# CHEMICAL COMPOSITION OF WHEAT FORAGE IN RELATION TO STOCKER DEATH SYNDROME

By

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1973

Submitted to the Faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE July, 1975

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#### ACKNOWLEDGEMENTS

I wish to express my appreciation to the Agronomy Department of Oklahoma State University for the use of their facilities and their financial assistance throughout the course of this study. My gratitude is expressed to Dr. L. I. Croy and the other members of my committee, Dr. E. Basler, Dr. R. M. Reed, and Dr. J. S. Kirby for their encouragement and guidance during my studies and the preparation of this thesis.

Deepest appreciation is expressed to my parents, Mr. and Mrs. Thomas N. Wilson, whose guidance, encouragement and assistance have been freely given throughout the course of my education.

Special appreciation is expressed to Carolyn Luthye for her help with the laboratory analyses and the typing of the first draft of this thesis and Mrs. David Jennings for typing the final copy.

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#### CHAPTER I

## INTRODUCTION

Wheat has long been an important cash crop in the Southern Great Plains region. In recent years livestock numbers in this region have increased, especially stocker cattle numbers. Wheat farmers are able to graze stocker calves on the high quality wheat forage until they reach ` approximately 700 pounds. With good management and normal weather conditions many farmers harvest respectable grain crops from fields which were grazed.

The shift from grain production alone to greater utilization of wheat forage has necessitated some management and cultural practice changes. Planting dates have been advanced 10-15 days with many farmers seeding wheat in mid to late September whenever weather conditions permit. Seeding rates are higher to furnish more forage and to allow for loss of seedlings due to uprooting and trampling by the grazing cattle. Seeding rates of 90 to 120 lbs/acre are not uncommon. Higher fertilization rates, especially nitrogen, are required for adequate fall forage growth before the wheat plant becomes dormant during colder winter weather. Usually forage is of sufficient quantity to permit grazing by November 1 at stocking rates of one to two animals per acre.

Wheat provides a high quality forage with high rates of animal gain. A major problem with stocker cattle grazing wheat pasture is that death losses in recent years have meant an increasing economic loss to

producers. Respiratory diseases are a distinct problem but the cause of many stocker deaths, i.e., losses due to the stocker syndrome (sudden death syndrome), are significant and of major importance.

Sudden death syndrome is a description coined by Kansas feedlot veterinarians during 1970 to describe deaths of feedlot animals that die acutely without any apparent struggle (50). Animals found dead on wheat pastures due to stocker syndrome exhibit many similar characteristics. Stocker animals are often noted to be healthy only hours before death. The greatest number of deaths occurred during November and March when an abundance of lush forage was present. These months are also marked by rapidly fluctuating weather conditions, with many cases of stocker syndrome reported immediately prior to or following a drastic change in weather. Most stocker syndrome deaths occur in the early morning or shortly after sundown.

Frothy bloat is thought to be a significant contributing factor to the loss of stocker cattle to sudden death syndrome. Plant proteins have been implicated in producing stable foams necessary for frothy bloat and thus stocker syndrome. The objective of this study was to chemically analyze forage samples throughout the growing season to determine the concentration of key chemical constituents which are postulated to be associated with stocker syndrome.

#### CHAPTER II

## LITERATURE REVIEW

The production of an abundance of high quality forage is essential to provide an adequate and economical supply of beef. In the U.S., beef consumption has steadily increased with the consumer preferring quality cuts of meat. Developments in the past year make it clear that the amount of grain fattened beef will be reduced in the future with more emphasis placed on forages. Considering the current high price of feed grains the seven to ten pounds of grain required to produce one pound of beef may become economically prohibitive. In the Southern Great Plains region, wheat is a high quality forage extensively used by farmers to graze stocker animals. However, the occurrence of stocker syndrome in recent years has meant a large financial loss to producers.

Deaths of animals on small grains forage have been attributed to stocker syndrome, hypomagnesemia, nitrate poisoning, bloat and other diseases. Stocker deaths due to stocker syndrome often have been confused with hypomagnesemia and nitrate poisoning and frothy bloat is almost always associated with stocker syndrome deaths. These disorders and possible implications with stocker syndrome will be discussed.

#### Hypomagnesemia

Hypomagnesemia (grass tetany, wheat pasture poisoning) is a magnesium deficiency of ruminants which results in considerable animal death

loss throughout the world's temperate regions. The symptoms of hypomagnesemia are distinctive and have been well documented (17,9,48). The occurrence of hypomagnesemia is seasonal as the chemical composition of forages fluctuates with periods of growth, and is obviously not a simple function of an absolute deficiency of magnesium (35).

Hypomagnesemia has been reported to occur in dry cows, heifers, and sheep. However, in animals grazing wheat forage Sims and Crookshank (48), observed this condition only in sexually mature cows which were pregnant or with calf at side, or both. Clay et al. (9) noted that most cases reported on wheat pasture in Oklahoma had been grazing on the pasture for 60 days or longer.

### Plant and Serum Mg Levels

Kemp (26) plotted serum-Mg values against the corresponding herbage-Mg values for 822 dairy cows. No cases of clinical tetany occurred at serum-Mg levels above 0.9 mg per 100 ml, or at herbage-Mg levels above 0.19%. Kemp's work established 0.20% as a safe level for herbage-Mg, above which the occurrence of hypomagnesemia is unlikely. In New Zealand predominately ryegrass pastures were sampled during an outbreak of grass tetany. The magnesium content ranged from 0.14-0.25% with a mean of 0.19% (30). In a study by Rook and Rowland (46) cattle on typical winter diets had serum-Mg levels within the normal physiological range of 1.8-3.0 mg/100 ml. When the animals were turned out to spring pasture, serum-Mg levels fell within a few days. Many levels remained above 1.8 mg/100 ml; however, some fell to as low as 0.2 mg/100 ml. Generally a minimum was reached within 1-2 weeks after grazing began.

In Oklahoma (8) animals grazing wheat forage which contained from

0.10-0.30% magnesium were affected. Sims and Crookshank (48) sampled wheat plants from pastures where wheat pasture poisoning had occurred and from pastures where this condition had not been observed. They found no significant differences in the content of magnesium, calcium, or phosphorus. Other investigations have reported no close association between magnesium content of the herbage and hypomagnesemia (51,47). Such variable values for an adequate level of Mg in plants have led some researchers to advocate that magnesium levels of 0.25% or 0.30% are necessary to insure against hypomagnesemia (49).

