; Crawford et al., 1966; Farra and Satter, 1971) milk production was not reduced. However, Nielson (1974) reported lower milk yields from cows on high nitrate forages.

Abortion

Abortions in pregnant cows, resulting from nitrate toxicity, have been reported in field investigations (Simon et al., 1958; Hibbs et al., 1978; Abbit, 1982; Hudson and Rawls, 1992). Most of these abortions occurred in the third trimester of pregnancy.

Simon et al. (1958) reported a nonspecific abortion in both beef and dairy cattle grazing lowland pastures in Wisconsin. Upon further investigation, they determined that these lowland pastures were populated by plant species that are high nitrate accumulators. When these pastures were sprayed and the nitrate accumulators killed, the incidence of abortions decreased. Abbit (1982) reported a case, in which 9% of the cows in a dairy herd had aborted over a 2 month interval. The abortions were generally confined to the third trimester of pregnancy. After complete necropsies, pathology and virology tests, no evidence of an infectious cause of abortion was detected. When the ocular fluid of two aborted calves was tested, it was high in nitrate. A check of the pens in which the cows were confined revealed an abundance of pigweed. When tested, the pigweed contained a high level of nitrates (2.7% on a dry matter basis). Shredding the weeds coincided with a cessation of abortions. Hibbs et al. (1978) reported a case of nitrate toxicosis in a 390 head beef cow herd in Nebraska. Cows were fed hay from stacks containing sorghum and kochia. Cows began to exhibit symptoms of nitrate toxicity. Death losses amounted to 217 cows, 5 bulls and 4 calves. Additionally, 42 of the remaining cows aborted, starting 48 h after the

acute episode and continuing for up to 3 weeks. Hudson and Rawls (1992) reported a case of nitrate toxicosis in a 140 cow beef herd. Cows were fed ground sorghum hay, which contained 3100 ppm nitrate according to analysis at harvest. After feeding, three cows expired. Abortions occurred beginning 4 d after initial exposure to the feed and continued for 15 d. Thirty-one cows aborted; seven, which had been treated, and 24, which had not been treated, for toxicity. Further analysis of the feed indicated levels of nitrate ranging from 4000 to 5000 ppm. Ocular fluid samples from aborted fetuses contained high levels of nitrate upon analysis.

In controlled studies, sublethal levels of nitrate have been reported to cause abortions by some researchers (Simon et al., 1959; Winter and Hokanson, 1964), but others have not observed the same results (Crawford et al., 1966).

Simon et al. (1959) was able to cause abortions in dairy heifers, given 100 gm of KNO_3 per day through a rumen fistula, after 3, 5 and 13 doses. Winter and Hokanson (1964) fed heifers a balanced ration containing sodium nitrate from the time they were two months pregnant until they calved or aborted. The dosage of nitrate fed one group of heifers was adjusted to maintain methemoglobin levels at 20 to 30% of total hemoglobin; methemoglobin of the other group was maintained at >40%. Three abortions occurred in heifers fed high nitrate, but one was due to vibriosis. The other two occurred in the eighth month of gestation, and a bacteriologic examination was negative. The authors indicated that nitrates may have caused these abortions. Crawford et al. (1966) were not able to cause abortions with nitrate.

Our research has focused on the abortion issue of nitrate toxicity. We used twelve crossbred beef cows in the second trimester of pregnancy. Six cows were assigned to be

controls. Seven cows were orally administered KNO_3 at the rate of 25 g per 100 lb of body weight in an attempt to cause acute toxicity. Acute toxicity was exhibited in those cows administered KNO_3, and these animals were treated with methylene blue. No abortions occurred in any of the animals. Seventeen days following the episode of acute toxicity, fetal viability was assessed using an ultrasound. Movement and/or pulses could be detected on all fetuses.

In another study, nineteen crossbred beef cows in the third trimester of pregnancy were utilized. Cows were assigned to one of three treatments based on expected calving date, body condition, weight and age. In the original design, six cows were assigned to the control group (no KNO_3 administered). Seven cows were assigned to a "chronic" nitrate toxicity group. These cows were to be administered 20 g/cwt of KNO_3 daily. Six cows were assigned to an "acute" nitrate toxicity group. These cows were to be administered 30 g/cwt of KNO_3. The three levels of KNO_3 administered were roughly equivalent to feeding a forage containing 16,000, 24,000 and 32,000 ppm nitrate (NO_3), respectively.

During the course of treatment, five of the cows assigned to the chronic toxicity group exhibited acute toxicity. Therefore, we discontinued this treatment. For purposes of statistical analysis, cows were designated as controls; cows that aborted following administration of KNO_3 were designated as abortive; and cows that did not abort after exhibiting symptoms of acute nitrate toxicity were designated nonabortive. To achieve balanced numbers for statistical analysis, hormone levels reported represent three cows from each treatment.

Cows were maintained on native range at the Eastern Colorado Research Center, Akron, CO, throughout the winter. Five days prior to the experiment, cows were

transported to the East Ag Campus in Fort Collins, where they received grass hay ad libitum. Blood samples were taken daily via jugular venipuncture from the cows beginning 3d prior to dosing and continuing until calving or abortion occurred. Nitrate toxicity was induced by orally administering KNO_3. No cows exhibited signs or significant levels of blood methemoglobin, indicative of nitrate toxicity after the initial dose. The following day, all cows administered KNO_3 exhibited elevated circulating concentrations of methemoglobin. Ten of the thirteen cows exhibited clinical symptoms of acute toxicity and were treated intravenously with a 1% methylene blue solution. One of these cows died. Table 1 lists maximum recorded methemoglobin levels of the cows and whether or not they were treated with methylene blue for acute nitrate toxicity.

Three of the remaining twelve cows that were administered KNO_3 aborted fetuses. Cows aborted 3, 4, and 12 d after the second day of KNO_3 administration. Ocular fluid from aborted fetuses was tested for nitrate concentration by the CSU Diagnostic Laboratory. Results are reported in Table 2. According to guidelines from the CSU Diagnostic Lab, all of these levels were potentially lethal. Necropsies on the fetuses indicated that the calves died in utero 12 to 24 hr prior to expulsion.

Differences in hormone concentrations among the three groups were noted immediately prior to and following dosing (Table 3). Surprisingly, cortisol levels were different ($P<.05$) between controls and abortive and nonabortive cows prior to administration of KNO_3. Cortisol levels within control and abortive groups were not different prior to or following dosing, but were different ($P<.05$) in the nonabortive group.

Estradiol levels were similar between treatments prior to dosing. After dosing, controls and those cows which aborted had higher levels ($P<.05$) of estradiol, than did cows

in the nonabortive group. The higher levels in controls may have been observed because they began calving three weeks after the beginning of the experiment. Cows in the nonabortive group did not begin calving until five weeks after the beginning of the experiment. Estradiol levels rise in the three weeks prior to normal parturition.

Progesterone levels were similar between groups prior to dosing. Following dosing, levels were significantly lower ($P<.05$) in abortive cows, when compared to controls and non abortive cows. This suggests that progesterone levels were dropping in the cows that aborted.

Differences in hormone concentrations between groups during the nine days prior to calving are shown in Table 4. Cortisol levels were higher in cows that aborted ($P<.05$), when compared to those that calved normally. Estradiol and progesterone levels are lower in cows that aborted ($P<.05$), compared to those that calved normally.

Normally, the fetus initiates its own parturition. In the weeks prior to parturition, the fetus will mature and begin to produce fetal cortisol. The cortisol will act on the placenta to increase production of estradiol and decrease production of progesterone. Cortisol will also act on the endometrium to produce prostaglandinF2 alpha, which will regress the corpus luteum and progesterone production will decrease. This allows the parturition process to begin.

In those cows which aborted, it appears that although cortisol levels did rise in the circulation, they were not able to cause increased production of estradiol and prostaglandinF2 alpha, or decreased production of progesterone. This suggests that either the cortisol is coming from the dam or that the placenta is not responding to the increased cortisol secretion. From our results, there appears to be two different mechanisms of action

of abortion due to nitrate toxicity. The mechanism of action by which abortion occurs immediately following nitrate toxicity may be due to the death of the fetus and/or placenta. The abortions, which occur from a week to two weeks following toxicity, appear to be caused by the death or impaired function of the placenta.

There is some speculation on how nitrate toxicosis may cause abortions. A recent report (van 't Klooster et al., 1990) showed a decrease in oxygen concentration in fetal arterial blood in dams fed nitrate. They speculated that this was due to decreased oxygen carrying capacity of the maternal blood and a decrease in maternal blood pressure, which would lower the perfusion pressure and transfer of oxygen to the fetus. Malestein et al. (1980) reported that the formation of methemoglobin in fetal blood is not greatly increased by regular nitrite dosing of the dam for upwards of four hours.

Further research is needed to define the mechanism of action by which abortions occur following nitrate toxicity.

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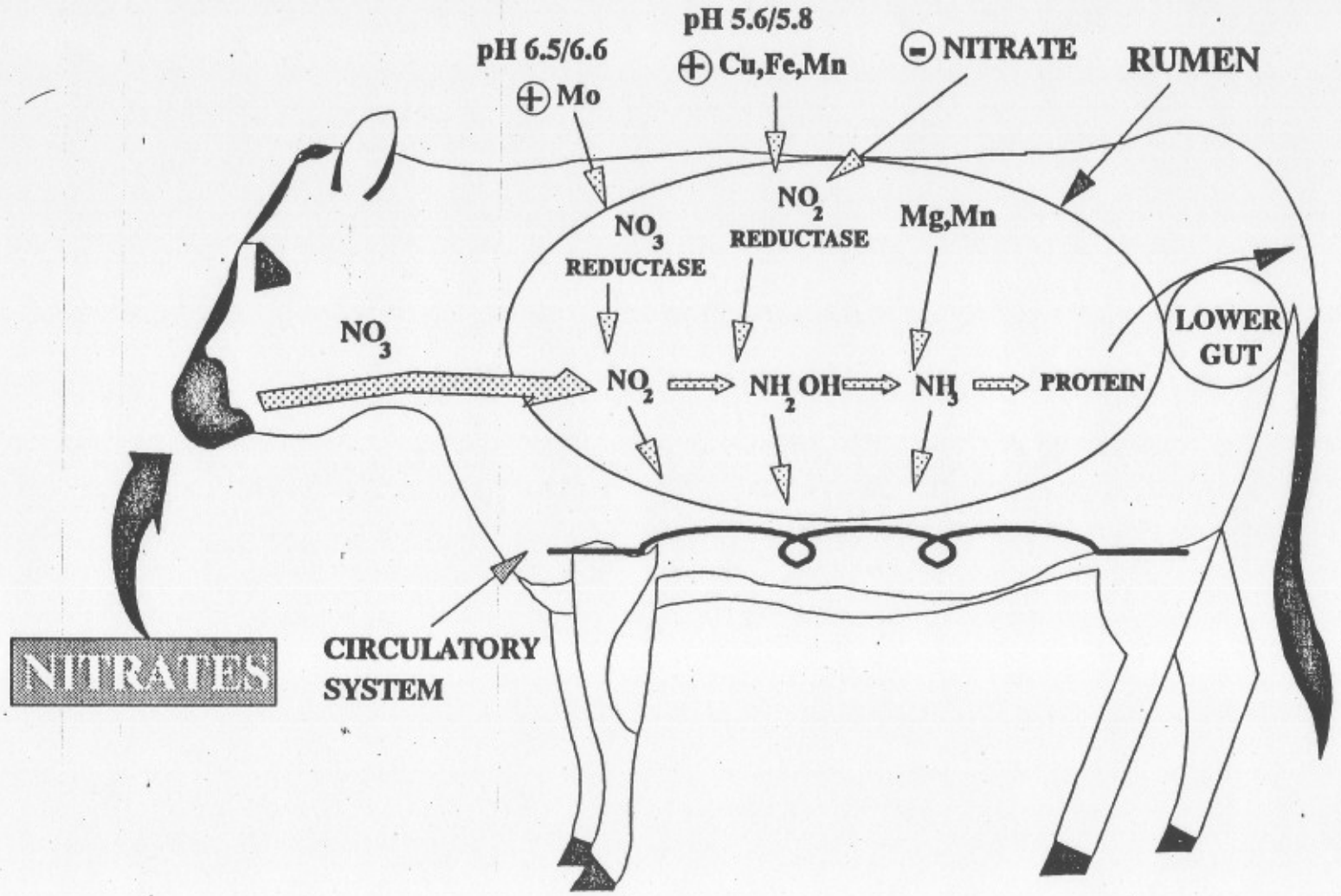

Figure 1. Nitrate metabolism by ruminant bacteria.

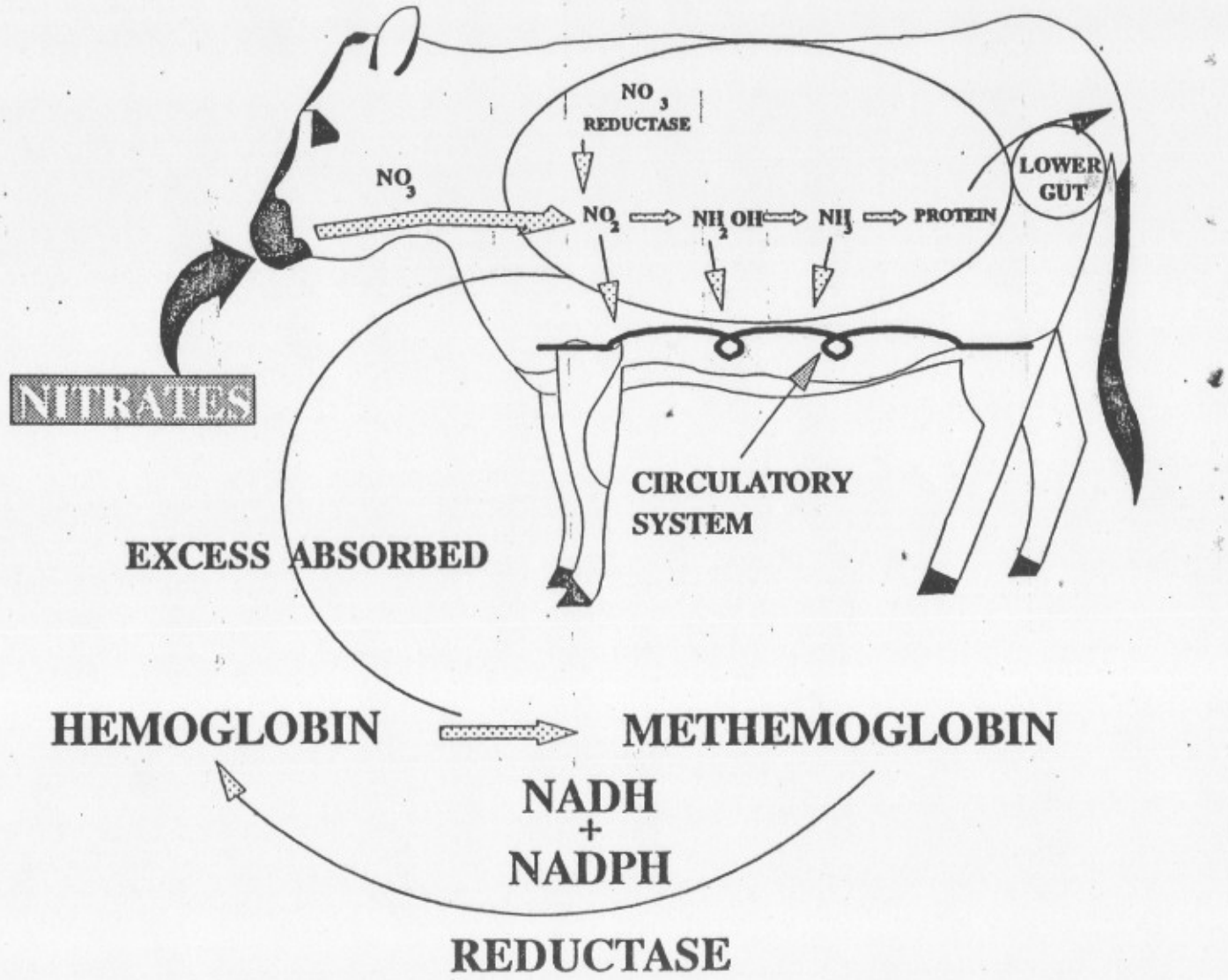

Figure 2. Nitrite (NO_2) is absorbed from the rumen into the blood and causes conversion of hemoglobin to methemoglobin, resulting in reduced oxygen transport. Elevated methemoglobin may lead to death.

Table 1
Maximum recorded blood methemoglobin concentrations[a]

Cow ID	Time to maximum % methemoglobin (h)	Maximum % methemoglobin	Methylene blue treatment	Recovered
20 g/cwt level				
0111	6	22	No	Yes
0608	8	34	No	Yes
2087	6	46	Yes	Yes
4039	6	67	Yes	Yes
9054	8	51	No	Yes
9077	6	57	Yes	Yes
9120	8	40	No	Yes
30-40 g/cwt level				
0029	8	60	Yes	Yes
2203	5	63	Yes	Yes
3093	8	63	Yes	Yes
5116	6	67	Yes	Yes
9025	6	78	Yes	No
9057	6	68	Yes	Yes

[a]Methemoglobin levels >40% may cause symptoms of nitrate toxicity.

Table 2
Nitrate concentration of ocular fluid from aborted fetuses[a]

Cow ID	Interval from dosing to abortion (d)	Nitrate (ppm)
4039	3	100
5116	12	50
9054	4	240

[a] > 40 potentially lethal?

