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## **Chemical composition and yield of tall fescue (*Festuca arundinacea* Schreb.) as influenced by nitrogen and potassium fertilization**

D. B. Hannaway

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To the Graduate Council:

I am submitting herewith a thesis written by D. B. Hannaway entitled "Chemical composition and yield of tall fescue (*Festuca arundinacea* Schreb.) as influenced by nitrogen and potassium fertilization." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant, Soil and Environmental Sciences.

John H. Reynolds, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by David Byron Hannaway entitled "Chemical Composition and Yield of Tall Fescue (Festuca arundinacea Schreb.) as Influenced by Nitrogen and Potassium Fertilization." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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CHEMICAL COMPOSITION AND YIELD OF TALL FESCUE  
(FESTUCA ARUNDINACEA SCHREB.) AS INFLUENCED  
BY NITROGEN AND POTASSIUM FERTILIZATION

A Thesis  
Presented for the  
Master of Science  
Degree  
The University of Tennessee

David Byron Hannaway

June 1975

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## ABSTRACT

Two one-year experiments were conducted to determine the effects of fertilization and season on the chemical composition of Kentucky 31 tall fescue. Samples were taken monthly for determination of K, Ca, Mg, P, crude protein and yield. Freeze-dried samples were used in determining malic acid, citric acid, and total ash alkalinity concentration. Nitrogen fertilization rates were 0 and 112 kg N/ha for the first experiment and 56, 112, and 168 kg N/ha for the second experiment. Potassium fertilization levels were employed only in the second experiment; the rates were 0 and 168 kg K/ha. The soil was Etowah silt loam.

Nitrogen fertilization generally increased the concentration of K. Increases in the concentration of Ca and Mg due to N fertilization were noted in some months. Nitrogen fertilization had little affect on the P concentration. Crude protein and dry matter yield were increased with N fertilization. Malic acid and total ash alkalinity concentrations were increased with N fertilization, while citric acid concentration was decreased.

Potassium fertilization increased the K concentration on all harvest dates. Decreases in Ca and Mg were associated with K fertilization. The P concentration was not affected by K fertilization. Crude protein values were increased in some months due to K fertilization. Dry matter yields were slightly increased in some months due to K fertilization.

Malic acid and total ash alkalinity concentrations were decreased by K fertilization, while citric acid concentration was increased.

Seasonal variation resulted in highest concentrations of K and organic acids in the cooler months, while highest Ca, Mg, and P concentrations were observed in the warmer months. Little forage material was produced during January and February, but production was possible the remainder of the year with split applications of N fertilization. It is suggested that K fertilization be applied after the spring growth period.



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## CHAPTER I

### INTRODUCTION

Demand for greater production from pasture acreage has resulted in the use of greater quantities of nitrogen and potassium fertilizers. The addition of these fertilizers not only increases production of dry matter, but is also fundamental in changing the composition of the forage material.

The purpose of this study was to evaluate the effect of fertilization variables and season on tall fescue (Festuca arundinacea Schreb.) forage. Of primary concern was the Mg concentration of forage as related to "grass tetany," or hypomagnesemia. The mineral composition (K, Ca, Mg and P), crude protein, and dry matter yields were determined monthly.

Nutrient imbalances have been shown to cause changes in the level of organic acids of plant extracts. These acids serve not only as reservoirs of potentially useful carbon chains, but also as anions essential for maintaining ionic balance. Concentration of organic acids in forage material has been implicated in grass tetany incidence. Citric and malic acids have been shown to be the organic anions of greatest concentration in tall fescue. In an attempt to characterize production of organic anions in response to nutrient uptake and season, malic acid, citric acid, and total ash alkalinity concentrations were determined monthly.



## CHAPTER II

### LITERATURE REVIEW

A summary of the changes in mineral composition of forage crops following fertilizer application has been presented by Whitehead (1966): (1) the addition of a nutrient to the soil can be expected to cause an increase in the level of that element unless growth had previously been limited by a deficiency of that element; (2) addition of a nutrient can cause secondary effects on plant composition, e.g. by dilution or by antagonistic or synergistic effects; (3) the degree of change in plant mineral composition may be affected by the time period between application and harvesting or grazing; (4) the effects of a given fertilizer treatment on plant mineral uptake and concentration may be significantly related to the effects which the treatment has on soil pH.

#### I. NUTRIENT UPTAKE IN PLANTS

Those factors which determine the ionic status of a plant are important in terms of forage quality. The ionic status of a plant is the result of those factors responsible for nutrient uptake. The basic assumption which governs these uptake mechanisms is the maintenance of cell electroneutrality.

Various investigators have demonstrated the existence of two distinct systems of nutrient absorption (Hagen and Hopkins, 1955; Fried and Noggle, 1958; Epstein, 1966, 1969). These two systems are defined

by the range of concentration at which they operate. System I approaches maximal rates at ion concentration of 0.1 to 1.0 mM. System II contributes to ion uptake when ion concentrations exceed 1.0 mM. When salt concentrations exceed 1.0 mM, both systems are operable. Reisenauer (1966) has shown that the normal range of ion concentration is below the concentration at which System II makes significant contributions to ion absorption. System I has been characterized as a hyperbolic relationship resembling Michaelis-Menten enzyme kinetics reactions in which the limiting rate of absorption is approached asymptotically (Hiatt, 1970).