## Plant - Carbohydrate Balance in Forage

Kemp (26) found that the higher the N and K contents of forage, the greater the Mg level needed to be to prevent hypomagnesemia. In New Zealand, Metson (35) found the nitrogen level to be extremely high in tetany prone pastures. Nitrogen content of the forage ranged from 4.2% to 6.3% (equivalent to 26% to 39% crude protein), with an average of 5.28% (33% crude protein). He suggests that this is why the 0.19% Mg content present in forage samples was not adequate to prevent tetany. Such a high level of dietary crude protein, especially if the soluble carbohydrate level is low may decrease the amount of available Mg to the animal (17,9,31,51,35). Rapid rumen ammonification of high N forage in the absence of adequate energy material may increase the pH in the digestive tract, thus decreasing Mg solubility.

Dishington (15) noted the food intake of cows on a high protein forage was almost the same as animals on normal diets. However, he estimated that in order to cover their energy requirements with a high protein grass the animals would have to consume about three times as much

protein as they needed; this they were usually unable to do. Metson (35) found the water-soluble carbohydrate averaged 9.7% with a range of 3.6% to 15.5% on ryegrass pastures. He considered these values low in relation to the high nitrogen values he obtained. Such a dietary imbalance between N and readily available energy may result in the rapid build up of  $NH_4$ -N in the rumen. In work with crested wheatgrass in the western U.S. Mayland et al. (31) concluded that the sudden change in the nitrogen/total water soluble carbohydrate ratio rather than its absolute level may be the important criterion in precipitating grass tetany.

Robertson and Hawke (45) found peak concentrations of ammonia in the rumen occurred 2 to 4 hr. after commencement of feeding. Diets having adequate levels of readily fermentable carbohydrates reduced rumen  $NH_3$  resulting from high protein forages. Concurrently, the additional carbohydrate enhances the synthesis of volatile fatty acids. The overall effect is the lowering of the rumen pH values and thus increase the absorption and effective availability of Mg and Ca (35).

High levels of K have often been associated with tetany-inducing pastures (35,20). Kemp and t'Hart (25) in the Netherlands noted that the percentage of tetany cases increased as the ratio of K/(Ca + Mg) in the herbage, expressed in milliequivalents, increased. At ratios above 2.2 a sharp increase in tetany cases was noted and this figure is often used as an index of the tetany proneness of pastures. Calcium also is often low in pastures where tetany cases are reported. Clay et al. (9) reported that a deficiency of calcium frequently accompanies magnesium deficiencies of cows on wheat pasture. In lactating cows 0.30% Ca in the dry matter is barely adequate for maintenance plus lactation (35). Metson (35) suggests that a Ca deficiency may act as a stress factor in the

development of hypomagnesemia.

Hypomagnesemia occurs most frequently in the fall or spring of the year. Conditions for rapid plant growth usually exist during these periods and temperatures are mild. Grunes (18) found that Mg and Ca concentrations were much higher in crested wheatgrass plants grown at 20° C than at 10° C. Potassium levels were fairly stable which resulted in an increased K/(Ca + Mg) ratio at lower temperatures. Kemp and t'Hart (25) indicated that tetany often occurred in the Netherlands 5 days after the temperature began to rise. In work with perennial ryegrass Dijkshoorn and t'Hart (14) grew plants at 10° C for 16 days then transferred them to a 20° C temperature for 8 days. The ratio of K/(Ca + Mg) was higher for those plants grown continuously at 10° or 20° C.

#### Frothy Bloat

Acute ruminal tympany (bloat) is considered one of the major factors involved in animal deaths due to stocker syndrome (50,8,9). Bloat is caused by retention of gas in the stomach of ruminants. The imbalance of gas production and removal that leads to bloat results most commonly from excessive foaming of the contents of the rumen. Foam production is the primary cause of bloat in animals feeding on legumes or lush young grass (7,10).

Bloat occurs most frequently on legume forages, but there are also reports of its occurrence on succulent grasses and small grains pastures (9). Bloat is associated with lush growth and environmental factors especially temperature and moisture conditions which affect both plant and animal are important. Clay et al. (9) reported that the largest number of bloat cases in cattle grazing small grains occur when the small

grains are growing rapidly and are high in protein.

## Relationship of Plant Protein to Bloat

Foamy bloat is due to the development of a stable foam in the contents of the rumen which traps gas. It is considered more serious than free-gas bloat due to the inability of the animal to expel foam by eructation (7).

Mangan (30) stated that cytoplasmic protein of red clover is of major importance as a foaming agent in bloat. In work with red, white, and subterranean clovers, which cause bloat and lotus major which is not a bloat producing forage, Jones et al. (21) postulate that the higher levels of tannins in lotus major prevent bloat. The soluble leaf proteins are precipitated by tannins and are therefore not available for foam production. Several workers have suggested that the foaming system in the rumen may be composed of salivary mucoproteins, saponins, soluble leaf proteins, and lipid constituents; surface active materials which would enter the rumen of an animal feeding on clover (19,24,27).

McArthur and Miltimore (31) separated the crude protein extract of alfalfa into two major fractions, Fraction I and Fraction II, on agar gel columns. The first peak proved to be a single protein and studies on its foaming properties indicated it was the foaming agent in Mangan's (30) preparation. The sedimentation coefficient identified the foaming agent as 18-S protein, also known as Fraction I. Other researchers have characterized Fraction I as a homogeneous protein of high molecular weight which exists as the major protein in the chloroplasts of green leaves (29). McArthur and Miltimore (34) found the amount of 18-S protein was much greater in bloat producing than in nonbloat producing forages.

Nitrogen and protein content generally tended to be higher in the bloating than in the nonbloating forages. The relative quality of the amino acid fraction, soluble protein fraction, and nonprotein nitrogen fraction of alfalfa plants remained constant over the growing season (36). The total nitrogen content of the plants did decrease with increasing maturity as did the occurrence of bloat. Fraction II protein comprises a heterogeneous mixture of all proteins other than Fraction I. Jones and Lyttleton (22,23) concluded that Fraction II as well as Fraction I is largely denatured by foaming and thus contributes to foam production. Fraction I, however, is generally thought to be primarily responsible for the foaming capability of the leaf protein extract (23,33).

Foam denaturation studies on clover protein have shown that foaming causes a change in the composition of leaf protein (23). The process of the formation of a stable foam from denatured Fraction I protein is discussed by McArthur et al. (33).