Table 3
Effect of nitrate toxicity on circulating concentrations of maternal cortisol, estradiol and progesterone

Hormone, time	Controls	Abortive	Nonabortive
Cortisol (ng/ml)			
Predosing[e]	7.7 ± 0.9[a]	10.7 ± 0.9[b]	10.3 ± 0.9[bc]
Postdosing[f]	7.0 ± 1.0[a]	11.2 ± 1.0[b]	13.5 ± 1.0[bd]
Estradiol (pg/ml)			
Predosing	4.6 ± 2.7[ac]	3.6 ± 2.7[ac]	3.2 ± 2.7[a]
Postdosing	11.2 ± 3.0[ad]	12.7 ± 3.1[ad]	5.3 ± 3.0[b]
Progesterone (ng/ml)			
Predosing	6.3 ± .4[a]	6.7 ± .4[ac]	5.3 ± .4[a]
Postdosing	5.7 ± .4[a]	4.2 ± .5[bd]	5.7 ± .4[a]

[ab]Means within a row, without a common superscript are different (P<.05)
[cd]Predosing mean is different from postdosing mean (P<.05)
[e]Average concentration of daily samples for five days prior to dosing.
[f]Average concentration of daily samples for four days following dosing.

Table 4
Differences in circulating concentrations of maternal cortisol, estradiol, and progesterone during the 9 d precalving or preabortion

Hormone	Controls	Abortive	Nonabortive
Cortisol (ng/ml)	6.8 ± .6[a]	11.4 ± .6[b]	7.2 ± .6[a]
Estradiol (pg/ml)	42.7 ± 2.8[a]	16.7 ± 2.9[b]	62.7 ± 2.8[c]
Progesterone (ng/ml)	4.0 ± .3[a]	4.9 ± .3[b]	3.0 ± .3[c]

[abc]Means within a row, without a common superscript are different (P<.05).

Nitrate Toxicity: Diagnosis and Treatment

Robert Smith, DVM and Glenn Selk

Clinical Signs

Nitrate poisoning in ruminants is an acute or subacute condition. Clinical signs generally are seen within 6 hours following ingestion of high nitrate forage, though as much as a week may pass. Signs are usually related to anoxia resulting from methemoglobinemia. The nitrite ion in blood converts hemoglobin to methemoglobin, and is a vasodilator. The signs of nitrite poisoning appear suddenly due to the tissue hypoxia and low blood pressure resulting from the vasodilation. Rapid, weak heart beat; subnormal body temperature; muscular weakness; ataxia; and brown mucous membranes develop rapidly. Exercise may accentuate the clinical signs and often results in marked dyspnea or collapse or both. Affected animals may die suddenly without evincing any premonitory signs, in convulsions within an hour, or after a clinical course of 3-4 hours. Depression and a cyanotic or brown cast to mucous membranes along with a rapid, weak pulse are often present. Animals occasionally show behavioral changes, muscular tremors, ataxia, and weakness. If not treated, animals may die within several hours to a day after onset of clinical signs. Abortions can result a few days after an episode of acute nitrate intoxication, even in animals that were not obviously affected.

Chronic nitrate intoxication has been reported. Most often this is associated with decreased weight gains and lactation, reproductive failure, and deranged vitamin A and thyroid metabolism. To date these reports are field observations. Nitrate readily passes through the placenta and causes methemoglobinemia in the fetus. Controlled studies to document chronic effects from nitrate have not been reported.

Diagnosis

The blood is chocolate brown because of its methemoglobin content. This characteristic color of the blood is suggestive of the appropriate diagnosis. The submucosa of the rumen, reticulum, and omasum, and the mucosa of the abomasum usually are congested. Petechiae on the serosal surfaces are commonly observed. The dark brown discoloration evident in moribund or recently dead animals is pathognomonic. Animals affected with nitrate intoxication have elevated methemoglobin levels. The per cent methemoglobin can be used to evaluate the condition of animals. Methemoglobin is not stable in refrigerated, heparinized blood for more than a few hours. Laboratory determination of methemoglobin must be done within this time or the methemoglobin must be preserved. One part blood may be mixed with 20 parts phosphate buffer (pH 6.6) to preserve the methemoglobin. A 1:20 dilution with distilled water has also been effective in preserving methemoglobin. This sample may then be refrigerated or frozen and delivered to the laboratory. The postmortem lesions are limited to the chocolate brown cast of the blood, mucous membranes, viscera, and muscles, especially if the postmortem investigation is conducted soon after death. Other findings such as pulmonary edema and

emphysema, or agonal hemorrhages associated with respiratory distress, may occasionally be present. Aborted fetuses may be examined for increased nitrate concentrations by diphenyalmine testing of the aqueous humor of the eyeball.

Chemical confirmation of elevated nitrate or nitrite levels is required for a firm diagnosis, even if clinical signs, history, and successful treatment all are strongly suggestive of nitrate intoxication. Forage, hay, or feed samples should be analyzed for nitrate content. A field test using diphenylamine should be available to all practitioners. One-half gm of diphenylamine* is added to 20 ml of water, then brought to a volume of 100 ml with sulfuric acid. This stock solution should be stored in a brown glass bottle. The working solution is made by mixing equal parts of the stock solution and 80 per cent sulfuric acid. The working solution should also be stored in a brown glass bottle. Plant material may be tested by placing a drop of working solution on the inside of the split stem at a node or joint. Several plants from different locations should always be tested. A deep blue color will develop within about 10 seconds if 2 per cent or greater nitrate is present. The diphenylamine test can be used readily in the field for testing drinking water, plant material, stomach contents, and urine. While it is not specific, a positive reaction can help to confirm a tentative diagnosis.

Distinguishing between nitrate and cyanide toxicosis

Differentiation between nitrate and cyanide toxicosis is crucial as antidotes for cyanide poisoning exacerbate nitrate toxicity. Livestock poisoning from cyanide is almost always due to the ingestion of plants containing cyanogenic glycosides that liberate cyanide gas upon hydrolysis in the rumen content or the acid media of the stomach. All species of farm animals may be affected with cyanide toxicosis, although cattle, sheep, and horses are most often involved.

Numerous plants contain cyanogenic glycosides at one or all stages of their growth process. Of those, the ones most commonly involved with livestock poisoning are Johnsongrass, Sudan grass, common sorghum, arrowgrass and choke cherry. A venous blood sample provides a quick, accurate test. Cyanide prevents the transfer of oxygen from the blood to its place of molecular utilization in the mitochondria of tissue cells. The circulating blood becomes hyperoxygenated and bright red in color. The venous blood from an animal with cyanide toxicity is bright red while that of an animal with nitrate toxicity is dark or even brownish in color.

Treatment for nitrate toxicity

Methylene blue is the principal therapeutic agent. Methylene blue causes reduction of ferric iron in hemoglobin to the ferrous state so that hemoglobin can again accept and transport oxygen. The suggested dose is 4.4 (Ruhr and Osweiler, 1986) or 9 mg/kg (Merck, 1986) body weight administered slowly via intravenous injection of a 1 to 4-percent solution. The treatment may be repeated if clinical signs recur. Treatment may be repeated in 20-30 min. if the initial response is not satisfactory. Mineral oil (1 L/400 kg)

*Available from Sigma Chemical Company, St. Louis, MO 63166.

orally or saline cathartics (sodium sulfate 0.5 kg/400 kg) orally have been suggested for lessening the time the high nitrate material remains in the gastrointestinal tract and is available for conversion to nitrite. (See Table 1 for a summary of drug dosages.) Nitrate intoxication usually results from feed or water sources, and many animals in a herd will be exposed. As initially affected animals are treated, others may be developing signs.

Removal of the suspected source of nitrate should also be undertaken. Chemical confirmation of the nitrate source allows recommendations to be made for preventing recurrences.

If treatment is prompt and continues for sufficient time and the high nitrate source can be removed, the prognosis is good, though abortions may still occur within a few days.

Table 1. Common Drug Dosages

Name of Drug	Available From	Species	Dose
Methylene blue	Mallinckrodt Chemical Co., St. Louis, MO	Bovine	4.4 mg/kg IV (2 to 4 percent solution; Ruhr and Osweiler, 1986)
			9 mg/kg IV (1 percent solution Merck, 1986)
Mineral Oil	Standard Oil Company Chicago, IL	Bovine	1 L/400 kg orally
Sodium sulfate orally	Mallinckrodt Chemical Co., St. Louis, MO	Bovine	0.5 kg/400 kg

Sources:

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Utilization of High Nitrate Forages by Beef Cows, Dairy Cows and Stocker Calves

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Nitrates in the ruminal environment

The nitrate ion (NO_3), itself, is not toxic to animals. In the rumen, however, microorganisms convert nitrate to nitrite (NO_2: Sapiro et al., 1949) or hydroxylamine (NH_2OH). Nitrite is then absorbed through the ruminal wall into the blood stream where it converts hemoglobin to chocolate-colored methemoglobin. This compound is incapable of carrying oxygen so as methemoglobin concentrations increase, oxygen supply to tissues decreases and asphyxiation occurs. Clinical signs of nitrate toxicity occur when methemoglobin concentrations exceed 40% of total hemoglobin (Deeb and Sloan, 1975). Death occurs when methemoglobin concentrations exceed 70-80% of total hemoglobin. Methemoglobin is naturally converted back to hemoglobin by NADPH reductase in the body but this process may be too slow when blood nitrite concentrations are too high. Under these circumstances, an intravenous dose of methylene blue (2-7 mg methylene blue/lb body weight) reverses this process and restores the oxygen status of the animal (Burrows, 1984; Merck, 1986; Ruhr and Osweiler, 1986).

In the rumen, bacteria convert nitrate to nitrite with the enzyme nitrate reductase. Many ruminal microorganisms have the genetic capability to produce this enzyme so nitrites can accumulate rapidly (within 4-6 hours) when large quantities of nitrate are consumed. Nitrite can be converted to ammonia (Lewis, 1951) or other nontoxic compounds by many ruminal microorganisms (Cheng et al., 1988) although ammonia appears to be the major end product (Kaspar and Tiedje, 1981). Ammonia can then be used as a nitrogen source by ruminal bacteria to produce bacterial protein.

The key enzyme in the process of nitrite detoxification is nitrite reductase. This is an inducible enzyme which means that microorganisms exposed to nitrite will increase nitrite reductase activity and their ability to detoxify ruminal nitrite. Alaboudi and Jones (1985) demonstrated that nitrite reduction is 3 to 5 times higher in sheep adapted to nitrate. Ruminal microorganisms will begin to adapt as quickly as four hours after the initial nitrate exposure, although three to six days are required for optimal adaptation (Allison and Reddy, 1984). Thus, adaptation of the normal ruminal microflora to nitrates may not be rapid enough to avoid toxicity. Also, producers may not always have enough time to adapt the ruminal microflora to nitrate prior to exposure to high nitrate feeds. In addition, the nitrite detoxifying ability of ruminal microorganisms can be overcome when nitrate intake is too high. For example, when dietary nitrate exceeds 210 mg nitrate/lb body weight (equivalent to 18,000 ppm nitrate in the diet), nitrate reduction has been shown to be two fold greater than nitrite reduction (Allison and Reddy, 1984). Thus, nitrite accumulates.

Ruminal microorganisms will maintain the ability to detoxify nitrate as long as nitrate is present in the diet or water. These microorganisms will de-adapt, however, by reducing the quantity of these enzymes when nitrate is removed from the diet, sometimes as quickly

as four days after nitrate withdrawal. Consequently, animals with previous exposure to high nitrate feeds may not be protected.

The enzymatic conversion of nitrate to forms of nontoxic nitrogen requires a variety of cofactors. These cofactors include certain minerals such as copper, iron, magnesium and manganese. Consequently, animals routinely exposed to high nitrate feeds should have access to these important minerals. In addition, the process of nitrate detoxification is speeded by available energy. Consequently, another strategy to minimize the toxic effects of nitrites is to ensure adequate ruminal energy.

A third strategy for reducing ruminal nitrite concentrations is to establish a population of bacteria in the rumen capable of reducing nitrite to nontoxic nitrogen forms. Some strains of propionibacteria are capable rapidly detoxifying nitrite. Consequently, a component of a nitrate management system could include the use of propionibacteria.

Recognizing nitrate toxicity

Symptoms of nitrate intoxication include staggered gait, accelerated pulse, frequent urination, labored breathing and collapse (Deeb and Sloan, 1975). The most effective diagnostic tool is a blood sample analyzed for methemoglobin or nitrite. Upon sampling, affected blood will be chocolate brown in color. In addition, nonpigmented skin and vaginal membranes may show a brownish discoloration. At this point, treatment should be rapidly initiated because coma and death can occur within 2-3 hours after symptoms appear.

Chronic, low grade nitrate toxicity is more difficult to recognize. Symptoms include decreased weight gain, decreased milk yield and/or abortion (Wright and Davison, 1964; Deeb and Sloan, 1965). Lethargy and reduced eating time are frequently noticed with moderate nitrate intakes and may explain the reduced productivity. In some cases, decreased productivity can be explained by decreased feed intake due to the unpalatable nature of high levels of nitrate (>20,000 ppm) in the diet. Nitrates have been shown to reduce cellulolytic, xylanolytic and total microbial populations and cellulase and xylanase activities (Marais et al., 1988) which could decrease feed utilization. In addition, nitrates alter ruminal VFA profiles with increased acetate and reduced propionate as the most important changes (Allison and Reddy, 1984). Because propionate is a more energetically efficient end product than acetate, this change could reduce the energetic efficiency of the animal.

Nitrate intake: What is toxic?

Nitrate risk is usually characterized by the nitrate concentration in livestock feeds (Table 1). However, we have all heard accounts of cattle consuming feeds much higher in nitrate (12-15,000 ppm) with no observable toxicity. It is important to remember that these concentrations (Table 1) are for the total diet and not for a smaller portion of the total diet. For example, a 500 lb steer might consume 12-14 lb of a 10,000 ppm nitrate sorghum/sudan hay and succumb to nitrate toxicity. But, if the intake of this 10,000 ppm nitrate hay was limited to 5 lb/day, nitrate intake would be decreased to the extent that toxicity symptoms would not be expected.

Table 1. Effect of nitrate concentration in feeds on livestock.

Nitrate (ppm)	Comment
0-1,500	Safe for pregnant cattle
1,500-5,000	Potential early term abortions Reduced breeding performance
0-5,000	Safe for nonpregnant cattle
5,000-10,000	Mid to late term abortions Weak newborn calves Decreased growth Reduced milk yield
10,000 +	Abortions Acute toxicity symptoms and death

A second concern is that the toxic nitrate concentrations (Table 1) do not consider other dietary alterations that can reduce the impact of nitrate on the animal. For example, energy feeds stimulate the conversion of nitrate to nontoxic nitrogen compounds and lessen the potential for toxicity (Burrows et al., 1987). In addition, rate of ingestion of high nitrate forage along with source of nitrate (green forage, dry hay or water) also affect the toxicity of consumed nitrate. Finally, previous exposure to nitrate helps both microbes and the animal to adapt to higher levels of nitrate intake.

A more appropriate expression for nitrate toxicity is mg nitrate/lb body weight (Table 2). This expression combines the nitrate concentration in the feed and the intake of that feed by the animal. In addition, it removes the variation due to animal size or weight. This expression still ignores adaptation or energy intake. Actual nitrate intake (mg nitrate/lb body weight) is important, however, because 10 lb of 20,000 ppm nitrate hay would be much more toxic to a 500 lb steer (400 mg nitrate/lb body weight) than a 1,000 lb steer (200 mg nitrate/lb body weight). The values for a lethal dose of nitrate range from 90 to 454 mg nitrate per lb body weight. This variation is due to the fact that these values were generated with both nitrate salts and nitrate in feeds. Generally, nitrate salts are immediately available in the rumen and are much more toxic than nitrates contained in feeds. Consequently, we have chosen 450 mg nitrate/lb body weight as the toxic level for nitrates consumed in feeds. Nitrates in water are probably toxic at much lower levels (150-200 mg nitrate/lb body weight).

Table 2. Toxic dose of nitrate (LD_{50}) for ruminants.

Dose (mg nitrate/lb body weight)	Source
90-454	O'Hara and Fraser, 1975
148	Bradley et al., 1940
454	Crawford et al., 1966
321-449	Wright and Davison, 1964
140	Deeb and Sloan, 1975
227	Ruhr and Osweiler, 1986
150-450	Faulkner and Hutjens, 1989

Using these values and the nitrate concentrations from Table 1, nitrate intake guidelines were developed (Table 3). The value of these numbers is that they allow consideration of: 1) nitrate content of feeds, 2) level of feed intake, 3) contributions from water or other nitrate sources, and 4) body weight.

Table 3. Effect of nitrate intake (mg nitrate/lb body weight) on livestock.

Nitrate (ppm)	Comment	mg nitrate/lb BW
0-1,500	Safe for pregnant cattle	0-20
1,500-5,000	Potential early term abortions Reduced breeding performance	20-60
0-5,000	Safe for nonpregnant cattle	0-60
5,000-10,000	Mid to late term abortions Weak newborn calves Decreased growth Reduced milk yield	60-120
10,000 +	Abortions Acute toxicity symptoms and death	120

These comments help explain some of the variation in livestock responses to nitrate intake. There remains, however, a significant amount of animal to animal variation. For example, some light beef heifers received a toxic dose of nitrate (Winter, 1962; Table 4). After 6 hours, blood methemoglobin ranged from 18.6 to 70.0% of total hemoglobin. Of these animals, three were probably safe (0-40% methemoglobin), two were borderline (40-60% methemoglobin) while one animal was very susceptible (70.0% methemoglobin). Very simply, some animals can tolerate much more dietary nitrate than others. Unfortunately, it is difficult to predict which animals are more nitrate tolerant so we must design management programs that protect all animals.