The release of chloroplasts from the plant leaf cells by mechanical or chemical rupture of the cell wall is followed by rapid release of 18-S protein. In the rumen liquor, the protein is in solution as an almost spherical particle. Molecules which diffuse to the surface, without being subjected to proteolytic activity, immediately uncoil and become insoluble. This surface-denatured protein will stabilize the liquid films in a foam. However, the cohesive forces between the protein molecules will promote coagulation. In this form the protein is not surface-active and will not stabilize foam. The coagulation process is enhanced by mechanical agitation of the surface films (p. 204).

Reid et al. (46) have found that 65% of the soluble protein in the feed can be released during mastication. The observations on denaturation were supported by the findings of Jones and Lyttleton (22). The pH at which plant proteins are foamed is a major factor in foam strength. Bloat has been observed to occur at rumen pH values of 5.7-6.3 (30). The production of strong foams over this pH range has been noted.

Mangan (30) found that the foam strength of red clover cytoplasmic protein varied with pH, being greatest at pH values between 5.4 and 6.0. Above 6.0 and below 5.0 foam production is still significant (30) but foam strength is very low (53). Buckingham (5) also working with red clover found the maximum foam strength occurred at a pH of 5.5. Below pH 5.5 the protein began to precipitate out of solution with the result that the concentration of protein remaining in solution was reduced. Jones and Lyttleton (23) report a definite foam strength maxima for white clover Fraction I protein at pH 5.8-5.9. Fraction I protein is rapidly foam denatured at this pH. In alfalfa the optimum pH for strong foam production is 5.5 (16).

In studies on rumen liquor, Jones and Lyttleton (22) suggested that leaf proteins and salivary mucoproteins contribute to strong foam formation. The concentration of Fraction I protein varied from 0.008% to 0.025% weight/volume in rumen samples (22). However, no correlation between the concentration of Fraction I and bloat was found. The persistence of rumen foam was the greatest in the pH range 5 to 6. Research conducted by McArthur and Miltimore (32) indicated that low rumen pH alone does not produce bloat, the Fraction I protein foaming agent must also be present. Mangan (30) reported difficulties in attempts to obtain a correlation between the foaming properties of rumen liquor and bloat. He concluded that proteins are readily surface denatured and once foam is produced in the rumen any attempts to refoam rumen samples in the laboratory are unsatisfactory.

### Effect of Protein Concentration on Bloat

Bloating forages are higher in soluble protein than nonbloating forages (32), thus protein concentrations as related to foam production has been investigated. Miltimore et al. (37) found the Fraction I protein concentration varied from 0.4% to 6.1% but bloat did not occur between concentrations of 0.4 to 1.8% Fraciton I protein. Wright (53) obtained a good relationship between the volume of foam produced and the amount of protein in solution in red clover. He showed a straight line relationship up to 5.5 ml of foam with about 3 mg of protein in 10 ml of solution. In research with white and red clovers Jones et al. (22) concluded that the minimum concentrations of Fraction I protein and Fraction II protein required to produce persistent foams at pH 5.8 were 0.02% W/V and 0.03% W/V, respectively. Buckingham (5) found that in red clover cytoplasmic protein above 0.05% protein foam strength increased rapidly with increasing protein concentration and then leveled off at 0.1% and above for both pH 5.5 and 5.7.

#### Effect of Temperature on Bloat

Buckingham (5) reported that foam strength at all concentrations of red clover protein studied rose rapidly and then leveled off as the temperature was reduced. The very rapid onset of strength occurs over a narrow temperature range ( $37^{\circ}$  to  $40^{\circ}$  C) in all concentrations greater than 0.04% protein. Jones and Lyttleton (22) showed the same decrease in foam strength regardless of concentration as temperatures rose from 2° to  $50^{\circ}$  C. Foam strength remained fairly constant through  $30^{\circ}$  C. Above  $40^{\circ}$  to  $50^{\circ}$  C, all foams possessed little strength and were very unstable. Rumen temperatures are normally about  $39^{\circ}$  C (6) thus there would not need to be a great drop in temperature to stabilize any protein foams present in the rumen.

#### Rumen Gas Production

The production of an adequate amount of rumen gas to form the foam-gas phase is imperative in foamy bloat. According to Conrad et al. (11) the initial rapid gas production in the rumen of cattle grazing legumes comes from the pectin-containing fibrous fraction of the plant. Pectic substances make up 14 to 15% of the dry weight of legumes but only 6 to 8% of the grasses (42,11). The difference in either water soluble or total pectins, however, could not be correlated with bloat (42). Boda and Johns (4) showed that the rapid gas production which follows the ingestion of legumes results from fermentation of plant juice rather than fiber.

## CHAPTER III

#### GENERAL MATERIALS AND METHODS

Wheat forage samples for this study were taken at the Agronomy Research Station, Stillwater, Oklahoma, and North Central Research Station at Lahoma, Oklahoma.

The Stillwater study was conducted on Kirkland silt loam soil. Drill strips of Danne wheat 10 ft. wide and 30 ft. long were planted in a randomized complete-block experimental design with 3 replications. Anhydrous ammonia at a rate of 40, 80, 160 lbs. N/A was applied approximately 1 week prior to planting. A plot receiving ammonium nitrate at 80 lbs. N/A was included in each replication as a check. Wheat was seeded on October 10, 1973 at a rate of 120 lbs./A.

The wheat at the Lahoma station was grown on a Pond Creek silt loam. Danne wheat was seeded on September 25, 1973 in a randomized completeblock design with four replications. Ammonium nitrate was applied at 90 and 120 lbs. of N/A.

Forage samples of entire plants were clipped and packed in ice. They were stored at  $-20^{\circ}$  C in the laboratory until chemical analyses were made.

Percent dry weight was determined on oven dry leaf tissue.

# Extraction of Forage for Carbohydrate and Alpha Amino Nitrogen Determination

Fifty ml of 80% ethyl alcohol was added to 1 gram fresh weight of leaf tissue in 600 ml beakers and refluxed for 1 hour. Leaf samples were chopped into approximately 1/4 inch lengths to facilitate extraction. The exudate was filtered through number 31 filter paper on a buchner funnel and plant residue and filter paper were washed with additional 80% ethanol. The extracts were heated on a hot plate placed under a hood until approximately 10 mls of liquid remained. This was transferred to centrifuge tubes with washes of distilled water and all tubes were brought to equal volume with distilled water. The plant extract was tested for carbohydrate by the anthrone method (2), and the alpha amino nitrogen was determined by the method of Moore and Stein (38) using ninhydrin.

# Extraction of Forage for Nitrate and Water Soluble Protein Determination

Ten milliliters of distilled water was added to 1 gram fresh weight of leaf tissue and homogenized for two minutes in a motorized Vitris homogenizer. The homogenate was strained through a double layer of cheesecloth and the suspension cleared by centrifugation at 13,000 rpm (20,850 X g) for 15 minutes at 0° C. The homogenate was kept in an ice bath during preparation.

Nitrate content was determined on the cleared solution by the method of Woolley et al. (52). Water soluble protein content of the plant extract preparation was determined on 5% TCA precipitable material by the Lowry et al. (28) procedure.

Forage nitrogen was determined on oven dry tissue by microkjeldahl procedure.

Precipitation and temperature data for the Stillwater and Lahoma stations are given in Tables I and II.

# TABLE I

	Rainfall (in)
October	2.44
November	3.06
December	1.05
January	0.51
February	2.12
March	3.16
April	2.48
May	5.76

# PRECIPITATION DURING FALL AND SPRING 1973-74 AT STILLWATER

## TABLE II

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# PRECIPITATION DURING FALL AND SPRING 1973-74 AT LAHOMA

	Rainfall	(in)
September	8.20	
October	5.47	
November	1.35	
December	1.59	
January	0.85	
February	1.05	
March	0.00	
April	2.60	
May	4.35	

## CHAPTER IV

EFFECT OF SAMPLING DATE, NITROGEN LEVEL, AND SEED RATE ON CHEMICAL CONSTITUENTS OF WHEAT FORAGE AT LAHOMA

#### Materials and Methods

The Lahoma Research Station is located in an area where large numbers of stocker animals are annually grazed on wheat forage. In recent years a significant number of stocker deaths have been attributed to stocker syndrome in the general area from which samples were taken.

Wheat forage samples were taken on November 8, November 29, January 4, January 22, February 12, March 8, and March 29. The forage was sampled between 11:00 and 12:00 a.m. in order to minimize diurnal variations in plant chemical content. Weather conditions at the time of sampling, however, may have a marked effect on some chemical constituents.

### Results and Discussions

# Effect of Sampling Date on Chemical Composition

## of Wheat Forage

Percent dry weight and all chemical constituents of the forage samples were significantly different between sampling dates. Growth patterns of wheat varied over the growing season and the levels of plant chemical constituents varied as the wheat plants were going through

periods of rapid growth and dormancy.

<u>Dry Weight</u>. In the late fall, forage grew rapidly and had a high moisture content. The percent dry weight was lowest on the November 8 date. Extended periods of cold temperatures during December, January, and February cause drastic reduction or cessation of growth resulting in higher percent dry weight; the highest percent dry weight was obtained on January 4. New spring growth again resulted in decreased dry weight with 15 to 20% not uncommon for either lush fall or spring growth (Figure 1).

<u>Nitrate</u>. Young forage was the highest in nitrate in the fall, containing about 4 mg NO<sub>3</sub>-N/g fresh weight (Figure 2). Nitrates declined throughout the fall and early winter. In January and February nitrates fluctuated slightly but remained low during the colder temperatures. Nitrate levels rose as spring growth resumed; however, the high level obtained on March 8 is thought to be due to weather conditions. The weather was cloudy with light rain on this date and this may have allowed nitrates to accumulate due to reduced nitrate reductase activity.

<u>Alpha Amino Nitrogen</u>. The alpha amino nitrogen content has been shown to decline with plant maturity. Alpha amino nitrogen did show a general decline throughout the sampling period from 5.5 mg alpha amino N/g fresh weight on November 8 to 1.3 mg on March 29. The January 4 date does not conform to this trend and is exceptionally high (Figure 3).

<u>Carbohydrates</u>. Carbohydrate (CHO) content of wheat forage was moderately low in the fall and spring (Figure 4). Forage samples contained about 4% CHO on the two November dates and only 2 to 3% during March. Carbohydrate levels were higher during the winter months when



Figure 1. Seasonal Patterns of Dry Matter Content of Wheat Forage From Lahoma



Figure 2. Seasonal Patterns of Nitrate Content of Wheat Forage From Lahoma



Figure 3. Seasonal Patterns of Alpha Amino Nitrogen Content of Wheat Forage From Lahoma



Figure 4. Seasonal Patterns of Carbohydrate Content of Wheat Forage From Lahoma

there was little growth. However, CHO levels were shown to fluctuate during the winter months possibly in response to periods of temperature above 40° F where plant growth and subsequent reduction in CHO levels may occur. This would account for the lower level on January 22.

<u>Water Soluble Protein</u>. The water soluble protein (WSP) content of fall and spring forage was comparable at 6 to 8 mg protein. The high level of WSP on January 22 is not fully understood (Figure 5). High levels of WSP are generally associated with an increase in metabolic activity. Temperatures were low on the January 4 date and a high metabolism rate would not be expected.

<u>Kjeldahl Nitrogen</u>. Kjeldahl nitrogen (KN) was found to be somewhat higher (approximately 5%) in fall and spring forage than in forage during colder temperatures (Figure 6). The lower KN content on March 29 is due to the maturity of the forage. Kjeldahl nitrogen content on November 8 and March 8 was significantly different due to nitrogen rate - seeding rate interaction (Table V, Appendix). Rao (43) has found N levels generally decline with plant maturity.

## Effect of Nitrogen Level on Chemical Composition

#### of Wheat Forage

Dry Weight. Percent dry weight was significantly different for the two nitrogen rates (90 and 120 lbs/A) when averaged across sampling dates. March 29, however, was the only individual date that a significant difference due to the level of nitrogen was indicated (Table VI, Appendix). Wheat forage was rapidly growing by the last of March thus plant response to different nitrogen levels was more apparent.



Figure 5. Seasonal Patterns of Water Soluble Protein Content of Wheat Forage From Lahoma



Figure 6. Seasonal Patterns of Kjeldahl Nitrogen Content of Wheat Forage From Lahoma

Nitrate. The nitrate content of the forage was significantly different at the two levels of nitrogen on November 29 and March 29 (Table VI, Appendix). A significance in nitrate content due to interaction was found on January 22 and March 29 (Table III, Appendix). Baker and Tucker (3) found the nitrate content of wheat plants increased as rates of nitrogen increased. Growing conditions, however, have a marked effect on nitrate content. On the November and March sampling dates one would expect conditions for plant growth to be good and thus higher rates of nitrate uptake from the soil. The nitrate content on November 8 is well above the level considered safe for grazing animals (Figure 2). Researchers place the potentially toxic level of nitrates to cattle between 2 and 3 mg of  $NO_z$ -N/g fresh weight; however, nitrate toxicity is not considered a major problem in the early fall. Even though little growth occurs during January, wheat plants will respond to several days of temperature above 49° F. Weather records indicate daytime temperatures were in the 49°, 50°, and 60° F range for a week prior to the sampling date. Plants had initiated new growth accounting for increased nitrate uptake.