Table 4. Blood methemoglobin content (6 h postdosing) of six beef heifers receiving an oral dose of 21.0 g nitrate/cwt (Winter, 1962).

Animal	Methemoglobin (% of total hemoglobin)
665	18.6
689	32.6
690	70.0
691	40.1
696	58.5
697	28.8

Factors that predispose ruminants to nitrate toxicity

Certain factors or circumstances appear to predispose animals to nitrate toxicity. Other factors or circumstances offer some protection. It is important to understand the difference.

Hunger. Hungry animals eat more feed. Thus, hungry animals released onto or fed a marginally toxic forage might become poisoned because of higher than expected intake (Kretschmer, 1958). Dollahite and Holt (1970) demonstrated that a calf consuming 1.1 g nitrate-N over 24 hours showed no toxicity but a calf force fed 0.32 g nitrate-N died within four hours. Environmental conditions also impact hunger in that cold weather or snow or ice cover may create circumstances where hungry cattle are capable of consuming extremely large quantities of feed.

It is also important to realize that many high nitrate forages are palatable and digestible and thus promote high consumption. In studies at Stillwater, 500 lb beef heifers were fed chopped prairie hay for one week prior to an abrupt conversion to high nitrate (20,000 ppm) pearl millet hay. Intake increased from 1.3% BW on the prairie hay to 1.8% BW on the pearl millet. Because animals cannot sense high nitrates in feed, intake will be controlled by other factors such as palatability and digestibility.

Adaptation. Because the ability of ruminal microorganisms to detoxify nitrate/nitrite is an inducible process, prior exposure to nitrate can help to protect animals from nitrate toxicity. Exposure to nontoxic levels of nitrates (< 6,000 ppm) for three to ten days is required to induce the nitrate detoxifying ability of ruminal microorganisms. This exposure must be continuous, however, until the animals are fed the high nitrate feed because the ability to detoxify nitrate can be lost as rapidly as it is developed.

In addition to the microbial adaptation to nitrate, the physiological processes of the animal also adapt. For example, animals exposed to a continuous source of nitrate have increased hemoglobin, hematocrit and blood volume (Jainudeen et al., 1964). Increased hemoglobin helps the animal compensate for the proportion of methemoglobin created by nitrite in the blood. Increased blood volume is an adaptive response to the vasodilation and resulting low blood pressure associated with increased blood nitrite concentrations. With these adaptations, the animals can adapt to, and tolerate, some blood nitrite.

Diet. Feeds that are high in energy, such as the starchy cereal grains, will stimulate ruminal microorganisms to convert nitrate to nontoxic nitrogen compounds at a faster rate. Burrows et al. (1987) showed a dose-related response to level of corn fed to cows dosed with nitrate (Table 5). Approximately 0.8% of body weight of corn reduced ruminal nitrite by 75% and blood nitrite and methemoglobin by approximately 50%. Thus, previous feeding of cereal grains such as corn or milo have the dual benefit of diluting the intake of high nitrate feed and stimulating the utilization of nitrate by ruminal microorganisms.

Table 5. The effect of corn supplementation on rumen and blood nitrite concentrations.

	Corn, % body weight		
	0	0.4	0.8
Rumen nitrite, ug/ml	1.89	1.45	0.5
Blood nitrite, ug/ml	0.07	0.07	0.04
Methemoglobin, %	25.3	23.7	12.4

Forage form. Differences in the toxicity of different types of forage may also affect potential nitrate toxicity. For example, the plant cells in dry hays become more permeable during dehydration. When consumed by ruminants, ruminal fluid rapidly saturates these cells and quickly releases nitrate into the ruminal environment (up to 80% of cellular nitrate can be released into ruminal fluid within 20 minutes). In contrast, green, growing forages are composed of intact plant cells. Although a portion of these cells are ruptured during chewing and swallowing, many plant cells reach the rumen intact. In the rumen, intact plant cells release their cell contents, including nitrate, more slowly which may help to minimize the potential toxicity (only 30% of cellular nitrate released within 20 minutes). Thus, dry hays are potentially more toxic to livestock than lush, green forages (Geurink et al., 1979).

Baling method may also affect potential toxicity. For example, the potential for toxicity may be greater when forage is harvested in large, round bales. The reason for this is that nitrate concentrations vary significantly across a given field. Thus, "hot spots" of increased nitrate concentration exist. With large, round bales, there is more potential to concentrate a large quantity of high nitrate forage into a single feeding unit (Edwards and McCoy, 1980).

Ruminal environment. Characteristics such as ruminal pH may affect a potential toxicity. The pH optimum for nitrate reductase is 6.5 while the optimum for nitrite reductase is 5.6 (Tillman et al., 1965). Because most forage diets create a ruminal environment with a pH from 6.2 to 6.5, nitrate reduction to nitrite is favored. Nitrite reduction, however, may be retarded. Consequently, normal ruminal pH may increase the likelihood of nitrite accumulation. Dietary alterations to reduce ruminal pH such as feeding cereal grains may help activate nitrite reductase.

Because ammonia is one of the normal end products of nitrite reduction, excessive ruminal ammonia concentrations may reduce nitrite reduction because of negative feedback mechanisms. Thus, forages high in protein or feeds high in nonprotein nitrogen sources such as urea may exacerbate the nitrite problem.

Sources of nitrates

Livestock consume nitrates from a variety of sources. The most common source is from forage with cultivars from the sorghum family (sorghum, sudan, pearl millet and their crosses) being particularly high accumulators of nitrate. Under certain conditions, a wide variety of plants can accumulate potentially toxic quantities of nitrate (Table 6). In addition to common livestock feeds, certain weeds that commonly inhabit pastures or crop fields can also accumulate nitrates. Cereal grains and protein concentrates rarely contain appreciable nitrate concentrations.

Table 6. Feeds known to accumulate nitrate under certain circumstances.

Barley forage	Alfalfa	Dock
Beet pulp	Annual brome	Goldenrod
Corn forage	Clovers	Jimson weed
Kale	Fescue	Johnsongrass
Molasses	Kikuyugrass	Kochia (Fireweed)
Oat forage	Orchardgrass	Lamb's quarter
Rape	Pearl millet	Nightshade
Turnips	Sorghum	Pigweed
Wheat forage	Sunflower	
	Sweetclover	
	Switchgrass	
	Timothy	
	Wheatgrasses	
	Wild rye	
	Witchgrass	

Nitrates also tend to be concentrated in certain parts of plants. For example, nitrates are typically higher in stems, lower in leaves (more nitrate reductase activity) and extremely low in grain (Fjell et al., 1991; Pfister, 1988). In pearl millet, stems contained three times more nitrate than leaves (Krejsa et al., 1987).

Forage maturity plays an important role in that nitrates are typically higher in young growth (or regrowth) but lower in mature plants (Pfister, 1988; Fjell et al., 1991). Sunlight is also important because nitrate reductase activity is low during shade or dark (Pfister, 1988). Thus, hay should be cut or animals released in the afternoon of a sunny day.

Soil moisture affects uptake and utilization of nitrates by plants. In contrast to popular belief, however, short or moderate drought creates more nitrate accumulation than extended drought. This is because moderately drought-stressed plants continue to take up nitrate but have reduced nitrate reductase activity because leaves are stressed (Pfister, 1988).

From the standpoint of drought, a greater concern is the nitrate uptake that occurs after a drought-ending rain. For example, Krejsa et al. (1987) and Pfister (1988) reported large accumulations of nitrate in plants shortly after a severe drought. Stem nitrate

concentrations in pearl millet increased from approximately 5,000 ppm to 9,000 ppm within two days after eliminating drought stress by irrigation (Krejsa et al., 1987). In addition, seven to 14 days are required for nitrate levels to return to normal after a drought-ending rain (Fjell et al., 1991)

Other factors such as soil mineral content and herbicide treatment also affect nitrate accumulation (Pfister, 1988).

The contribution of nitrates in drinking water is sometimes overlooked. Either we don't analyze the nitrate content of water resources or we fail to recognize its significance. Nitrates in water are more toxic than plant nitrates because they are immediately available in the rumen while plant nitrates must be released from the plant cell. Consequently, toxic levels for water nitrate are lower (150-200 mg/lb body weight) than toxic levels for forage nitrate (450 mg/lb body weight).

Across Oklahoma, the nitrate content of water ranges from 0.5 to 26.5 ppm (Judy Duncan, State Environmental Laboratory, personal communication). Although these nitrate levels may not seem very high, they can contribute to total nitrate intake and may aggravate a diet that already contains moderate levels of nitrate. The combination of daily water intake and water nitrate content have a significant effect on the quantity of nitrate actually consumed by the animal (Table 7).

Table 7. Effect of water intake and nitrate content (ppm) on total nitrate intake (g nitrate/day).

Water	Water nitrate, ppm						
intake (gal)	10	25	50	100	200	400	800
	g nitrate/day						
0.5	0.02	0.04	0.08	0.16	0.32	0.64	1.27
1.0	0.03	0.08	0.16	0.32	0.64	1.27	2.54
2.0	0.06	0.16	0.32	0.64	1.27	2.54	5.09
5.0	0.16	0.40	0.80	1.59	3.18	6.36	12.71
10.0	0.32	0.80	1.59	3.18	6.36	12.71	25.42
20.0	0.64	1.59	3.18	6.36	12.71	25.42	50.85
40.0	1.28	3.18	6.36	12.71	25.42	50.85	101.70

To produce 100 lb of milk per day, a 1,500 lb Holstein consumes approximately 40 gallons of water. If her drinking water contains 25 ppm nitrate, her nitrate intake from water alone would represent 2 mg/lb body weight. This represents 10% of the quantity of nitrate that could cause reproductive problems (Table 3). Drinking water containing 100 ppm nitrate (common in California) would contribute 8 mg nitrate/lb body weight which is 40% of the quantity of nitrate that could cause reproductive problems. Based on these calculations, water containing 235 ppm nitrate could cause reproductive problems in dairy cows. Although this level of nitrate in water would be unusual in Oklahoma, cows with access to water contaminated by feedlot effluent or runoff from heavily fertilized fields, septic tanks or manure piles could be exposed to high levels of water nitrate.

Management strategies - proactive vs reactive

Nitrate toxicity can be managed in either of two ways. The first method involves the release of livestock onto a forage of unknown nitrate content. If the animals survive, we breath easier and go about our business. If some of the animals die, we call the veterinarian to confirm the cause of death and then lament our misfortune. This approach is a "reactive" method that involves some degree of risk. An example of a reactive nitrate management program is when 390 beef cattle were released onto high nitrate forage in Nebraska and 226 (58%) died (Hibbs et al., 1978).

The risk associated with a "reactive" management approach could be minimized if producers had immediate access to methylene blue, the antidote for nitrate intoxication. Unfortunately, most producers don't have methylene blue on hand.

The second method is a proactive approach to nitrate management. The specifics of a proactive nitrate management system are outlined below. Basically, a proactive approach involves: a) using our knowledge of varietal, environmental and harvesting strategies to minimize the production of high nitrate forage, b) analyzing the nitrate content of the forage to assess risk, and c) implementing a cattle management program to minimize the effects of nitrate consumption on livestock productivity and health.

What are the possibilities of producing a high nitrate forage?

Production of high nitrate forages should be minimized by recognizing and managing the factors that potentially create situations where nitrates accumulate. For example, some types of forage (sorghum vs millet) and some varieties within type accumulate more nitrate than others (Selk, 1993). Nitrogen fertilization is highly correlated with forage nitrate (Selk, 1993). Planting date can be adjusted so that grazing and haying do not occur during commonly drougthy times. At the time of harvesting, weather can be monitored and cattle turnout or haying timed to minimize forage nitrate content. Harvesting can be delayed until 4 to 7 days have elapsed after a drought-ending rain. Harvesting height can be increased to minimize the quantity of high nitrate stems in hay and ensiling can be used to reduce forage nitrate content by microbiological action.

Recognition and management of these factors should minimize the nitrate content and the quantity of high nitrate forage produced. If we successfully reduce forage nitrate concentrations, then livestock management is simplified. Unfortunately, it is virtually impossible to eliminate the nitrate problem. Good forage management, however, should help to minimize nitrate problems.

What is potential nitrate intake?

Before proactive nitrate management strategies can be implemented, the magnitude of the potential nitrate problem must be determined with an estimate of potential nitrate intake. To determine nitrate intake (g nitrate/day or mg nitrate/lb body weight), the nitrate content of the feed must be combined with the projected intake of that feed which is then divided by the body weight of the animal (Table 8).

Table 8. Calculation of nitrate intake assuming a 1,200 lb beef cow consumes 10 lb (4,540 g) of a 10,000 ppm nitrate hay.

Nitrate intake (g/day) = Forage intake (g/day) X nitrate concentration (ppm)

= 4,540 g/day X 0.01 (ppm converted to decimal)

= 45.4 g nitrate/day

Nitrate intake (mg/lb body weight) = 45.4 g/day X 1,000 mg/g / 1,200 lb

= 37.8 mg nitrate/lb body weight

In this example, a 1,200 lb beef cow consuming 10 lb of a 10,000 ppm nitrate hay would consume 38 mg nitrate/lb body weight. Using the nitrate concentration column of Table 1, we might conclude that this cow is at risk. The high nitrate hay, however, only represents a portion (10 lb) of her total daily intake. Thus, her actual nitrate intake (38 mg/lb BW; see Table 3) is within the safe range for nonpregnant cattle although she may be a candidate for early term abortion.

Nitrate content of feeds is determined by accurate sampling of those feeds and accurate analysis of nitrate concentration. Forage samples must be obtained so that they represent the variation in nitrate concentrations in the feed. With dry hay, this sampling involves collection of individual core samples from 20 to 40% of the bales. Although this is a labor intensive process, the results will help us determine the most appropriate management strategy.

Sampling a field is more difficult. On June 30, 1992, we obtained 48 forage samples from a five acre area of a sorghum/sudan pasture that had received 80 lb actual N/acre plus had rainfall within the previous three days. The nitrate content of these samples ranged from 7,930 to 43,600 ppm (Figure 1). Because the nitrate content of standing forage varies significantly, it is difficult to determine the average nitrate content of a pasture.

Nitrate Map (5 acres)

34,600 31,000	33,700 37,800	12,200 22,500	7,930	
20,200 22,800	20,100 30,600 26,700 26,100 43,600	11,700 17,200 21,100 10,400 33,100	9,610 13,400 21,300 11,800 33,400	16,100 23,700 25,400
30,000 22,200 36,400	20,200 28,300 26,500 23,300	9,590 19,700 9,010 25,300	22,200 27,400 13,500 13,300 15,400	25,600 22,900 35,500 18,400 10,700

Figure 1. The variation in nitrate concentration of samples collected from a 5 acre sorghum/sudan pasture.

Haliburton and Edwards (1978) also noted that nitrate tends to accumulate unevenly across a field with "hot spots" that potentially contain an extremely high concentration of nitrate. One of the major problems with these "hot spots" is that hay packaged in large round bales from these fields may have large quantities of nitrate concentrated in a few bales. When these bales are then fed, the potential for nitrate toxicity is very high even though hay from the same field may have been fed for some period of time with no detrimental effects.

The second step in this process is to determine the actual nitrate content of the feed samples. The diphenylamine blue "drop" test has been the standard screening tool for many years. Recent evidence (Selk, 1993) helps to validate the "drop" test as an indicator of nitrate content because high nitrate forages tend to turn blue or black. The major concern is the number of false negatives determined with this method. False negatives are samples that contain high levels of nitrate but are declared low nitrate because the "drop" test didn't change color. Approximately 5% of samples in excess of 10,000 ppm nitrate did not react with diphenylamine (false negative) while 61% of the low nitrate forages showed a positive reaction (false positive). A false negative reading can be dangerous to the livestock.

Most commercial labs perform nitrate testing with an ion specific electrode or a nitrate meter. The cost of these tests ranges from $5-8/sample. Care must be taken to determine the method of expressing nitrate content of feeds. In this paper, nitrate concentration has been expressed as the actual nitrate ion. Certain labs may report nitrate-N, potassium nitrate or % nitrate. For conversion factors, see Kilgore (1993).

A special note of caution. Even during "normal" Oklahoma summers, many forage samples contain high nitrate concentrations. Consequently, all susceptible forage should be considered toxic unless proven otherwise by a reputable analytical laboratory.

The next step is to estimate the quantity of high nitrate feed that the animals can be expected to consume. As general guidelines, young animals (500 lb) will consume approximately 2.5% of their body weight (dry matter intake) of a green, succulent forage (Table 9). This amounts to 12 to 15 lb of dry forage.

Table 9. The effect of forage quality on intake.

Forage type	DM intake (% BW)
Low quality forage - dormant grass - wheat straw	1.5
Average quality forage - native grass - bermuda in late summer	2.0 - 2.5
High quality forage - alfalfa - wheat pasture	2.5 - 3.0

To calculate actual nitrate intake, feed nitrate concentration is multiplied by feed intake (Table 8). This result can be divided by body weight to determine the mg nitrate/lb body weight. This number can be compared to the values in Table 3 to determine the risk of nitrate toxicity. If nitrate effects on livestock appear imminent, proactive nitrate management strategies should be implemented to minimize the impact of nitrate consumption.

The relationship between nitrate content of the diet, daily intake and total nitrate intake is presented in Table 10. A cow fed 10 lb of a 5,000 ppm nitrate hay would have a nitrate intake of 22.7 g/day which is equivalent to 22.7 mg nitrate/lb body weight. Values in excess of 20 mg/lb body weight could affect rebreeding performance (Table 3). Thus, even low intake of a hay with moderate nitrate content could present problems.

Identification and Application of a Propionibacteria Strain for Nitrate and Nitrite Reduction in the Rumen

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Introduction

The feeding of specific viable microorganisms to livestock with the intent to alter the microbial balance within the gastrointestinal tract has been in practice for many years (1). Although the mode of action of these direct-fed microbial products and their beneficial effect has not always been scientifically demonstrated, the important role microorganisms play in fermentation and digestion is well recognized (15). In the case of nitrate toxicity, it is clearly been shown that nitrite is the toxic intermediate resulting from nitrate reduction by rumen microorganisms. Therefore, it was our hypothesis that the effects of nitrate toxicity could be reduced by increasing the reduction of nitrite. One way to accomplish this would be to feed a viable microorganism capable of nitrite reduction. In this paper, we present the results of the identification and application of a propionibacteria strain capable of nitrate and nitrite reduction. Strain selection, establishment and *in vivo* nitrate toxicity trials are summarized in the following sections. The previous papers in this symposium have reviewed important factors such as plant varieties and environmental conditions that lead to nitrate accumulation. In addition, the toxic effect of nitrite in ruminants has been described. Understanding these issues is critical for developing good management practices to avoid the deleterious effects of acute and subclinical nitrate toxicity. A propionibacteria direct-fed microbial capable of nitrite reduction could be a useful management tool to aid in this endeavor.

A more detailed account of these studies will be presented by Mr. Swartzlander at the Annual Meeting of American Society for Microbiology in May and the American Society of Animal Science Annual Meeting in July. Papers from these presentations will be submitted for publication to Applied and Environmental Microbiology and the Journal of Animal Science.

Strain Selection

Screening the culture collection for in vitro denitrification activity.

The propionibacteria are an industrially important group of organisms primarily used by the dairy industry as starter cultures for Swiss-type cheeses (6). Other industrial applications of propionibacteria have been described including their use as a direct-fed microbial (5, 7, 8, 9, 11, 16 and 17), as an inoculant for silage and grain and in the

production of vitamin B_{12} (12) and propionic acid (13). Of the alternate applications of the propionibacteria, the use of these organisms as direct-fed microbials holds great promise, however little has been reported to date. Here, we report the results of a strain screening program to identify those strains capable of nitrate and nitrite reduction.

Seventeen of the 154 strains in the *Propionibacterium* culture collection were found to reduce nitrate to N_2O or N_2 (denitrification). Eleven of these strains were phenotypically identical and, based on chromosomal DNA analysis, had identical DNA finger-prints. A representative strain from this group and the six unique strains were tested for the ability to reduce high levels of nitrate (15,000-20,000 ppm). Only two strains, P5 and P42, were capable of growth and reduction of high levels of nitrate. The reduction of nitrate and nitrite in growth medium by strain P5 is shown in figure 1. When broth medium was inoculated with strain P5 at 1% (10^5 CFU/ml), 50% of the nitrate was reduced in 24 h. Nitrite accumulation started at 12 h and continued for the next 60 h, after which time nitrite was reduced. Strain P42 reduced nitrate and nitrite at a slower rate. Therefore, strain P5 was chosen for further work.

The time course of nitrate/nitrite reduction shown in figure 1 may indicate a common regulatory control system of the nitrate and nitrite reductase enzymes. The nitrate/nitrite reduction was also found to be inducible as reported by other researchers (3, 4). These findings are critical for the performance of the culture and have been exploited to increase activity in the production culture.

Since ruminal levels of nitrate are not expected to reach these high levels, a second in vitro experiment was conducted to compare nitrate and nitrite reduction in growing and non-growing cells which were induced or noninduced for nitrate reduction (figure 2). Induced, growing cells were able to reduce 80% of the nitrate in 6 h. Noninduced, growing cells required 16 h to reduce an equivalent amount of nitrate. All non-growing cells (chloramphenicol added) were unable to reduce nitrate to any significant degree. This may indicate that growth is required for nitrate reduction.

Establishment of propionibacteria in the rumen of beef cattle.

Monitoring Propionibacterium populations in the rumen.

The ability to track the population of a direct-fed microbial is necessary to more completely understand, interpret and predict beneficial interactions of the microorganism with the animal hosts. However, in complex microbiological systems such as the rumen, populations of single strains are difficult to monitor. A prerequisite for tracking populations of propionibacteria in the rumen is the development of a selective medium to eliminate or greatly reduce the competing microflora. Using this strategy, a selective-differential medium was developed for the quantitative determination of propionibacteria in the rumen.

Currently, there are no biochemical or serological tests to differentiate between strains within the same species of propionibacteria. Therefore, it was impossible to determine if the enumerated population represented native propionibacteria or the

Figure 1. DENITRIFICATION BY STRAIN P5

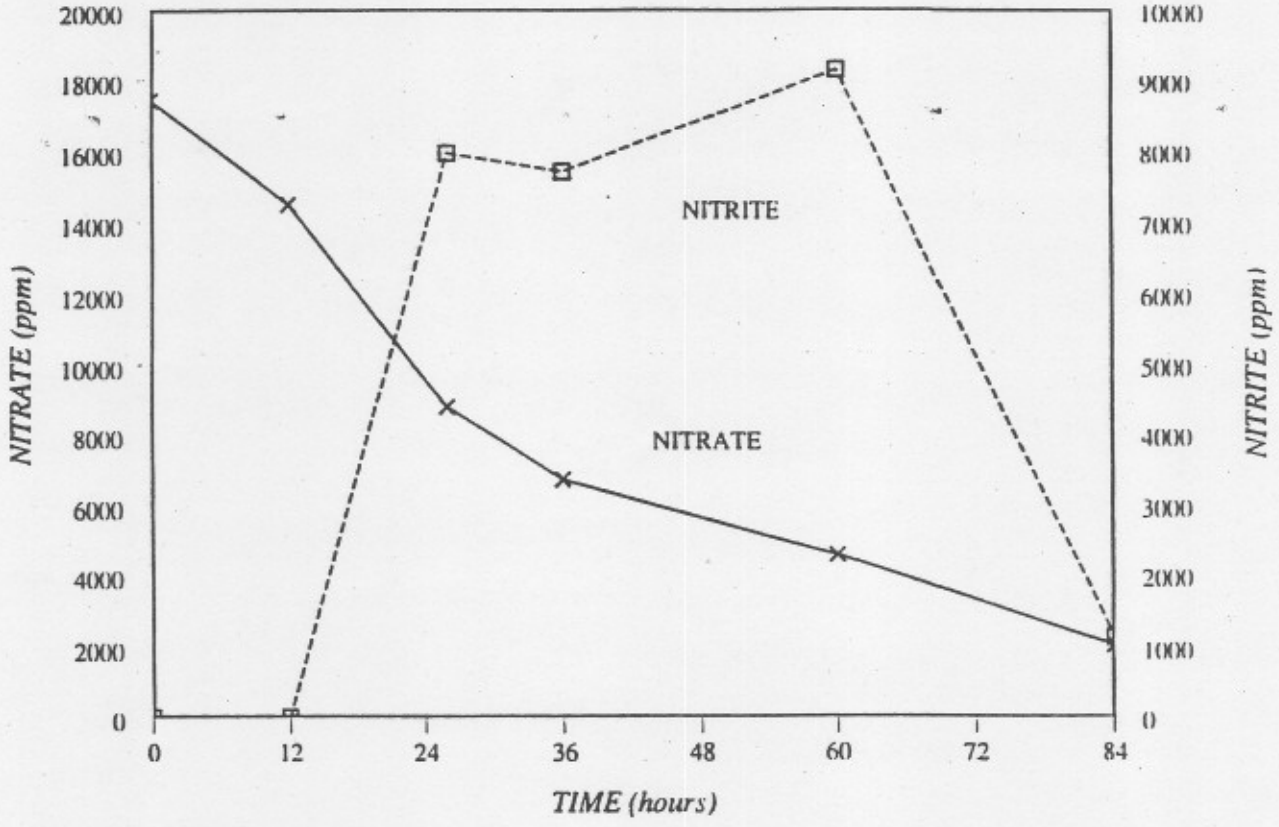
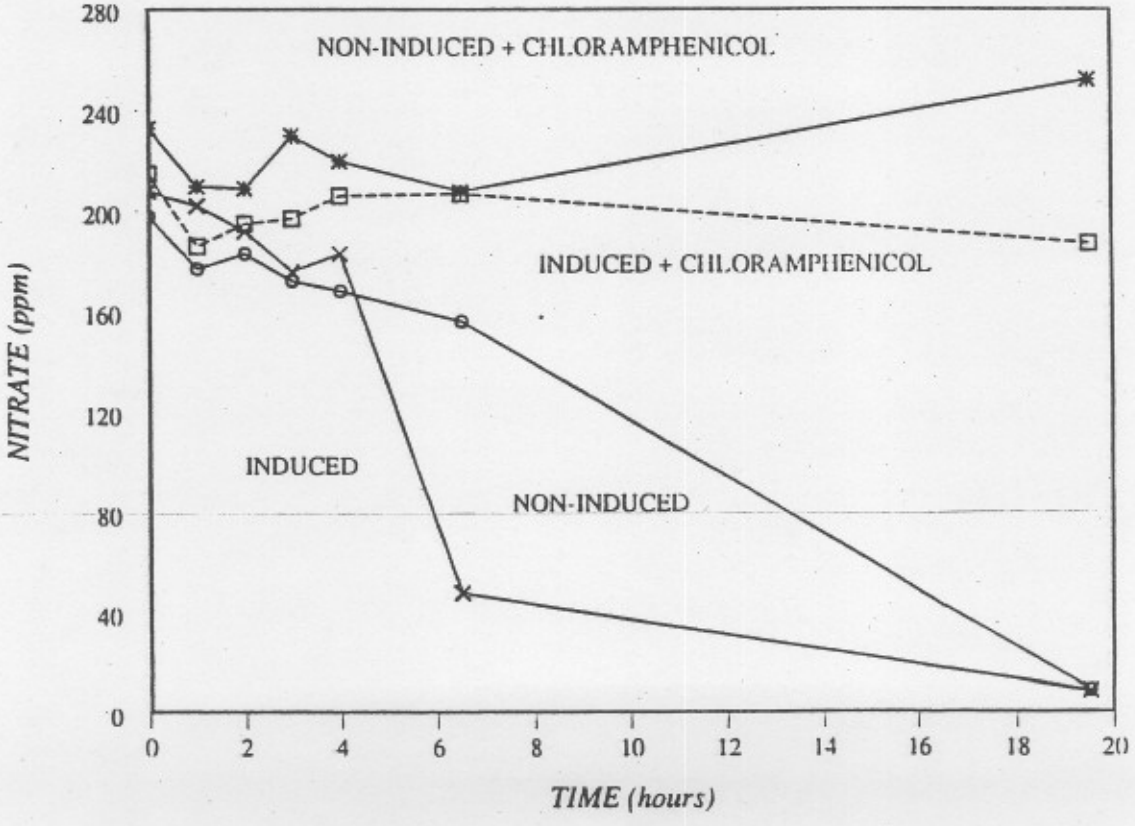


Figure 2. IN VITRO NITRATE REDUCTION



establishment of the strain being fed. Newer techniques for strain identification utilizing plasmid DNA profiles (2, 14) and chromosomal DNA finger-prints (10) were utilized to identify specific strains in the propionibacteria populations isolated from the rumen.

Establishment trials

With the development of techniques to monitor the population of specific strains of propionibacteria in the rumen, animal trials were conducted to determine if strain P5 could be established in the rumen. Strain P5 was fed to eight crossbred beef heifers fitted with ruminal cannulas. In the first trial, heifers were fed strain P5 at a dose of 10^5 CFU/ml of rumen contents daily for a 30-day period. In the second trial, heifers were fed strain P5 at a dose of 10^7 CFU/ml daily for 30 days. In both trials, two heifers which did not receive the culture served as controls. All animals were fed a 50:50 concentrate:roughage diet balanced for protein, minerals and vitamins. Ruminal samples were collected on days 0, 1, 2, 3, 4, 5, 7, 10, 14, 18, 21, 24, 29 and 32. Samples were plated (10^{-3}, 10^{-4}, 10^{-5}) on selective medium and incubated for 7-10 days under anaerobic conditions at 32 C. Following incubation, typical colonies of propionibacteria were enumerated and identified using plasmid DNA profiles.

Five of the eight animals fed strain P5 at 10^5 CFU/ml of rumen contents had detectable levels of propionibacteria (>10^3 CFU/ml) by day 18. By day 30, all eight treated heifers had propionibacteria counts greater than 10^4 CFU/ml. Controls had no detectable propionibacteria counts. Propionibacteria counts of samples taken 2 and 10 days following the trial (no culture was fed) indicated the propionibacteria populations were not reduced in heifers in which the organisms had established.

In trial 2 (10^7 CFU/ml daily), propionibacteria were detected earlier than trial 1. On day 10, all eight heifers had counts greater than 1,000 CFU/ml. At the end of the trial, all eight heifers had propionibacteria counts greater than 100,000 CFU/ml. Controls had no detectable propionibacteria.

Nitrate Toxicity Trials

The results of animal trials indicate that a viable population of strain P5 could be established in the rumen. Nitrate toxicity trials were conducted to determine if the established population was capable of reducing the toxicity of a high dose of nitrate.

Nine crossbred beef heifers (500 lb) fitted with ruminal cannulas were used to evaluate the effect of feeding propionibacteria on ruminal nitrate/nitrite and blood nitrite. Five heifers were dosed daily with the propionibacteria culture (10^7 cfu/ml) while the remaining four heifers served as controls. Heifers were fed coarsely chopped low-quality native grass hay for seven days prior to the nitrate challenge. The nitrate challenge was accomplished with the use of a coarsely chopped pearl millet hay that contained approximately 20,000 ppm nitrate.