<u>Carbohydrates</u>. Nitrogen rates did not significantly affect carbohydrate levels in the plant samples (Table IV, Appendix). Samples from plots receiving the higher rate were generally higher in carbohydrates during the winter months.

<u>Alpha Amino Nitrogen</u>. The content of alpha amino nitrogen was not significantly affected by the rate of nitrogen (Figure 4). Alpha amino nitrogen content of plants at the same stage of growth is generally fairly constant (39). <u>Water Soluble Protein and Kjeldahl Nitrogen</u>. Water soluble protein and Kjeldahl nitrogen levels were not significantly different due to nitrogen fertilization rates (Table VII, Appendix). However, a significant difference in WSP content due to nitrogen rate - seeding rate interaction occurred on March 8 and KN was significant on November 8 and March 8 (Table V, Appendix).

# Effect of Seeding Rate on Chemical Composition

#### of Wheat Forage

<u>Dry Weight</u>. Percent dry weight was significantly different at the two seeding rates (60 and 120 lbs/A) on only the January 4 date (Table VIII, Appendix). The 60 lb rate has the lowest dry weight. Extended periods of cold with frozen soil conditions may greatly restrict water uptake. Plots with thinner stands have more area available for water uptake.

<u>Water Soluble Protein</u>. WSP on January 22 and March 8 showed a significant difference due to seeding rates (Table IX, Appendix). On March 8 a significant difference due to nitrogen rate - seeding rate interaction was also obtained (Table V, Appendix). The WSP content is highest for the 60 lb rate on January 22 with the 120 lb rate giving the highest value on the January 4 date.

Nitrate, Carbohydrates, Alpha Amino Nitrogen and Kjeldahl Nitrogen. Nitrate content on March 29 was significantly different between the 60 and 120 lb seeding rate (Table VIII, Appendix). Levels of carbohydrates, nitrates, alpha amino nitrogen, or Kjeldahl nitrogen were not significantly different due to seeding rate. A significant difference in CHO levels was obtained due to nitrogen rate - seeding rate interaction on January 4 (Table IV, Appendix). The levels of plant chemical constituents were only slightly affected by seeding rates in that nutrient, water, and possibly light levels to the plant are altered.

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#### CHAPTER V

EFFECT OF SAMPLING DATE, NITROGEN LEVEL, AND NITROGEN SOURCE ON CHEMICAL CONSTITUENTS OF WHEAT FORAGE AT STILLWATER

#### Materials and Methods

Fertilization rates are usually higher when wheat forage is to be grazed. Both anhydrous ammonia and ammonium nitrate are used extensively as sources of nitrogen and rates up to 160 lbs of N/A are not uncommon.

The Stillwater test was designed to study selected plant chemical changes when anhydrous ammonia and ammonium nitrate were used as nitrogen sources. Also, the effect of different levels of nitrogen was evaluated.

Wheat forage samples were taken on November 9, December 3, January 2, January 25, February 20, and March 22. Samples were taken between 1:00 and 3:00 o'clock in the afternoon to minimize variations in plant chemical content.

Results and Discussion

#### Dry Weight

Higher percent dry weight was noted during the winter with characteristicly lower dry weight during fall and spring growth (Figure 7). Percent dry weight was not significantly different due to rate of nitrogen on any sampling date. Growing conditions were good at Stillwater



Figure 7. Seasonal Patterns of Dry Matter Content of Wheat Forage From Stillwater

with adequate moisture the entire growing season.

#### Nitrates

Nitrate levels decreased throughout the growing season as was shown in the Lahoma study. Differences in nitrate levels were statistically significant on the March 22 sample due to nitrogen fertilization (Table XV, Appendix). The LSD .05, however, showed only the 160 lb anhydrous and 80 lb  $\text{NH}_4\text{NO}_3$  treatments significantly different. The February 20 sample showed similar results, however, for this date the F-test was not significant. The forage on November 9 contained potentially toxic levels of nitrate (Figure 8).

#### Carbohydrates

Carbohydrate levels were low in fall and spring with higher winter levels (Figure 9). This response was consistent with previously reported research (8) and with the results from the Lahoma study.

Nitrogen rate was significant on January 25 (Table XIII, Appendix). The 160 lb anhydrous treatment contained the highest level of CHO. No other dates were significant for nitrogen rate and the 160 lb anhydrous gave the highest CHO level on only one other date, November 9.

Nitrogen source was not significant for any date.

#### Alpha Amino Nitrogen

Alpha amino nitrogen decreased throughout the growing season as in the Lahoma study and was significantly different between nitrogen treatments in forage samples on February 20. However, rates of nitrogen were not significant for any date. Amino N levels were not higher for the



Figure 8. Seasonal Patterns of Nitrate Content of Wheat Forage From Stillwater



Figure 9. Seasonal Patterns of Carbohydrate Content of Wheat Forage From Stillwater

160 lb anhydrous application on any sampling date.

On the February 20 date alpha amino N levels were significantly different between the 80 lb anhydrous and 80 lb  $\text{NH}_4\text{NO}_3$  treatments (Table XIV, Appendix). The  $\text{NH}_4\text{NO}_3$  sample was lowest in alpha amino N on this date. However, no consistent pattern was found as the levels fluctuated widely (Figure 10).

Forage content of alpha amino N was not expected to show drastic changes during the growing season. Other researchers (36) have found alpha amino nitrogen levels to remain fairly constant in plants.

#### Water Soluble Protein

The WSP content of the wheat forage was highest on the first sampling date, November 9. High WSP levels were also found on January 2 in forage receiving anhydrous ammonia (Figure 11). WSP content did not vary appreciably on the other sampling dates, ranging between 5 and 9 milligrams protein.

Water soluble protein was found to be statistically significant by an F-test on the January 25 and February 20 samples. Nitrogen rate was significant with the 40 lb anhydrous treatment having the highest levels of WSP on both dates (Tables XIII and XIV, Appendix).

The LSD .05 showed a significant difference between sources of nitrogen; however, the F-test was not significant. Source of nitrogen is not expected to influence WSP content and the lack of a significant F-test tends to negate any real difference.



Figure 10. Seasonal Patterns of Alpha Amino Nitrogen Content of Wheat Forage From Stillwater



Figure 11. Seasonal Patterns of Water Soluble Protein Content of Wheat Forage From Stillwater

## Kjeldahl Nitrogen

Percent Kjeldahl nitrogen varied little over sampling dates (Figure 12). The lowest levels occurred on January 2. Kjeldahl nitrogen was statistically significant on the January 2 and March 22 dates. Nitrogen rate and nitrogen source were significant on both dates (Tables XII and XV, Appendix).