Figure 3. RUMINAL NITRATE CONCENTRATIONS

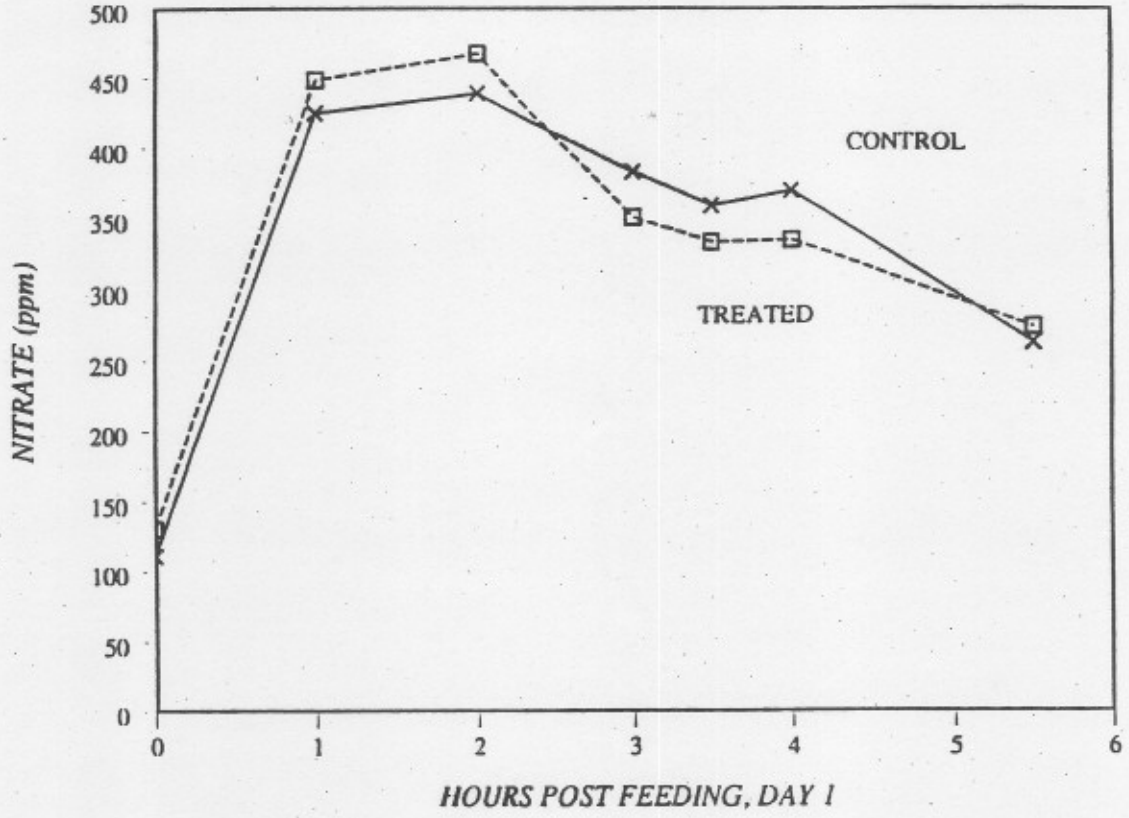


Figure 4. RUMINAL NITRITE CONCENTRATIONS

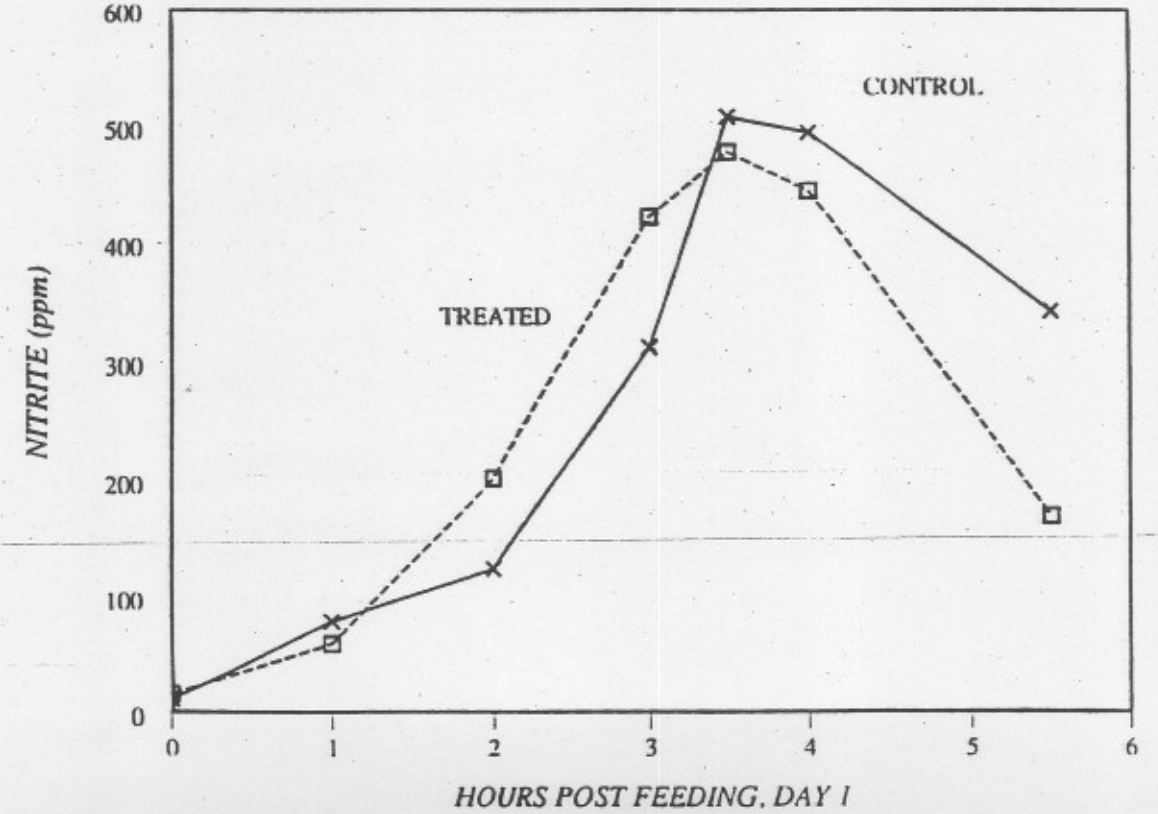
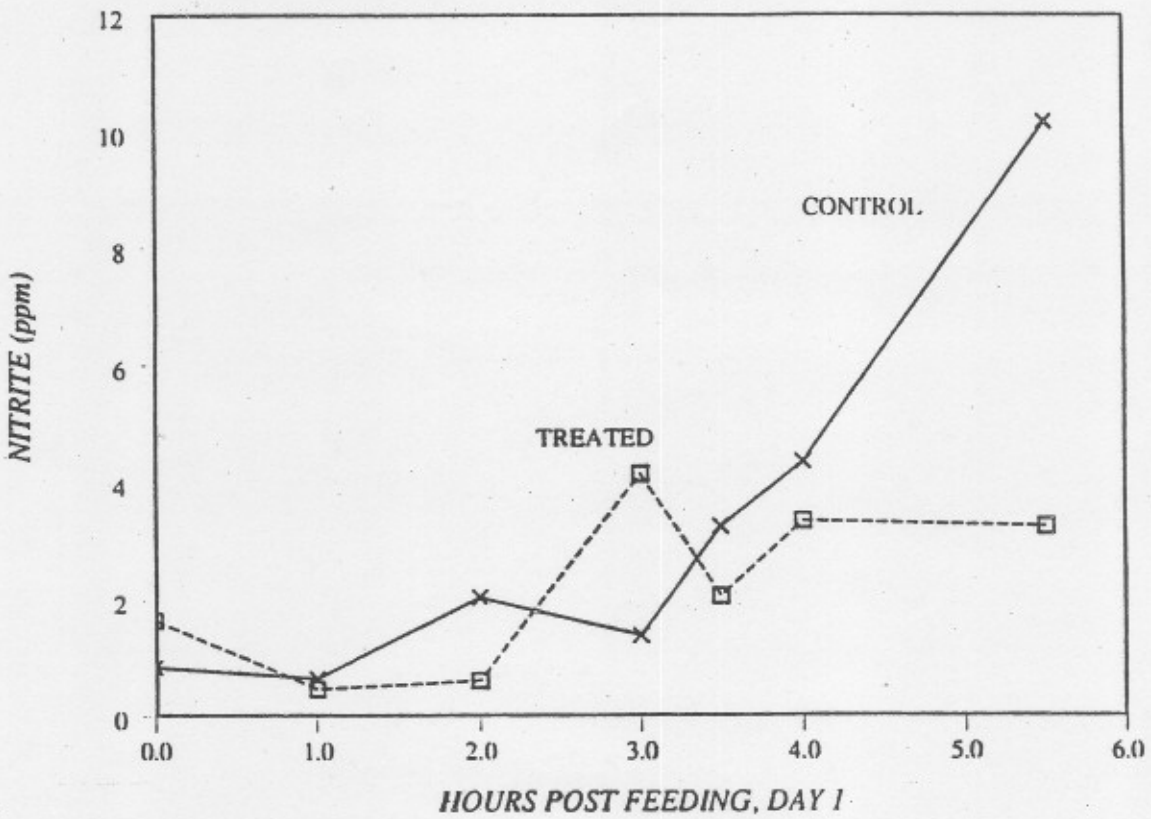


Figure 5. BLOOD NITRITE CONCENTRATIONS



Trial 1 - 20,000 ppm Nitrate Challenge:

Following 14 days on a low nitrate diet and seven days on low-quality native grass hay, all heifers were offered straight pearl millet hay (20,000 ppm nitrate) as a high nitrate challenge. Frequent rumen and blood samples were collected to monitor nitrate/nitrite status. Ruminal nitrate concentrations (figure 3) increased rapidly and peaked two hours post-feeding. Inoculated heifers had ruminal nitrate concentrations that were similar to the controls even though hay intake was higher (21%) for the inoculated heifers. Ruminal nitrate concentrations peaked at approximately 450 ppm which was 3-fold higher than ruminal nitrate concentrations observed when animals were fed 10,000 ppm nitrate (data not shown). Compared to the control, ruminal nitrite concentrations appeared to increase sooner for the inoculated heifers but also declined more rapidly (figure 4). The impact of this response can be considered two ways. First, by extrapolation of the nitrite curves to zero, the area under the curve, or total quantity of ruminal nitrite was reduced by 43% for the inoculated heifers compared to the controls. Alternately, if we arbitrarily choose 200 ppm as a toxic threshold, control heifers will be exposed to ruminal nitrite concentrations in excess of 200 ppm 40% longer than inoculated heifers. Regardless of the method of expression, the ruminal nitrite load was significantly reduced with propionibacteria inoculation.

Blood nitrite concentrations (figure 5) verify the differences noted in ruminal nitrite. Blood nitrite concentrations increased slowly until three hours post-feeding and then plateaued. In contrast, blood nitrite concentrations for the control heifers began to increase at 3 hours post-feeding and continued to increase for the remainder of the sampling period. These data suggest that control heifers were exposed to a significantly higher blood nitrite load than heifers inoculated with propionibacteria.

Trial 2 - 20,000 ppm Nitrate Challenge:

In order to obtain additional data to confirm the findings of trial 1, a duplicate high nitrate challenge trial was conducted. Following 14 days on a low nitrate diet and seven days on low-quality native grass hay, all heifers were offered straight pearl millet hay (20,000 ppm nitrate) as a high nitrate challenge. Rumen samples were collected to monitor nitrate/nitrite status. Blood samples were collected to monitor nitrite, and in addition, methemoglobin and total hemoglobin concentrations. Ruminal nitrate concentrations (figure 6) increased rapidly and peaked two hours post-feeding. Inoculated heifers had ruminal nitrate concentrations that were similar to the controls even though hay intake was higher (17.5%) for the inoculated heifers. Ruminal nitrate concentrations peaked at approximately 750 ppm which was 67% higher than ruminal nitrate concentrations observed in trial 1. Ruminal nitrite concentrations (figure 7) appeared to increase later for the inoculated heifers and peaked at a much lower level (525 ppm). Ruminal nitrite concentrations in control heifers increased more rapidly and peaked at 1075 ppm. Compared to the control, total rumen nitrite was reduced by 46% for the inoculated heifers. This was nearly identical to the 43% reduction in total ruminal nitrite observed in trial 1.

Blood nitrite concentrations (figure 8) mimicked the differences noted in ruminal nitrite concentrations. Blood nitrite concentrations increased slowly in both groups until three hours post-feeding. Blood nitrite concentrations for the treated heifers peaked at 13 ppm and decreased rapidly. In contrast, blood nitrite concentrations for the control heifers peaked at 22 ppm and decreased more slowly. These data suggest that control heifers were exposed to a significantly higher blood nitrite load than the heifers inoculated with propionibacteria. Methemoglobin concentrations (figure 9) remained low until three hours post-feeding. Concentrations of methemoglobin for the treated heifers increased from three to six hours post-feeding and then plateaued at 1.75 g/100 ml of blood. In contrast, methemoglobin concentrations for control heifers continued to increase from three to seven hours post-feeding to a concentration of 2.75 g/100 ml of blood. After seven hours post-feeding, the mean methemoglobin concentration for the control group appears to decrease however, this was due to the elimination of methemoglobin concentrations from blood samples not collected from a heifer that was treated for symptoms of acute nitrate toxicity.

Summary and Conclusions - Trials 1 and 2:

Ruminal nitrate/nitrite and blood nitrite increased significantly when high nitrate hay (20,000 ppm) was fed to unadapted heifers. Inoculation with propionibacteria reduced ruminal nitrite load by 40-46% and minimized changes in blood nitrite and methemoglobin. In contrast, blood nitrite and methemoglobin concentrations in control heifers continued to increase throughout the sampling period. When the increased hay intake with propionibacteria feeding is also noted, this response becomes even more remarkable.

In conclusion, these studies suggest that continuous inoculation with propionibacteria may have a significant prophylactic value when feeding high nitrate hay. If the 40%-46% reduction in ruminal nitrite is accurate, this suggests that hay containing 16,600 ppm nitrate could be fed with the same confidence as 10,000 ppm nitrate hay. Consequently, the risk of nitrate toxicosis would be significantly reduced.

Additional Considerations for the Development of a Direct-fed Microbial to Reduce Nitrate Toxicity.

A number of other issues important to the function of *Propionibacterium* strain P5 in reducing ruminal nitrite levels have been examined. These include the susceptibility of strain P5 to common antibiotics used in the industry, long-term ruminal establishment, retention of nitrite reductase activity and determination of the minimum effective dose. A brief summary of some of the experiments conducted to address these issues is presented.

Susceptibility of P5 to Antimicrobials

Figure 8. BLOOD NITRITE CONCENTRATIONS

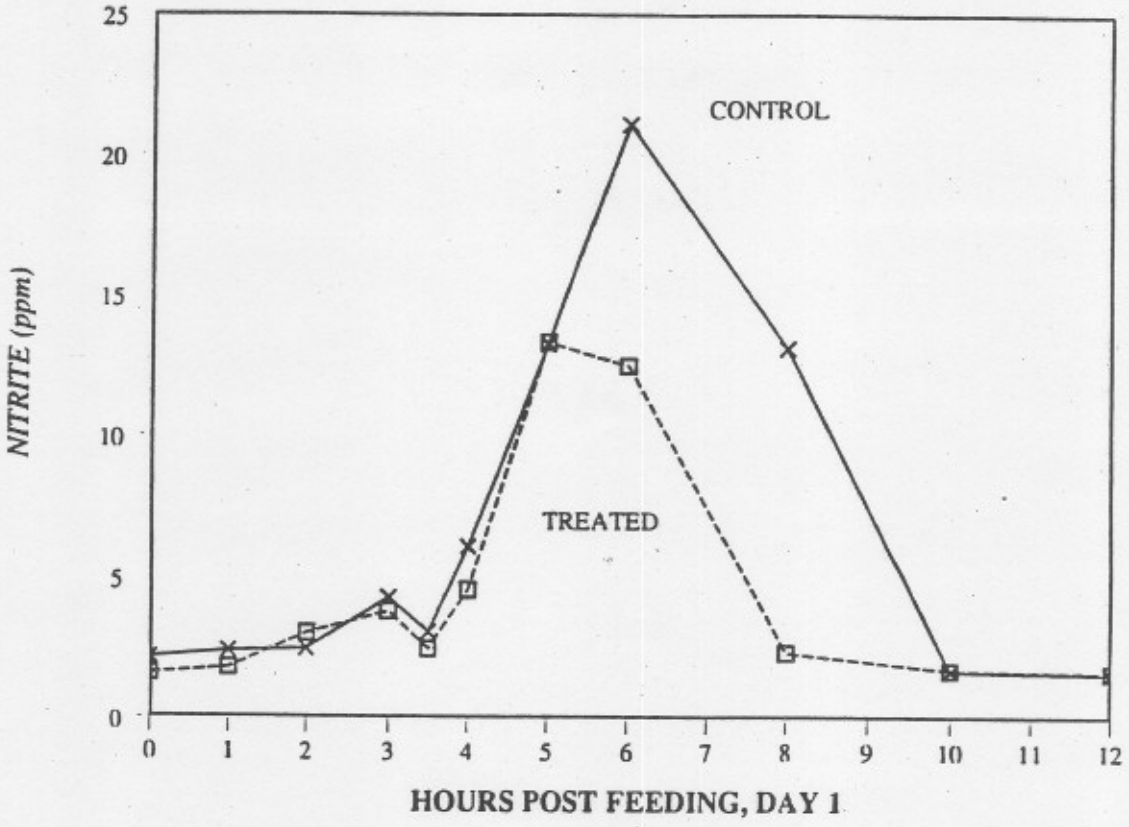
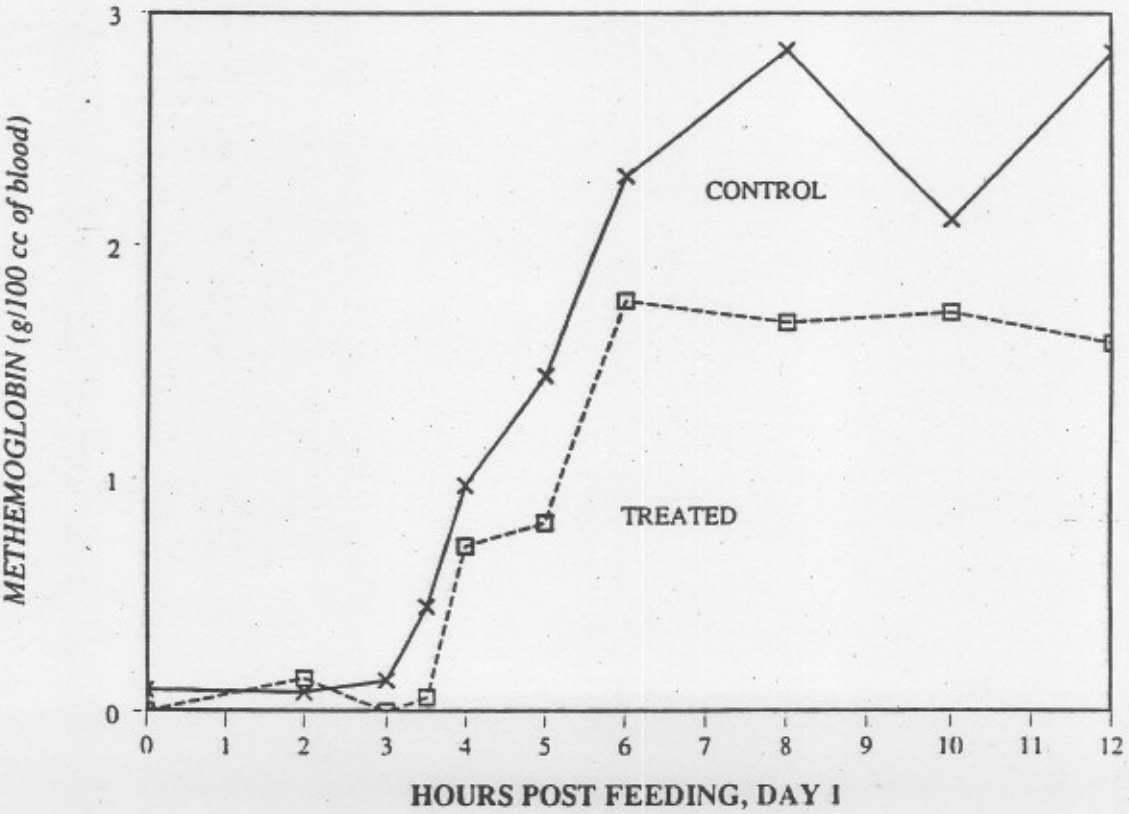


Figure 9. METHEMOGLOBIN CONCENTRATIONS



The survival of strain P5 in rumen fluid was monitored in vitro in the presence of various antibiotics commonly used as animal health products in the cattle industry. RUMENSIN (monensin, sodium salt), AUREOMYCIN (chlortetracycline) and DECCOX (decoquinate) were obtained as feed-grade antibiotics from commercial sources. Antibiotics were added individually at recommended levels and at 2 times the recommended levels to rumen fluid samples. In order to test for effects on both established populations and a fresh inoculum, rumen fluid consisted of composite samples from animals established with strain P5 and propionibacteria-free composite rumen samples to which a fresh inoculum of freeze-dried strain P5 was added. Rumen samples were incubated at 39 C for 48 hours. Samples were taken every 12 hours to enumerate the propionibacteria populations. Controls consisted of both types of rumen fluid samples without antibiotics added.