Figure 12. Seasonal Patterns of Kjeldahl Nitrogen Content of Wheat Forage From Stillwater

#### CHAPTER VI

# EFFECT OF TIME OF DAY ON CHEMICAL CONSTITUENTS OF WHEAT FORAGE

### Materials and Methods

Deaths of stocker animals due to stocker syndrome have been observed to occur most frequently in late evening or early morning. To determine if differences occurred in the levels of several chemical constituents of wheat forage, samples were taken at Lahoma at 7:00 and 12:00 a.m. This study was conducted during the 1972-73 growing season. The forage was sampled on March 17, March 23, and April 5, 1973. The plots from which samples were taken were not arranged in a valid experimental design, thus significance values may not be statistically correct.

#### Results and Discussion

The percent dry weight varied greatly over the sampling dates. Precipitation which occurred prior to the 12:00 sampling but after the 7:00 sample was taken may account for the higher moisture content at 12:00 on April 5 (Figure 13). Sampling date was significant for all plant analyses, which follows the results of the 1973-74 Lahoma data.

The interaction of time of sampling and sampling date was significant for percent dry weight, nitrate content, and alpha amino nitrogen. However, time of sampling was not significant. Samples were taken when the forage was growing rapidly. Nitrate levels for the two March dates



Figure 13. Seasonal Patterns of Dry Matter Content of Wheat Forage From Lahoma for 1972-73

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were comparable at 7:00 and 12:00 but the 12:00 sample on April 5 was excessively high (Figure 14). Weather conditions were not recorded on this date thus extensive cloud cover may partially account for a build up of nitrates.

Alpha amino nitrogen levels fluctuated very little over the sampling dates (Figure 15).

Time of sampling and time by date interaction were significant for carbohydrates and water soluble protein.

Figures 16 and 17 show an inverse relationship between carbohydrate content and WSP at the 12:00 sampling time. On March 17 and April 5 the plants may not have been reducing metabolites at a high level thus energy requirements by the plant would be low.



Figure 14. Seasonal Patterns of Nitrate Content of Wheat Forage From Lahoma for 1972-73



Figure 15. Seasonal Patterns of Alpha Amino Nitrogen Content of Wheat Forage From Lahoma for 1972-73



Figure 16. Seasonal Patterns of Carbohydrate Content of Wheat Forage From Lahoma for 1972-73



Figure 17. Seasonal Patterns of Water Soluble Protein Content of Wheat Forage From Lahoma for 1972-73

#### CHAPTER VII

#### SUMMARY AND CONCLUSIONS

Wheat forage samples were taken at Stillwater and Lahoma during the spring of 1973, the fall of 1973, and the spring of 1974 to study the possible relationship between plant chemical composition and stocker syndrome. Forage samples were analyzed for percent dry weight, nitrates, alpha amino nitrogen, carbohydrates, water soluble protein, and Kjeldahl nitrogen.

Percent dry weight is lowest in the fall and spring, times when the greatest losses of animals due to stocker syndrome occur. Wheat forage at these times is only about 18 to 20% dry matter, requiring animals to ingest large quantities of forage to provide adequate amounts of dry matter.

The nitrate content is highest in the fall rising only slightly in the spring after maintaining low levels during the winter. Young fall forage contained potentially toxic levels of nitrate, above the 2 to 3 mg of  $NO_3$ -N considered toxic to animals. However, nitrate toxicity is not a major problem in the early fall, since there is not enough forage present at this time for animals to consume a large enough quantity for nitrate poisoning to occur.

Carbohydrate levels were extremely low in both fall and spring forage. Only about 3 to 4% of the fresh weight of wheat forage during these periods was carbohydrate. Animals grazing wheat forage that is low

in carbohydrate, but relatively high in protein may have difficulty consuming enough forage to adequately meet their energy requirements.

Levels of alpha amino nitrogen generally declined throughout the grazing season. Differing rates of nitrogen and source of nitrogen did not significantly affect plant alpha amino nitrogen.

Kjeldahl nitrogen percentage of the forage dropped during the winter months of December and January with higher levels in spring and fall. Spring and fall levels of Kjeldahl nitrogen at Stillwater and Lahoma were between 4 and 5 percent which corresponds to 25.0 and 31.25% crude protein. Wheat forage is noted as high protein, high quality forage for grazing animals.

Levels of water soluble protein in forage from Stillwater were higher in the fall remaining relatively constant throughout the rest of the season. Lahoma data showed fall and spring WSP quantities relatively constant at about 6 mg/g fresh weight. Researchers in animal bloat have postulated the soluble protein fraction as stabilizing foam in the rumen. WSP quantities need not be excessively high for stable foam production; however, the lack of a dramatic change in WSP level during the winter months when animal deaths decline indicates other factors are involved.

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APPENDIXES

# TABLE III

# MEAN VALUES OF DRY WEIGHT AND NITRATES FOR NITROGEN BY SEEDING RATE INTERACTION AT LAHOMA

**************************************		Dry Weight (%)			Nitrates (mg/g fr. wt.)			
Date	Seeding Rate (#/A)	Nitrogen R 90	ate (#/A) 120	LSD.05	Nitrogen Rate 90	e (#/A) 120	LSD .05	
11-8	60 120	13.97 15.40	15.15 14.35	NS*	3.26 5.62	4.52 3.13	NS	
11-29	60 120	23.55 21.50	21.00 20.57	NS	1.64 2.14	3.04 3.12	NS	
1-4	60 120	38.87 44.60	39.77 41.25	NS	0.41 0.42	0.29 0.33	NS	
1-22	60 120	35.97 31.67	29.50 28.12	NS	0.65 0.93	0.71 0.41	0.39	
2-12	60 120	31.40 32.52	32.97 31.37	NS	0.18 0.14	0.24 0.24	NS	
3-8	60 120	20.80 21.50	20.15 20.65	NS	2.39 0.81	1.69 1.37	NS	
3-29	60 120	23.95 22.52	20.37 20.90	NS	0.62 0.48	0.45 1.60	0.66	

\*NS - denotes nonsignificance.