None of the antibiotics tested decreased the propionibacteria population of the established or freshly inoculated rumen fluid. Therefore, it appears that these antibiotics will have no adverse effects on establishment or maintenance of strain P5 populations in the rumen.

Maintenance of P5 in the Rumen

Initial ruminal establishment studies of strain P5 focused on the establishment time and level of P5 populations achieved with daily dosing. Counts taken at two and ten days post-inoculation indicated that the propionibacteria populations were maintained however, long term ruminal establishment of strain P5 was not initially examined.

Long term establishment/maintenance

Three crossbred cows fitted with ruminal cannulas were used to evaluate the long term survival and maintenance of populations of strain P5 in the rumen. Each cow was dosed daily (10^7 CFU/ml) with strain P5 for a period of 21 days. Following this establishment period, dosing to all cows ceased. Ruminal samples were taken monthly to determine the viable propionibacteria populations.

At the end of the 21 day establishment period, all cows had populations greater or equal to 10^5 CFU/ml. Results of monthly sampling indicate that populations fluctuate from 10^4 to 10^5 CFU/ml but have been maintained at these levels for 180 days.

Maintenance of Denitrification Activity

The consistent results of enumeration studies indicate the long term establishment and maintenance of strain P5 in the rumen. While this is encouraging, the dentrifying activity of these populations has never been confirmed. Given the fact that denitrification activity has been reported to be inducible in propionibacteria, the question remained as to whether an established culture is still capable of denitrification. Further, if denitrification activity is present, is it enough to protect the animal from nitrate toxicity?

Effects of withdrawal time on dentrification.

Six heifers fitted with ruminal cannulas were used to evaluate the effect of time of withdrawal on dentrification activity. Two heifers were dosed daily (10^7 CFU/ml) throughout the study, two heifers were withdrawn from daily dosing at day 0 and the remaining two heifers were not given any culture and had last received culture in April 1992. At 4, 7 and 11 days withdrawal from daily dosing, rumen fluid was collected from all heifers and used in an in vitro dentrification assay.

In vitro ruminal nitrate concentrations on day 4, 7 and 11 of withdrawal were higher than animals dosed daily or withdrawn for 7 months. Thus the rate of nitrate reduction was lower for these animals. Ruminal nitrite concentrations on day 4, 7 and 11 of withdrawal were significantly lower than animals dosed daily or withdrawn for 7 months. Taken together, these results suggest that during the withdrawal time examined here, heifers manage higher levels of nitrate more effectively. This is probably the direct result of reduced nitrate reduction. At extended withdrawal times (7 months), nitrate reduction increases; however, nitrite reduction activity still remains. If this activity is enough to protect the animal from nitrate toxicity will be addressed in future in vivo studies.

The addition of freeze dried cells of P5 to rumen fluid from animals withdrawn for 7 months tended to decrease nitrate reduction and increase nitrite reduction. These results provide preliminary evidence to support a one time dose for increasing the efficiency of managing high nitrate concentrations in the rumen.

The unexpected and interesting results observed in the withdrawal studies will be confirmed by an in vivo nitrate toxicity trial using heifers withdrawn at various times.

Dose Response

The daily dose of P5 found to significantly reduce the effects of a high nitrate diet was 10^7 CFU/ml of rumen fluid. Therefore, in a 350-500 lb animal (typical of stocker cattle) which has a 25 liter rumen, the effective daily dose is 2.5×10^{11} CFU. Based on establishment trials, feeding this daily dose for 7-10 days will establish the effective population. Lower doses of P5 have not been examined. In order to more accurately determine the minimal effective dose, a dose response trial was conducted.

Dose Response in Vitro

Freeze-dried strain P5 was added to flasks of propionibacteria-free rumen fluid to provide a 10^5, 10^7, and 10^9 CFU/ml inoculum. Nitrate was added to each flask to a level of 1500 ppm and the flasks were incubated at 39 C. Nitrite levels were monitored over a 30 hour period. Duplicate flasks were prepared for each inoculum level.

Nitrite concentrations in rumen fluid treated with 10^9 CFU/ml increased slower and remained lower than samples treated with lower levels of strain P5 (figure 10). Nitrite concentration decreased faster in rumen samples treated with 10^7 or 10^9 CFU/ml compared to samples treated with 10^5 CFU/ml. These data suggest that there is a significant dose response effect.

While the in vitro assays indicate there was a significant dose response effect, the level necessary to prevent nitrate toxicity in vivo is still unknown. Animal trials have been

conducted to determine the minimum effective dose. At the time of this publication, the results from these trials had not been completely analyzed.

Implications

The nitrate toxicity studies suggest that *Propionibacterium* strain P5 appears to exert a measure of prophylaxis when cattle consume large quantities of nitrate. Consequently, production losses to nitrate toxicity should be significantly reduced when producers use this strain as a direct-fed microbial at effective doses. Strain P5 is stably maintained in the rumen without continuous feeding and retains nitrite reduction activity. The viability of strain P5 is unaffected by antimicrobials such as monensin, chlorotetracycline and dequinate. Therefore, a direct-fed microbial product containing strain P5 fed at effective doses will be an effective component of a nitrate management program. In addition, increased hay intake with propionibacteria inoculation suggests that these organisms exert some physiological effect to stimulate appetite. We do not know whether this response is due to increased fermentation of the diet or animal to animal variation. Consequently, the validity and physiological basis for this intake response must be determined.

Figure 10.

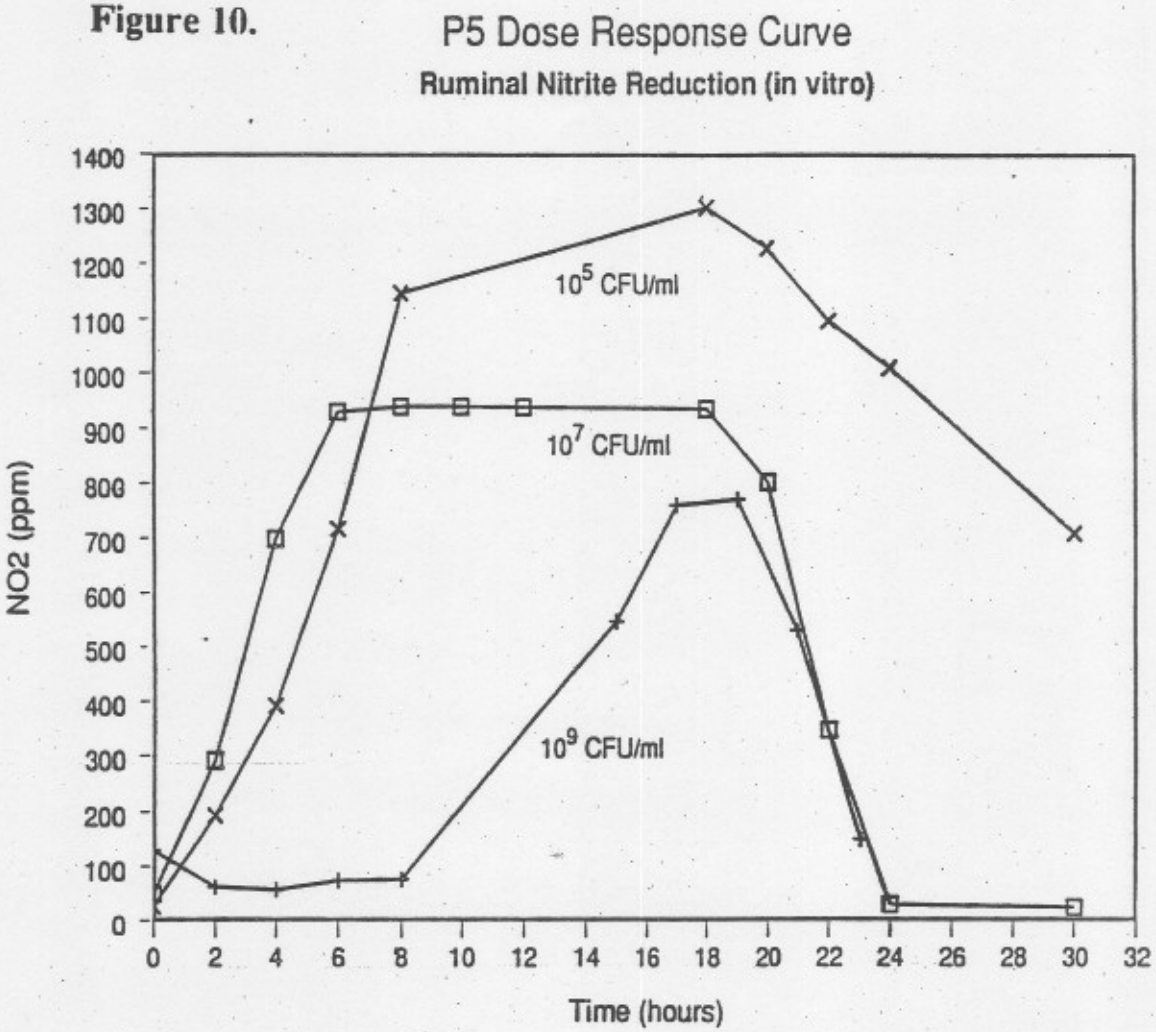

P5 Dose Response Curve
Ruminal Nitrite Reduction (in vitro)
NO2 (ppm)
Time (hours)
10^5 CFU/ml
10^7 CFU/ml
10^9 CFU/ml
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
0
2
4
6
8
10
12
14
16
18
20
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Table 10. Relationship between nitrate concentration (ppm), hay intake (lb/day) and total nitrate intake (g nitrate/day).

Feed intake, lb/d	Forage nitrate, ppm				
	1,000	5,000	10,000	20,000	50,000
	g nitrate/day				
5	2.27	11.35	22.7	45.4	113.5
10	4.54	22.7	45.4	90.8	227
15	6.81	34.05	68.1	136.2	340.5
20	9.08	45.4	90.8	181.6	454
25	11.35	56.75	113.5	227	567.5

What steps can be taken prior to, or during, preliminary exposure to nitrate?

1. Fill hungry cattle prior to release. If cattle are hungry, take the time (1-3 days) to make sure they are consuming a significant quantity of a bulky forage such as good quality grass hay. Then, release the cattle in the afternoon when they are not as hungry.

2. Adapt cattle to nitrate. The objective is to give the ruminal microorganisms the opportunity to adapt to high nitrate intake. With high nitrate hay, this can be accomplished by blending with low nitrate feeds such as grass hay or concentrates. Grain feeding has the additional benefit of providing ruminal energy to stimulate the conversion of nitrate to nontoxic nitrogen compounds. With grazed high nitrate forages, palatable, low nitrate hay or concentrates can be used. Another alternative with grazed forage is to limit graze for the first 6 to 8 days by increasing the grazing time each day. For example, cattle might be allowed to graze high nitrate forage for 2 hours on day 1 and increase by 2 hours each day through day 6 after which cattle could be released full time onto the high nitrate forage. Another strategy with grazed forage would be to feed the animals several times per day (3-5x/day) to disrupt grazing periods and provide ruminal fill to decrease the rate and extent of consumption of the high nitrate forage.

3. Dilute high nitrate feeds with low nitrate feeds. Dilution is one method that can be used to help ruminal microorganisms adapt to high nitrate feeds. But, it may also be the only practical method that can be used to feed extremely high nitrate forage (>20,000 ppm). Dilution is most effective when the low nitrate feed can be blended or mixed directly with the high nitrate feed.

4. Utilize propionibacteria. Some strains of propionibacteria are capable of rapidly reducing nitrite to nontoxic nitrogen compounds. These bacteria can be established in the rumen by feeding them for a minimum of eight days prior to a nitrate challenge. Once established, they have the capability of reducing ruminal and blood nitrite concentrations by 40 to 50%. Although the propionibacteria can effectively reduce the probability of nitrate toxicity, other methods of nitrate management should also be employed to minimize nitrate exposure.

5. Release cattle in afternoon when night-time nitrate accumulations have subsided. In addition, avoid release shortly after a drought-ending rain.

6. Stock lightly so animals can choose lower nitrate leaves over higher nitrate stems (Fjell et al., 1991)

7. Provide large quantities of fresh drinking water. Water dilutes nitrate concentrations in the rumen and reduces the potential of toxicity (Fjell et al., 1991).

Nitrate management scenarios

The objective of this section is to present five scenarios selected to illustrate the major concerns relative to a specific situation and the types of management that could be applied to minimize the effects of nitrate consumption.

Scenario #1: High nitrate hay as an emergency feed for beef cows

Sorghum/sudan hay harvested in August is to be used as an emergency feed source for some spring-calving beef cows during winter.

Concerns:

What is the stage of production (month relative to calving date)?

- If cows are in early gestation even low nitrate levels could cause abortions.
- If cows are in late gestation, excess nitrate intake could kill or weaken the fetus so that calves may die at, or shortly after, birth (Broadmeadow et al., 1984).
- If cows are in early lactation, abortions are not a concern so attention must be focused on the effects of nitrate on the cow.

What is the nitrate content of the hay?

- To determine the nitrate content of the hay, representative samples must be collected and analyzed. During sampling, bales should be identified so that "hot" bales can be sorted.

How much hay will the cows eat?

- If snow or ice cover, cows can consume an extremely large quantity of hay (25-35 lb/cow).
- If hay is fed during cold, open weather, intake will likely be less (8-15 lb/cow) and dependent on feeding rate.

Management:

Determine susceptibility of cows based on stage of production.

Calculate potential nitrate intake from hay nitrate analysis and projected hay intake.

Sort bales by nitrate content:

- Feed low to moderate nitrate bales (<10,000 ppm) as emergency feeds.
- Feed higher nitrate bales (>10,000 ppm) as a supplemental feed (4-8 lb/cow/day).
- Consider discarding extremely high nitrate bales (>20,000 ppm)

Consider some combination of the following:

Watch weather and start feeding low to moderate nitrate hay prior to inclement weather to adapt ruminal microorganisms to nitrate. Slowly increase feeding level so that cows are adapted by the time the storm arrives.

Feed high energy grain cubes to dilute nitrate intake and provide energy to stimulate microbial detoxification of nitrate.

Establish propionibacteria (feed for 8 days) prior to nitrate exposure to minimize the effects of nitrate intake.

Scenario #2: High nitrate hay as a supplemental feed for wintering beef cows

Sorghum/sudan hay harvested in August is to be used through the winter as a supplemental feed source for spring-calving beef cows.

Concerns:

What is the nitrate content of the hay?

To determine the nitrate content of the hay, representative samples must be collected and analyzed. During sampling, bales should be identified so that "hot" bales can be sorted.

How much hay do the cows need to eat?

Consider stage of production, forage quality and forage quantity to determine appropriate feeding rate. Based on this feeding rate and the nitrate content of the hay, what is the nitrate exposure?

Management:

Determine the potential nitrate exposure based on hay intake and forage nitrate concentrations.

Sort bales by nitrate content:

Feed low to moderate nitrate bales (<10,000 ppm) as emergency feeds.

Feed high nitrate bales (>10,000 ppm) as a supplemental feed (4-8 lb/cow/day).

Discard extremely high nitrate (>20,000 ppm).

Consider some combination of the following:

Adapt cattle with low to moderate nitrate hay by slowly increasing the feeding level.

Feed high energy grain cubes to dilute nitrate intake and provide energy to stimulate microbial detoxification of nitrate.

Establish propionibacteria (feed for 8 days) to minimize the effects of nitrate intake.

Scenario #3: Summer grazing of sorghum/sudan by beef stockers

Beef stockers (4-600 lb) will be released onto sorghum/sudan in mid summer (July 1).

Concerns:

What is the nitrate potential?

Although it is difficult to determine the actual nitrate content of a field, a thorough knowledge of the circumstances or factors that promote nitrate accumulation in plants should help us predict when nitrates could be a problem. For example, fertilization rates, rainfall, variety, etc. can all affect the potential for nitrate accumulation. If nitrate accumulation is likely, take extra management precautions to minimize nitrate intoxication.

Have cattle recovered from shipping stress?

Stressed, hungry cattle should not be released directly on to potentially toxic forage. Use the opportunity to put the cattle through a recovery program so that ruminal function and health status are normal. Then release the cattle.

Management:

Evaluate the potential nitrate exposure based on environmental conditions and previous management decisions. If necessary, delay release.

Consider some combination of the following:

Adapt cattle to nitrate:

Use increasing levels of high nitrate feeds harvested in previous years.

Adapt cattle to sorghum/sudan forage by limit grazing for increasing hours for 5-7 days.

Feed a high energy receiving ration to stimulate ruminal recovery and increase ruminal energy prior to release.

Feed frequently to disrupt grazing patterns and provide fill.

Establish propionibacteria (feed for 8 days) to minimize the effects of nitrate intake.

Consider releasing in the afternoon when cattle are full and appetite is low.

Scenario #4: High nitrate forages in receiving programs

Newly received beef calves (4-800 lb) are fed sorghum/sudan-based receiving rations.

Concerns:

What is the nitrate potential?

Nitrate analysis of sorghum/sudan hay is essential. Bales should be sampled and identified to allow sorting into low and high nitrate groups. Design concentrate portion of diet so that nitrate is diluted adequately and energy is available to stimulate nitrate utilization by ruminal microorganisms.

How stressed are the cattle?

Moderately stressed cattle may consume large quantities of high nitrate hay because they are hungry and sorghum/sudan hay can be very palatable.

Heavily stressed cattle may not be hungry and their microbial activity may be low so they should not be as susceptible to nitrate upon arrival. As they recover from the stress and increase intake, nitrates may become more of a concern.

Management:

Minimize initial nitrate exposure for newly received cattle by using low nitrate hay first.

Consider some combination of the following:

Use increasing levels of high nitrate feeds to slowly adapt cattle to nitrate.