# TABLE IV

MEAN VALUES OF CARBOHYDRATES AND ALPHA AMINO NITROGEN FOR NITROGEN BY SEEDING RATE INTERACTION AT LAHOMA

	onden nie name in der Einen die eine der Geschnichen Stellten der Stellten Stellte	Carbohydrates (mg/g fr. wt.)			Alpha Amino Nitrogen (mg/g fr. wt.)			
Date	Seeding Rate (#/A)	Nitrogen R 90	ate (#/A) 120	LSD .05	Nitrogen Ra 90	ite (#/A) 120	LSD.05	
11-8	60 120	42.80 41.40	44.72 61.82	NS*	5.65 4.98	5.42 5.01	NS	
11-29	60 120	50.37 36.52	32.48 40.30	NS	2.93 3.26	3.52 2.50	NS	
1-4	60 120	82.25 93.00	99.30 79.97	14.96	8.67 7.89	7.65 8.08	NS	
1-22	60 120	58.05 63.82	66.15 56.90	NS	2.96 3.06	2.69 3.07	NS	
2-12	60 120	79.55 80.07	84.22 84.87	NS	2.58 2.37	2.44 2.33	NS	
3-8	60 120	18.12 18.57	16.25 19.07	NS	2.19 1.75	2.21 2.13	NS	
3-29	60 120	31.30 32.02	<b>3</b> 0.35 27.52	NS	1.32 1.40	1.31 1.51	NS	

\*NS - denotes nonsignificance.

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# TABLE V

# MEAN VALUES OF WATER SOLUBLE PROTEIN AND KJELDAHL NITROGEN FOR NITROGEN BY SEEDING RATE INTERACTION AT LAHOMA

		Water Soluble Protein (mg/g fr. wt.)			Kjeldahl Nitrogen (%)			
Date	Seeding Rate (#/A)	Nitrogen R 90	ate (#/A) 120	LSD .05	Nitrogen Ra 90	te (#/A) 120	LSD .05	
11-8	60 120	4.00 6.12	7.75 6.12	NS*	5.00 4.82	4.87 4.95	0.16	
11-29	60 120	10.22 7.62	8.25 7.79	NS	4.32 4.52	4.47 4.30	NS	
1-4	60 120	14.00 15.00	13.67 13.72	NS	3.80 3.70	3.67 3.85	NS	
1-22	60 120	4.40 3.10	4.05 3.60	NS	4.05 3.92	4.17 3.95	NS	
2-12	60 120	9.42 9.52	9.22 8.67	NS	4.32 4.22	4.35 4.22	NS	
3-8	60 120	7.92 6.47	7.72 7.40	0.78	4.92 4.87	4.95 4.87	0.15	
3-29	60 120	6.02 6.20	5.40 5.50	NS	3.65 4.07	4.15 4.15	NS	

\*NS - denotes nonsignificance.

# TABLE VI

# MEAN VALUES OF DRY WEIGHT, NITRATES, AND CARBOHYDRATES FOR NITROGEN RATE AT LAHOMA

Date	Nitrogen	Dry V (?	Veight %)	Ni (mg/g	trates fr. wt.)	Carboł (mg/g	nydrates fr. wt.)
	Rate (#/A)		LSD .05	<u></u>	LSD .05		LSD .05
11-8	90 120	14.68 14.75	NS*	4.44 3.82	NS	42.10 53.27	NS
11-29	90 120	22.52 20.78	NS	1.89 3.08	0.94	43.45 36.39	NS
1-4	90 120	41.73 40.51	NS	0.42 0.31	NS	87.62 89.63	NS
1-22	90 120	33.82 28.81	NS	0.79 0.56	NS	65.93 61.52	NS
2-12	90 120	31.96 32.17	NS	0.16 0.24	NS	79.81 84.55	NS
3-8	90 120	21.15 20.40	NS	1.60 1.53	NS	18.35 17.66	NS
3-29	90 120	23.23 20.63	2.02	0.55 1.03	0.47	31.66 28.93	NS

\*NS - denotes nonsignificance.

# TABLE VII

# MEAN VALUES OF ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN RATE AT LAHOMA

ate (#/A)	walkerpe selfender Selfert en der			a a construction of the state o		
		CO, UCI		LSD .05		LSD .05
90 120	5.32 5.21	NS*	5.06 6.93	NS	4.91 4.91	NS
90 120	3.09 3.01	NS	8.92 8.10	NS	4.42 4.38	NS
90 120	8.28 7.86	NS	14.55 13.70	NS	3.75 3.76	NS
90 120	3.01 2.88	NS	3.75 3.82	NS	3.98 4.06	NS
90 120	2.47 2.38	NS	9.47 8.95	NS	4.27 4.28	NS
90 120	1.92 2.17	NS	7.20 7.56	NS	4.90 4.91	NS
90 120	1.36 1.41	NS	6.11 5.45	NS	3.86 4.15	NS
	120 90 120 90 120 90 120 90 120 90 120 90 120	120   5.21     90   3.09     120   3.01     90   8.28     120   7.86     90   3.01     120   2.88     90   2.47     120   2.38     90   1.92     120   2.17     90   1.36     120   1.41	120 5.21 NS**   90 3.09 NS   120 3.01 NS   90 8.28 NS   120 7.86 NS   90 3.01 NS   90 3.01 NS   90 3.01 NS   90 3.01 NS   90 2.47 NS   90 2.38 NS   90 1.92 NS   90 1.36 NS   90 1.36 NS	120   5.21   NS**   6.93     90   3.09   NS   8.92     120   3.01   NS   8.10     90   3.01   NS   14.55     120   7.86   NS   14.55     120   7.86   NS   3.75     90   3.01   NS   3.75     120   2.88   NS   3.82     90   2.47   NS   9.47     120   2.38   NS   7.20     90   1.92   NS   7.56     90   1.36   NS   6.11     120   1.41   NS   6.11	120 5.21 NS** 6.93 NS   90 3.09 NS 8.92 NS   120 3.01 NS 8.10 NS   90 8.28 NS 14.55 NS   120 7.86 NS 13.70 NS   90 3.01 NS 3.75 NS   90 3.01 NS 3.75 NS   90 2.47 NS 3.82 NS   90 2.47 NS 9.47 NS   90 2.47 NS 7.20 NS   90 1.92 NS 7.56 NS   90 1.36 NS 6.11 NS	120 5.21 NS* 6.93 NS 4.91   90 3.09 NS 8.92 NS 4.42   120 3.01 NS 8.10 NS 4.38   90 8.28 NS 14.55 NS 3.75   120 7.86 NS 14.55 NS 3.75   90 3.01 NS 3.75 3.76   90 3.01 NS 3.75 3.98   120 2.88 NS 3.75 3.98   120 2.47 NS 3.75 NS   90 2.47 NS 9.47 NS 4.28   90 1.92 NS 7.20 NS 4.28   90 1.92 NS 7.56 NS 4.91   90 1.36 NS 5.45 NS 3.86

\*NS - denotes nonsignificance.