Feed a high energy receiving ration to stimulate ruminal recovery and increase ruminal energy.

Establish propionibacteria (feed for 8 days) to minimize the effects of nitrate intake.

Scenario #5: Dairy cows exposed to multiple sources of nitrate

Dairy cows are fed a diet that contains 20% corn silage (DM basis) harvested during a dry summer and consume water that is known to be high in nitrate.

Concerns:

What is the nitrate content of the corn silage?

The silage must be representatively sampled and analyzed for nitrate content. Nitrate analysis shows 8,000 ppm.

What is the nitrate content of the water?

Water nitrate must be determined to evaluate its contribution to nitrate intake. Nitrate analysis shows 200 ppm.

Management:

Calculate total nitrate intake to evaluate risk.

A 1,500 lb Holstein producing 100 lb milk/day will consume 58 lb dry feed and 40 gallons of water.

58 lb DM X 20% silage X 8,000 ppm =>	42.1 g nitrate/day
40 gal X 7 lb/gal X 100 ppm =>	12.7 g nitrate/day
Total nitrate intake	54.8 g nitrate/day

54.8 g nitrate X 1,000 mg/g /1,500 lb cow = 36 mg nitrate/lb BW

This quantity of nitrate (36 mg/lb BW) is within the range where rebreeding performance may be reduced and early term abortions may occur (Table 3). Acute toxicity, however, is not a concern.

Evaluate alternative roughage sources. To completely compensate for water nitrate, silage intake must be reduced to 2 lb DM/cow/day or 3.4% of diet DM. This level of corn silage may not be worth the trouble. Perhaps other, low nitrate, forages should be purchased for use with the high producing cows. The higher nitrate corn silage could be used with dry cows or other less productive animals.

Evaluate alternative water sources. If wells are the water source, consider drilling a deeper well to potentially draw lower nitrate water. Also evaluate other water

sources such as rural or city water. If surface water is used, determine the source of the nitrates (manure runoff, excess fertilizer, etc) and attempt to control the nitrate source.

Establish propionibacteria (feed for 8 days) to reduce nitrate effects in the rumen. Caution: Propionibacteria do not have a demonstrated effect on subacute nitrate toxicity.

Identify nitrate sensitive cows and cull. Some individuals are less able to physiologically manage nitrates and thus, are more susceptible to nitrate intake. These animals might be identified by blood nitrite or methemoglobin concentrations when they are first exposed to high nitrate diets. These animals should probably be culled.

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HYBRID SUDANGRASS PERFORMANCE TRIALS IN OKLAHOMA, 1990

S.E. Hawkins and G.L. Strickland

Each year, performance trials for hybrid sudangrasses are conducted in Oklahoma to provide producers with useful information in selecting hybrids for the next growing season. These trials, are conducted in various locations throughout the state to indicate which hybrids are adaptable to general areas and growing conditions. These trials are conducted in fulfillment of Oklahoma State Department of Agriculture seed regulations, section 8-112.

To stay updated on current hybrids and new releases, producers should consult yield trials conducted by OSU, seed companies and other sources. Producers are encouraged to plant some of the hybrids they presume will perform well in their location to evaluate their performance under producer conditions in small areas on their farms.

Hybrid sudangrasses and pearl millets are listed in Table 1. This table includes the company, hybrid name, and type of hybrid cross.

Seventeen sudangrass hybrids and three pearl millets were tested at three locations (Tables 2 to 5). The locations are: Eastern Research Station, Muskogee County; South Central Research Station, Grady County, Southwest Agronomy Research Station, Tillman County. A randomized complete block design was used at each location. At time of planting both soil and moisture conditions were good at all locations for germination and emergence. An ensuing dry period during the months of June and July relegated good early plant growth and utilization of available fertilizer. All locations were fertilized in accordance with OSU soil test recommendations. Each location was cut prior to seed head exertion and top dressed, to provide adequate nitrogen for secondary growth.

A tractor-powered cone planter was used to plant all tests. A seeding rate of approximately 300,000 seeds per acre (17-20 lbs/A) was used. Recommendations on seeding rates will vary with company and hybrid. To calculate the pure live seed rate desired, use the following equation; Recommended pure live seed per acre ÷ germination % = lbs of seed to plant. Plots were harvested with a Carter Forage harvester and the reported yields are based on 16% moisture to resemble normal hay production.

Small differences should not be overemphasized since these can be due to variations in soils, climate and uncontrollable experimental error (Table 2 to 5). Least significant differences (L.S.D.) are shown at the bottom of each table. Unless two entries differ by at least the L.S.D. shown, little confidence can be placed in one entry being superior to another. If differences between two entries exceed the L.S.D. (0.05) value given for that data, the chances are approximately 95 out of 100 that the apparent difference is real. The coefficient of variability is an estimate of the precision of the data, with the higher C.V. indicating greater variability. The C.V. and L.S.D. are directly relaated in that a higher C.V. will also generate a higher L.S.D.

Two and three year means have been provided in each table for the hybrids that have appeared in the test for two and three years. Producers interested in comparing consistency of hybrid yields over a 2 or 3 year period should review these mean yields. The yield levels may differ between years, but the relative rankings remain similar for most hybrids. Producers looking for hybrids with above average yield potential should consider the top 5 or 6 hybrids in a group.

All proteins contain nitrogen. One ton of forage testing 12.5% crude protein contains 40 lbs of nitrogen. Somem nitrogen will be available from the soil but without the addition of fertilizer nitrogen, production will be limited to about 1.5 tons per acre for the year.

Fifty pounds of actual nitrogen per acre applied at planting time plus 50 lbs of nitrogen topdressed after each cutting will allow near optimum forage production and reduce the risk of nitrate accumulations.

Sudan and sudan hybrids can vary a great deal in quality (protein and digestibility). Production and harvesting practices can control much of this variability.

Optimum yield and digestibility can be obtained by harvesting at the "boot" state of growth (just before the head appears). Digestibility drops rapidly as a forage plant starts to produce seed and only a small increase in tonnage occurs during that time.

Additional information can be found in OSU Extension Facts No. 2568, **Protein-Nitrogen Relationship in Forages** and at your local OSU County Extension Center.

Sudan and hybrid sudan grasses may contain potentially harmful levels of nitrate and prussic acid, while pearl millets may contain harmful levels of nitrates. Proper management of grazing, haying, and ensiling can reduce potential risks. Additional information on nitrate and prussic acid can be found in OSU Current Report No. 3272, **Nitrate and Prussic Acid Poisoning in Cattle** and at your local OSU County Extension Center.

This past growing season provided moisture stress that resulted in the accumulation of nitrates in sudans, hybrid sudans, and pearl millets in many areas of the state. All three types of hay should be sampled and tested for nitrates to be safe. For information on where this analysis may be performed contact your county extension office.

Contributors

The following people have contributed to this report by gathering data or assisting in its final preparation: Rocky Thacker, Mike Goodson, Toby Kelly, Larry Bull, Jimmy Gaither, Vernon (Bucky) Young, Alton Young, Lynn Halford, Jerry Walker, Tommy Pickard, Dwayne Miller, Don Hooper, Lawrence Hurt, William Rayburn, Randall Boldt, Jerry McLemore, Jimmie Wheeler, Barbara Herring.

Table 1. Hybrid Sudangrass Performance Entries, 1990.

COMPANY	ENTRY DESIGNATION	TYPE OF CROSS
AR-B SEEDS INC.	AR-B SWEET II	B
ASGROW SEED CO.	GRAZER SG	A
BIG CROP SEED	SWEETER-N-HONEY	A
CARGILL HYBRID SEEDS	SS111C	A
CARGILL HYBRID SEEDS	SWEET SIOUX V	B
CARGILL HYBRID SEEDS	HS33	C
CARGILL HYBRID SEEDS	X8530-145	C
DELTA AND PINE LAND CO.	DELTAPINE FP5	A
GARRISON SEED CO.	S.G. GAINER	A
GARRISON SEED CO.	SUGAR DAN	A
LEVELLAND DELINTING CO.	ALL-TEX	A
NC+ HYBRIDS	NC+ SWEETLEAF	B
NC+ HYBRIDS	NC+ 200	C
NC+ HYBRIDS	EXP MILL I (P.M.)	PM
NC+ HYBRIDS	EXP MILL II (P.M.)	PM
NORTHRUP KING CO.	MILLEX 24 (P.M.)	PM
OKLAHOMA SEED CO.	OKLAHOMA'S BEST	A
PIONEER HI-BRED INT'L.	979	A
VISTA	TRUE	C
WARNER SEED CO.	W-8493	B

HYBRID TYPES
A) SORGHUM-SUDANGRASS
B) SORGO - SUDAN
C) SUDAN X SUDAN
PM) Pearl Millet

Table 3. Grady County, Hybrid Sudangrass and Pearl Millet Forage Yields, South Central Research Station, Chickasha, OK.

Entry Designation	Hybrid Type	Yield reported @ 16% moisture Annual Total Yield 1989	Annual Total Yield 1990	1st Cut 1990	2nd Cut 1990	3rd Cut 1990
		*		(Tons/A)		
GRAZER SG	Sorghum-	–	14.87	6.38	4.09	4.41
SUGAR DAN	Sudangrass	–	12.53	6.77	3.63	2.12
SSIIIC	Hybrids	12.90 (9)	12.13	5.40	4.14	2.58
OKLAHOMA'S BEST		12.32 (11)	11.78	6.58	3.73	1.46
ALL TEX		11.13 (14)	11.74	5.12	4.56	2.05
979		14.93 (3)	10.76	5.33	4.03	1.41
SWEETER-N-HONEY		–	10.21	4.49	3.61	2.11
DELTAPINE FP5		13.93 (6)	10.05	4.73	4.09	1.22
S.G. GAINER		–	9.83	4.29	3.55	1.99
NC+ SWEETLEAF	Sorgo-	–	15.73	7.05	4.47	4.21
W-8493	Sudangrass	–	11.64	4.94	3.35	3.35
SWEET SIOUX V	Hybrids	12.62 (9)	11.07	5.22	3.87	1.98
AR-B SWEET II		13.39 (5)	9.18	4.03	3.00	2.14
TRUE	Sudan-	–	10.69	4.38	4.30	2.01
HS33	Sudan	–	10.22	4.30	3.27	2.64
X8530-145	Hybrids	–	9.95	4.27	3.25	2.42
NC+ 200		–	9.21	3.80	3.26	2.15
MILLEX 24	Pearl	11.96 (3)	6.61	4.35	1.47	0.80
EXP MILL II	Millets	–	6.25	3.35	1.73	1.17
EXP MILL I		–	5.88	3.15	1.62	1.10
Overall Mean			11.01	4.93	3.47	2.62
LSD (0.05)			3.22	1.54	1.14	2.06
C.V.			20.85			

Soil Name: Reinach silt loam Row Width: 12-inches

Monthly Rainfall (in.):

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
1.92	5.00	6.42	5.21	5.59	1.92	2.47	3.48	2.76	1.90

Ferilization: Preplant N: 50 lbs/A P_2O_5: 0 lbs/A K_2O: 0 lbs/A
Postemergenc N: 50 lbs/A applied after 1st and 2nd harvest

Planted: 5-23-90 Harvested: 7-11, 8-23, 10-12-90

*Number in parenthesis indicates the rank position of the hybrid for that year and hybrid type.

Table 2. Muskogee County, Hybrid Sudangrass and Pearl Millet Forage Yields, Eastern Research Station, Haskell, OK.

Entry Designation	Hybrid Type	Annual Total Yield 1988 *	Annual Total Yield 1989 *	Yield reported @ 16% moisture* Annual Total Yield 1990	1st Cut 1990	2nd Cut 1990	3rd Cut 1990
				(Tons/A)			
SUGAR GRAZE II	Sorghum-	–	–	6.45	1.58	2.71	2.15
ALL TEX	Sudangrass	9.25 (7)	9.48 (10)	6.41	1.57	2.93	1.91
GRAZER SG	Hybrids	–	–	6.29	1.99	2.40	1.90
DELTAPINE FP5		8.70 (12)	8.98 (13)	6.16	1.91	2.38	1.88
979		10.36 (2)	11.18 (2)	6.00	1.89	2.29	1.81
SSIIIC		9.56 (9)	10.47 (5)	5.79	1.53	2.50	1.76
OKLAHOMA'S BEST		–	9.56	5.37	1.23	2.70	1.44
SWEETER-N-HONEY		–	–	5.24	1.31	2.59	1.34
S.G. GAINER		7.65 (5)	–	4.68	1.08	2.04	1.56
NC+ SWEETLEAF	Sorgo-	–	–	7.13	2.02	2.90	2.21
SWEET SIOUX V	Sudangrass	10.70 (3)	12.16 (1)	7.04	2.62	2.36	2.06
AR-B SWEET II	Hybrids	8.89 (9)	9.28 (8)	6.55	1.70	2.66	2.19
W-8493		–	–	6.04	1.56	2.77	1.71
TRUE	Sudan-Sudan	–	–	6.93	1.89	2.92	2.12
X-8530-145	Hybrid	–	–	6.68	1.77	2.72	2.20
HS33		–	–	5.84	1.99	2.34	1.51
NC+ 200		–	–	5.07	1.71	2.35	1.01
EXP MILL I	Pearl	–	–	6.08	2.35	3.02	0.71
MILLEX 24	Millets	–	9.23 (2)	5.67	2.26	2.93	0.48
EXP MILL II		–	–	4.47	1.83	2.44	0.20
Overall Mean				5.86	1.72	2.53	1.62
LSD (0.05)				NS	NS	0.62	0.88
C.V.				26.95			

Soil Name: Taloka silt loam Row Width: 12-inches

Monthly Rainfall (in.):

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
4.07	3.09	5.44	7.64	7.38	.86	2.65	1.42	8.30	1.77

Fertilization: Preplant: N: 50 lbs/A P_2O_5: 0 lbs/A K_2O: 0 lbs/A
Postemergence: N: 50 lbs/A applied after 1st and 2nd harvest.

Planted: 5-29-90 Harvested: 7-11, 8-17, 10-19-90

*Number in parenthesis indicates the rank position of the hybrid for that year and hybrid type.

Table 4. Tillman Co., Hybrid Sudangrass and Pearl MilletForage Yields, Southwest Agronomy Research Station, Tipton, OK.

Entry Designation	Hybrid Type	Yield reported @ 16% moisture Annual Total Yield 1989	 1990	1st Cut 1990	2nd Cut 1990
		*	(Tons/A)		
S.G. GAINER	Sorghum-	–	10.34	3.88	6.46
OKLAHOMA'S BEST	Sudangrass	10.57 (1)	10.16	4.39	5.78
ALL-TEX	Hybrids	8.90 (6)	8.77	4.14	4.63
979		8.05 (9)	8.67	3.84	4.83
SUGAR DAN		–	8.61	4.25	4.36
SWEETER-N-HONEY		–	8.06	3.31	4.75
GRAZER SG		–	7.93	3.79	4.15
DELTAPINE FP5		10.55 (2)	7.69	3.52	4.17
SSIIIC		7.78 (11)	6.52	2.81	3.71
W-8493	Sorgo-	–	9.70	3.87	5.83
AR-B SWEET II	Sudangrass	6.79 (9)	9.49	4.34	5.15
SWEET SIOUX V	Hybrids	6.93 (7)	9.37	3.61	5.76
NC+ SWEETLEAF		–	8.88	3.20	5.68
HS33	Sudan-	–	10.22	3.67	6.55
X8530-1450	Sudan	–	9.72	3.32	6.39
TRUE	Hybrids	–	8.85	4.54	4.31
NC+ 200		–	8.42	3.78	4.63
EXP MILL I	Pearl	–	9.66	3.72	5.94
EXP MILL II	Millets	–	7.84	2.57	5.27
MILLEX 24		5.87 (3)	7.62	2.25	5.37
Overall Mean			8.91	3.80	5.11
LSD (0.05)			3.15	1.60	NS
C.V.			25.23		

Soil Name: Tipton silt loam Row Width: 12-inches

Fertilization: Preplant N: 50 lbs/A P_2O_5: 0 lbs/A K_2O: 0 lbs/A
Postemergence N: 50 lbs/A applied after 1st and 2nd harvest.

Planted: 6-7-90 Harvested: 8-6, 9-24-90
*Number in parenthesis indicates the rank position of the hybrid for that year and harvest type.

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Oklahoma State University

HYBRID SUDANGRASS PERFORMANCE TRIALS IN OKLAHOMA, 1991

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Each year, performance trials for hybrid sudangrasses are conducted in Oklahoma to provide producers with useful information in selecting hybrids for the next growing season. These trials are conducted in various locations throughout the state to indicate which hybrids are adaptable to general areas and growing conditions. These trials are conducted in fulfillment of Oklahoma State Department of Agriculture seed regulations, section 8-112.

To stay updated on current hybrids and new releases, producers should consult yield trials conducted by OSU, seed companies and other sources. Producers are encouraged to plant small areas of the hybrids they presume will perform well in their location to evaluate their performance under producer conditions.

Hybrid sudangrasses and pearl millets are listed in Table 1. This table includes the company, hybrid name, and type of hybrid cross.

Twenty nine sudangrass hybrids and five pearl millets were tested at four locations (Tables 2 to 6). The locations are; Eastern Research Station, Muskogee County; Perkins Research Station, Payne County; South Central Research Station, Grady County; Southwest Agronomy Research Station, Tillman County. A randomized complete block design was used at each location. At time of planting both soil and moisture conditions were good at all locations for germination and emergence.

Muskogee County experienced a dry period during the last part of the initial crop and the recurrent growth of the second crop lowering yields somewhat. Adequate rainfall was received for good 3rd harvest yields. Payne County had adequate rainfall for the initial crop but not for recurrent growth of the second crop.