# TABLE VIII

# MEAN VALUES OF DRY WEIGHT, NITRATES, AND CARBOHYDRATES FOR SEEDING RATE AT LAHOMA

Date	Seeding	Dry I	Weight %)	Ni (mg/g	Nitrates (mg/g fr. wt.)		Carbohydrates (mg/g fr. wt.)	
	Rate (#/A)		LSD .05		LSD .05		LSD .05	
11-8	60 120	14.56 14.87	NS*	3.89 4.37	NS	43.76 51.61	NS	
11-29	60 120	22.27 21.03	NS	2.34 2.63	NS	41.43 38.41	NS	
1-4	60 120	39.32 42.92	3.32	0.35 0.37	NS	90.77 86.48	NS	
1-22	60 120	32.73 29.90	NS	0.68 0.67	NS	67.10 60.30	NS	
2-12	60 120	4.33 4.22	NS	0.21 0.19	NS	81.88 82.47	NS	
3-8	60 120	20.47 21.07	NS	2.04 1.09	NS	17.18 18.82	NS	
3-29	60 120	22.16 21.71	NS	0.54 1.04	0.47	30.82 29.77	NS	

\*NS - denotes nonsignificance.

# TABLE IX

# MEAN VALUES OF ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR SEEDING RATE AT LAHOMA

Date	Seeding	Alpha Ami (mg/g	no Nitrogen fr. wt.)	Water Solı (mg/g	able Protein fr. wt.)	Kjeldah	l Nitrogen (%)
	Rate (#/A)		LSD .05		LSD .05		LSD °05
11-8	60 120	5.53 4.99	NS*	5.87 6.12	NS	4.93 4.88	NS
11-29	60 120	3.22 2.88	NS	9.23 7.78	NS	4.40 4.41	NS
1-4	60 120	8.16 7.99	NS	13.88 14.36	NS	3.73 3.77	NS
1-22	60 120	2.82 3.07	NS	4.22 3.35	0.74	4.11 3.93	NS
2-12	60 120	2.51 2.35	NS	9.32 9.10	NS	4.33 4.22	NS
3-8	60 120	2.16 1.94	NS	7.82 6.93	0.79	4.93 4.87	NS
3-29	60 120	1.31 1.45	NS	5.71 5.85	NS	3.90 4.11	NS

\*NS - denotes nonsignificance.

# TABLE X

## MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON NOVEMBER 9 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Pei	rcent	mg/g f	ř. wt.	mg/g fr	.Wt.
Anhydrous	40	25.00	4.73	13.27	30.20	5.23	10.00
Anhydrous	80	20.93	4.93	14.60	31.07	5.10	7.45
Anhydrous	160	23.93	4.93	14.97	37.07	4.84	9.70
NH4NO3	80	21.47	4.83	14.30	21.93	4.21	6.29
LSD .01		NS*	NS	NS	NS	NS	NS
LSD .05							

\*NS - denotes nonsignificance.

# TABLE XI

## MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON DECEMBER 3 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Per	cent	mg/g f	r. wt.	mg/g fr	. wt.
Anhydrous	40	19.80	4.50	8.57	77.57	4.38	4.43
Anhydrous	80	19.93	4.63	7.73	75.10	4.44	2.66
Anhydrous	160	20.17	4.87	7.40	53.73	3.73	3.89
NH4NO3	80	20.07	4.67	7.77	55.00	3.32	2.06
LSD .01		NS*	NS	NS	NS	NS	NS
LSD .05							

\*NS - denotes nonsignificance.

# TABLE XII

## MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON JANUARY 2 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Pei	cent	mg/g f	r. wt.	mg/g fr	.wt.
Anhydrous	40	34.93	3.00	10.63	88.60	2.81	0.54
Anhydrous	80	34.63	3.13	11.80	90.70	3.60	0.58
Anhydrous	160	35.83	3.20	11.33	89.57	3.18	0.45
NH4NO3	80	36.27	2.90	9.70	79.53	3.12	0.55
LSD .01		NS*	0.28			NS	NS
LSD .05			0.19	1.75	10.32		

\*NS - denotes nonsignificance.

# TABLE XIII

### MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON JANUARY 25 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Per	cent	mg/g f	fr. wt.	mg/g fr.	wt.
Anhydrous	40	36.00	3.67	11.33	80.53	2.61	0.17
Anhydrous	80	37.13	3.77	10.20	72.03	2.79	0.19
Anhydrous	160	36.33	3.77	8.53	87.80	2.39	0.20
NH4NO3	80	34.30	3.87	10.90	71.57	2.62	0.15
LSD .01		NS*	NS		9.57	NS	NS
LSD .05				1.96	6.32		

\*NS - denotes nonsignificance.

# TABLE XIV

## MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON FEBRUARY 20 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Рез	cent	mg/g f	r. wt.	mg/g fr.	wt.
Anhydrous	40	33.23	4.37	10.47	63.03	2.97	0.21
Anhydrous	80	30.0 <b>3</b>	4.50	7.46	66.03	2.71	0.25
Anhydrous	160	31.03	4.40	8.83	64.73	2.71	0.31
NH4NO3	80	31.83	4.13	7.40	71.93	2.07	0.04
LSD .01		NS*	NS	2.98	NS	0.76	
LSD .05				1.96		0.50	0.25

\*NS - denotes nonsignificance.

# TABLE XV

## MEANS FOR DRY WEIGHT, NITRATES, ALPHA AMINO NITROGEN, WATER SOLUBLE PROTEIN, AND KJELDAHL NITROGEN FOR NITROGEN SOURCE AND NITROGEN RATE ON MARCH 22 AT STILLWATER

Nitrogen	Nitrogen	Dry Weight	Kjeldahl Nitrogen	Water Soluble Protein	Carbohydrates	Alpha Amino Nitrogen	Nitrates
Source	Rate (#/A)	Per	cent	mg/g f	ir. wt.	Alpha Amino Nitrogen 	. wt.
Anhydrous	40	22.70	4.13	10.87	57.40	2.36	1.55
Anhydrous	80	22.00	4.27	10.13	43.87	3.62	1.23
Anhydrous	160	21.77	4.03	9.93	46.27	2.70	1.81
NH4NO3	80	23.77	3.37	7.87	46.80	4.16	0.19
LSD .01		NS*	0.66		NS		
LSD .05			0.43	2.80		1.47	1.48

\*NS - denotes nonsignificance.

# VITA Y

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