Only second cutting yields are reported for Grady County due to an error that compromised the accuracy of the first cutting yields. Good rainfall distribution at this location resulted in good second harvest yields. A higher C.V. is indicated for this location due to the fact that only the second cutting was analyzed and the regrowth variation that is encountered among hybrids during secondary growth. Tillman County had good rainfall distribution and optimum conditions for good yields. All locations were fertilized in acoordance with OSU soil test recommendations. Each location was cut prior to seed head exertion and top dressed to provide adequate nitrogen for secondary growth.

A tractor-powered cone planter was used to plant all tests. A seeding rate of approximately 413,000 seeds per acre (25 lbs/A) was used. Recommendations on seeding rates will vary with company and hybrid. To calculate the pure live seed rate desired, use the following equation: Recommended pure live seed per acre ÷ germination % = lbs of seed to plant. Plots were harvested with a Carter Forage harvester and the reported yields are based on 16% moisture to resemble normal hay production.

Small differences should not be overemphasized since these can be due to variations in soils, climate and uncontrollable experimental error (Table 2 to 5). Least significant differences (L.S.D.) are shown at the bottom of each table. Unless two entries differ by at least the L.S.D. shown, little confidence can be placed in one entry being superior to another. If differences between two entries exceed the L.S.D. (0.05) value given for that data, the chances are that approximately 95% of the time the apparent difference is real. The coefficient of variability is an estimate of the precision of the data, with the higher C.V. indicating greater variability. The C.V. and L.S.D. are directly related in that a higher C.V. will also generate a higher L.S.D.

Two year means have been provided in each table for the hybrids that have appeared in the test for two consecutive years. Producers interested in comparing consistency of hybrid yields over a 2 year period should review these mean yields. The yield levels may differ between years, but the relative rankings remain similar for most hybrids. Producers looking for hybrids with above average yield potential should consider the top 5 or 6 hybrids in a group.

All proteins contain nitrogen. One ton of forage testing 12.5% crude protein contains 40 lbs of nitrogen. Some nitrogen will be available from the soil but without the addition of nitrogen fertilizer, production will be limited to about 1.5 tons per acre for the year.

Fifty pounds of actual nitrogen per acre applied at planting time plus 50 lbs. of nitrogen topdressed after each cutting will allow near optimum forage production and reduce the risk of nitrate accumulations.

Sudan and sudan hybrids can vary a great deal in quality (protein and digestibility). Production and harvesting practices can control much of this variability.

Optimum yield and digestibility can be obtained by harvesting at the "boot" state of growth (just before the head appears). Digestibility drops rapidly as a forage plant starts to produce seed and only a small increase in tonnage occurs during that time.

Additional information can be found in OSU Extension Facts No. 2568, **Protein-Nitrogen Relationship in Forages** and at your local OSU County Extension Center.

Sudan and hybrid sudan grasses may contain potentially harmful levels of nitrate and prussic acid, while pearl millets may contain harmful levels of nitrate. Proper management of grazing, haying, and ensiling can reduce potential risks. Additional information on nitrate and prussic acid can be found in OSU Current Report No. 3272, **Nitrate and Prussic Acid Poisoning in Cattle** and at your local OSU County Extension Center. When a problem is suspected all three types of hay should be sampled and tested for nitrate. For information on where this analysis may be performed, contact your County Extension office.

Contributors

The following people have contributed to this report by gathering data or assisting in its final preparation: Rocky Thacker, Mike Goodson, Toby Kelly, Larry Bull, Jimmy Gaither, Alton Young, Lynn Halford, Rick Matheson, Burt Coffman, Jerry Walker, Tommy Pickard, Dwayne Miller, Don Hooper, Lawrence Hurt, William Rayburn, Randall Boldt, Jimmie Wheeler and Barbara Herring.

Table 1. Hybrid Sudangrass and Pearl Millet Performance Entries, 1991.

Company	Entry Designation	Type of Cross
Agripro Seeds	HY-PRO (PM)	PM
BWI Texarkana	JACKPOT	A
BWI Texarkana	TOPCUT	B
Cargill Hybrid Seeds	SS111C	A
Cargill Hybrid Seeds	SWEET SIOUX V	B
Cargill Hybrid Seeds	HS35	C
Coffey Seed Co.	SUGAR QUEEN II	B
Coffey Seed Co.	SUGAR GRAZE III	A
Dekalb Pfizer Genetics	DEKALB SUDAX SX-132	A
Garrison Seed Company	SUGAR DAN	A
Garrison Seed Company	S.G. GAINER	B
Garrison Seed Company	TRI-SWEET +	A
Garst Seed Co.	757 G	A
Garst Seed Co.	PEARL MILLET (PM)	PM
Garst Seed Co.	SUPERMILL (PM)	PM
Gayland Ward Seed	SUPER SUGAR	B
George Warner Seed Co. Inc.	W-8493	B
Hobart Seed Co.	EXPERIMENTAL 850	A
Hyperformer Seed Company	TASTEMAKER III	A
Hyperformer Seed Company	HSC M2D (PM)	PM
Hyperformer Seed Co.	HB91-M22 (PM)	PM
James Reneau Seed Co.	SUPERGRAZE II	A
Justin Seed Co.	ROYAL SWEET	A
Justin Seed Co.	ROYAL REDTOP	A
MBS Seed Inc.	HAYMAN I	A
MBS Seed Inc.	GOTCHA II	B
NC+ Hybrids	NC+ SWEETLEAF II	B
Richardson Seed Co.	XSSP II	B
Scott Seed Co.	PREFERRED STOCK	A
Sharp Brothers Seed Co.	GRAZEX	A
Sharp Brothers Seed Co.	GRAZEX II	A
Sunburst Seed Co.	SUNBURST SWEET	A
Taylor Evans Seed Co.	HAYGRAZER-S	A
Cal/West Seeds	MONARCH V (CHECK)	C

Hybrid Types: A) Sorghum-Sudangrass B) Sorgo-Sudangrass
C) Sudan-Sudan PM) Pearl Millet

Table 2. Muskogee County, Hybrid Sudangrass and Pearl Millet Forage Yields, Eastern Research Station, Haskell, Ok.

Entry Designation	Hybrid Type	Annual Total Yield 1990	Yield reported @ 16% moisture 1991	1st Cut 1991	2nd Cut 1991	3rd Cut 1991
		(*)	(Tons/A)			
GRAZEX II	Sorghum-	-	8.48	2.86	2.27	3.35
GRAZEX	Sudangrass	-	8.44	3.14	2.13	3.17
SS111C	Hybrids	5.79(6)	7.49	2.31	1.77	3.42
JACKPOT		-	7.07	2.64	1.53	2.90
SUGAR DAN		6.45(1)	7.07	2.27	1.81	2.99
757 G		-	7.05	2.68	1.49	2.88
SUGAR GRAZE III		-	6.96	2.68	1.62	2.66
EXPERIMENTAL 850		-	6.94	2.61	1.56	2.77
TASTEMAKER III		-	6.86	2.74	1.56	2.57
HAYGRAZER-S		-	6.85	2.46	1.74	2.65
PREFERRED STOCK		-	6.64	2.40	1.55	2.69
DEKALB SUDAX SX-132		-	6.41	2.23	1.17	3.01
TRI-SWEET +		-	6.29	2.57	1.29	2.43
HAYMAN I		-	6.27	2.15	1.43	2.70
ROYAL SWEET		-	5.92	2.31	1.25	2.36
SUPERGRAZE II		-	5.75	2.13	1.34	2.28
SUNBURST SWEET		-	5.69	1.95	1.06	2.68
ROYAL REDTOP		-	5.54	2.36	0.90	2.28
SWEET SIOUX V	Sorgo-	7.04(2)	7.68	2.83	1.69	3.17
S.G. GAINER	Sudangrass	4.68(9)	7.08	2.84	1.46	2.78
NC+ SWEETLEAF II	Hybrids	-	6.76	2.42	1.74	2.60
TOPCUT		-	6.62	2.49	1.53	2.61
W-8493		6.04(4)	6.24	2.15	1.36	2.73
SUPER SUGAR		-	6.19	2.75	1.51	1.93
XSSP II		-	6.09	2.26	1.11	2.72
GOTCHA II		-	5.25	1.83	0.96	2.46
SUGAR QUEEN II		-	5.23	1.94	0.96	2.33
HS35	Sudan-Sudan	-	4.84	2.02	1.24	1.58
MONARCH V (CHECK)	Hybrids	-	4.52	1.74	1.13	1.64
SUPERMILL (PM)	Pearl	-	5.79	3.21	1.02	1.56
HY-PRO (PM)	Millets	-	5.22	2.94	0.94	1.35
HB91-M22 (PM)		-	4.84	2.96	1.04	0.83
PEARL MILLET (PM)		-	4.34	3.17	0.65	0.52
HSC M20 (PM)		-	4.32	2.83	1.11	0.38
Overall Mean			6.3	2.5	1.4	2.4
L.S.D.(.05)			1.7	0.7	0.7	0.9
C.V.			19.5			

Soil Name: Taloka Silt Loam Row Width: 12 in.

Monthly Rainfall (in)

Jan.	Feb.	March	April	May	June	July	Aug	Sept.	Oct.
2.11	.54	1.26	2.10	2.48	5.72	.26	1.76	5.39	6.28

Fertilize: Preplant N: 50 lbs/A P: 80 lbs/A K: 80 lbs/A
Postharvest N: 50 lbs/A

Planted: 5-14-91 Harvest: 6-26, 8-9, 10-8-91

* Number in parenthesis indicates the rank position of the hybrid for that year and hybrid type.

Table 3. Payne County, Hybrid Sudangrass and Pearl Millet Forage Yields, Perkins Research Station, Perkins Ok.

Entry Designation	Hybrid Type	Yield reported as 16% moisture Annual Total Yield 1990	1991	1st Cut 1991	2nd Cut 1991
		(*)	(Tons/A)		
SUGAR DAN	Sorghum-		6.81	5.82	0.98
GRAZEX II	Sudangrass		6.59	5.38	1.22
ROYAL SWEET	Hybrids		6.18	5.22	0.96
SS111C			6.17	4.97	1.20
GRAZEX			6.16	5.19	0.98
EXPERIMENTAL 850			6.08	5.00	1.08
HAYMAN I			6.00	4.98	1.02
SUGAR GRAZE III			5.70	4.81	0.89
HAYGRAZER-S			5.56	4.73	0.84
DEKALB SUDAX SX-132			5.50	4.60	0.90
SUNBURST SWEET			5.45	4.70	0.74
JACKPOT			5.24	4.46	0.77
PREFERRED STOCK			5.20	4.31	0.89
TRI-SWEET +			5.13	4.27	0.86
TASTEMAKER III			5.10	4.20	0.90
757 G			4.85	3.83	1.02
ROYAL REDTOP			4.68	4.20	0.48
SUPERGRAZE II			4.64	3.81	0.83
NC+ SWEETLEAF II	Sorgo-		6.53	5.33	1.20
SWEET SIOUX V	Sudangrass		6.02	4.67	1.35
S.G. GAINER	Hybrids		5.96	4.93	1.03
SUGAR QUEEN II			5.83	4.66	1.17
SUPER SUGAR			5.74	4.87	0.88
TOPCUT			5.52	4.26	1.26
W-8493			5.47	4.53	0.94
XSSP II			4.75	3.79	0.96
GOTCHA II			4.60	3.62	0.97
HS35	Sudan-Sudan		5.13	4.30	0.84
MONARCH V (CHECK)	Hybrids		4.74	3.79	0.94
HB91-M22 (PM)	Pearl		5.06	4.48	0.58
HY-PRO (PM).	Millets		4.90	4.75	0.15
SUPERMILL (PM)			4.74	4.32	0.43
PEARL MILLET (PM)			4.64	4.49	0.14
HSC M20 (PM)			4.44	4.26	0.17
Overall Mean			5.4	4.6	0.9
L.S.D.(.05)			1.0	0.8	0.5
C.V.			12.6		

Soil Name: Teller Loam Row Width: 12 in.

Monthly Rainfall (in.)

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
.81	.06	1.10	2.36	6.56	3.51	.97	.51	5.15	4.59

Fertilize: Preplant N: 50 lbs/A P: None K: None
Postharvest N: 50 lbs/A

Planted: 5-17-91 Harvest: 7-8, 10-1-91

* No sudangrass trial was planted at this location in 1990.

Table 4. Grady County, Hybrid Sudangrass and Pearl Millet Forage Yields, South Central Research Station, Chickasha, Ok.

Entry Designation	Hybrid Type	Yield reported @ 16% moisture Total Annual Yield 1990	1991
		(*)	(Tons/A)
DEKALB SUDAX SX-132	Sorghum-	-	5.17
EXPERIMENTAL 850	Sudangrass	-	5.17
ROYAL REDTOP	Hybrids	-	4.61
TASTEMAKER III		-	4.60
JACKPOT		-	4.58
SUGAR GRAZE III		-	4.55
GRAZEX II		-	4.30
SUNBURST SWEET		-	4.21
HAYMAN I		-	4.18
GRAZEX		-	4.06
SUPERGRAZE II		-	3.82
SS111C		12.13(3)	3.78
PREFERRED STOCK		-	3.77
ROYAL SWEET		-	3.60
SUGAR DAN		12.53(2)	3.47
HAYGRAZER-S		-	3.24
TRI-SWEET +		-	3.03
757 G		-	2.99
SWEET SIOUX V	Sorgo-	12.62(3)	4.88
W-8493	Sudangrass	11.64(2)	4.08
NC+ SWEETLEAF II	Hybrids	-	3.88
S.G. GAINER		9.83(9)	3.63
GOTCHA II		-	3.57
SUPER SUGAR		-	3.57
TOPCUT		-	3.20
XSSP II		-	3.04
SUGAR QUEEN II		-	2.65
MONARCH V (CHECK)	Sudan-Sudan	-	3.16
HS35	Hybrids	-	2.71
HB91-M22 (PM)	Pearl	-	1.62
SUPERMILL (PM)	Millets	-	1.05
HY-PRO (PM)		-	1.01
HSC M20 (PM)		-	0.88
PEARL MILLET (PM)		-	0.82
Overall Mean			3.4
L.S.D.(.05)			1.7
C.V.			35.5

Soil Type: Reinach Silt Loam Row Width: 12 (in.)

Monthly Rainfall(in.)

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
1.49	.05	1.51	3.28	6.72	3.80	3.41	3.75	9.88	3.47

Fertilize: Preplant N: 50 lbs/A P: None K: None
Postharvest: 50 lbs/A

Planted: 5-28-91 Harvest: 10-4-91

* Numbers in parenthesis indicates the rank position of the hybrid for that year and hybrid type.

Table 5. Tillman County, Hybrid Sudangrass and Pearl Millet Forage Yields, Southwest Agronomy Research Station, Tipton Ok.

Entry Designation	Hybrid Type	Total Annual Yield 1990	Yield reported @ 16% moisture 1991	1st Cut 1991	2nd Cut 1991
		(*)	(Tons/A)		
GRAZEX	Sorghum-	-	12.00	7.24	4.76
ROYAL SWEET	Sudan	-	11.72	7.61	4.11
JACKPOT	Hybrids	-	10.97	6.07	4.90
SUGAR GRAZE III		-	10.87	6.35	4.52
PREFERRED STOCK		-	10.80	6.56	4.25
TRI-SWEET +		-	10.75	6.65	4.10
EXPERIMENTAL 850		-	10.66	6.29	4.37
HAYGRAZER-S		-	10.39	5.61	4.78
DEKALB SUDAX SX-132		-	10.17	5.71	4.47
TASTEMAKER III		-	9.99	5.02	4.97
SUGAR DAN		8.61(5)	9.97	6.02	3.96
757 G		-	9.95	5.52	4.43
HAYMAN I		-	9.75	5.28	4.47
ROYAL REDTOP		-	9.08	5.55	3.53
GRAZEX II		-	8.94	4.48	4.46
SUPERGRAZE II		-	8.73	4.99	3.74
SUNBURST SWEET		-	8.54	5.18	3.36
SS111C		6.52(9)	8.50	4.61	3.89
SWEET SIOUX V	Sorgo-	9.37(3)	10.95	6.93	4.02
W-8493	Sudangrass	9.70(1)	10.60	6.15	4.45
GOTCHA II	Hybrids	-	9.90	6.06	3.84
NC+ SWEETLEAF II		-	9.78	5.26	4.52
XSSP II		-	9.78	5.47	4.31
TOPCUT		-	9.61	5.23	4.38
S.G. GAINER		10.34(1)	9.43	5.52	3.91
SUPER SUGAR		-	9.39	6.41	2.98
SUGAR QUEEN II		-	7.67	4.65	3.02
MONARCH V (CHECK)	Sudan-Sudan	-	7.97	3.97	4.00
HS35	Hybrids	-	7.87	3.69	4.17
PEARL MILLET (PM)	Pearl	-	8.78	4.80	3.98
HB91-M22 (PM)	Millets	-	6.29	4.75	1.54
SUPERMILL (PM)		-	5.61	4.94	0.68
HSC M20 (PM)		-	5.15	4.47	0.68
HY-PRO (PM)		-	5.05	4.24	0.80
Overall Mean			9.3	5.5	3.8
L.S.D.(.05)			2.7	2.1	2.1
C.V.			20.9		

Soil Name: Tipton Silt Loam Row Width: 12-inches

Fertilize: Preplant N: 50 lbs/A P: None K: None
Postharvest N: 50 lbs/A

Planted: 5-29-91 Harvest: 8-1, 9-11-91

* Number in parenthesis indicates the rank position of the hybrid for that year and hybrid type. (S.G. GAINER was entered as a TYPE A CROSS in 1990)