## II. ION INTERACTIONS

Whether or not the absorption process involves the action of a mobile carrier, it seems evident that ions in some way interact with the cell membrane. This interaction is of electrostatic nature regardless of the mechanism of transport across the cell membrane. Certain physical properties of ions influence their interactions on membrane sites. The most important of these are charge and hydrated radii. Monovalent ions are absorbed faster than divalent or multivalent ions of similar hydrated radii.

Absorption rates for divalent cations are less than those for the monovalent cations. The presence of  $\text{Ca}^{2+}$  is considered to be essential to the integrity of selective ion transport mechanisms (Epstein, 1961; Handley, Metwally, and Overstreet, 1965; Solomon, 1960; Whittenbury, Sugino, and Solomon, 1960).



Magnesium absorption by excised roots is generally equal to or greater than  $\text{Ca}^{2+}$  absorption in single salt solutions. However, differences among tissues are observed for  $\text{Mg}^{2+}$  absorption in the presence of  $\text{Ca}^{2+}$ . Calcium has been shown to have an inhibitory effect on  $\text{Mg}^{2+}$  absorption in barley (Moore, Jacobson, and Overstreet, 1961) and in maize (Maas, 1969; Maas and Ogata, 1971).

Absorption rates of cations are influenced somewhat by the counter (associated anion) ion. However, the counter ion effect is primarily of significance in System II, which operates above normal physiological levels.

### III. FERTILIZATION EFFECTS ON NUTRIENT UPTAKE

The nitrate anion is usually associated with higher cation absorption rates due to its metabolic fate. The nitrate anion provides a mobile carrier for transport, and is then destroyed by reduction forming an organic anion. This destruction of  $\text{NO}_3^-$  maintains an anion gradient as well as providing a cation-binding site. The slower mobility and lower accumulation levels of  $\text{SO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  (or  $\text{HPO}_4^{2-}$ ) make them less effective than  $\text{NO}_3^-$  in providing a cation-binding site (Kirkby and Mengel, 1967).

Changes in the mineral composition of grassland forage induced by nitrogen fertilization were studied by Stewart and Holmes (1953). High levels of N were found to decrease the K concentration and increase the concentrations of Ca and Mg. Little effect was noted on the concentration of P. Grunes et al. (1974) have shown that fertilization with 448 kg N/ha

markedly increased the concentrations of Mg and Ca in tall fescue. Potassium fertilization has been shown to result in large increases in the K concentration of forage material and to depress the uptake of Mg and Ca (Stewart and Holmes, 1953). Kemp (1960) found a similar effect of K fertilization on grass pastures. Reith et al. (1964) found that N and K fertilization separately had a variable effect on Ca concentration, but decreased the Ca concentration when applied together. Magnesium concentration was increased by N fertilization but decreased with K fertilization. When both N and K were applied the depressing effect of K fertilization was less pronounced.

The form of nitrogen fertilization has been shown to affect the mineral composition of forage crops. Nitrate-nitrogen has been shown to increase the Mg concentration to a greater degree than ammonium nitrogen (Mulder, 1956; Wolten, 1960).

#### IV. SEASONAL AND TEMPERATURE EFFECTS ON NUTRIENT UPTAKE

Seasonal effects on the mineral composition of forage plants defoliated by grazing or cutting during the growing season have been shown by Reith et al. (1964). Marked increases in the concentration of Ca and Mg with the advance of the growing season were observed, with little seasonal effect on the P concentration. Significant increases in plant Mg concentration have been shown with the advance of the growing season (Stewart and Holmes, 1953; Reith, 1954; Todd, 1961). The K concentration of orchardgrass (Dactylis glomerata L.), was shown to decrease from a high level in April to a low point in June (Reynolds,



Lewis, and Laaker; 1971). Calcium and Mg concentrations increased from the April harvests to the June harvests, reflecting similar trends of these elements to trends reported by Reid, Post, and Jung (1970).

Temperature has been shown to have an affect on the uptake of nutrients. At lower temperatures,  $K^+$  is preferentially absorbed, resulting in higher concentrations of K and lower concentrations of Ca and Mg. Voisin (1963) has shown that a rapid rise in temperature following a cold period is accompanied by a rapid rise in the K concentration of plants. The Mg and Ca concentrations are not increased as rapidly.

#### V. ORGANIC ACID SYNTHESIS

Ulrich (1941) demonstrated that when  $K^+$  was absorbed in excess of the associated anion, the excess  $K^+$  entering the cells was balanced by an increase of organic anions within the tissues. Subsequently other investigators (Jacobson and Ordin, 1954; Jacobson, 1955; Jackson and Coleman, 1959; Hiatt and Hendricks, 1967; Hiatt, 1967; Hiatt and Leggett, 1974) have similarly reported that organic acid content of roots increased when cations were absorbed in excess of anions and decreased when anions were absorbed in excess of cations. Stoichiometric increases in organic acid concentration have been demonstrated when cations are absorbed in excess of anions (Jacobson and Ordin, 1954).

The assimilation of  $CO_2$ , during photosynthesis and the subsequent conversion of bicarbonate to the carboxylic group of phosphoglycerate results in no long term accumulation of anion, because phosphoglycerate is quickly reduced to phosphoglyceric aldehyde (Hatch and Slack, 1970).

However, the dark fixation of  $\text{CO}_2$  with phosphoenolpyruvate or enolpyruvate as  $\text{CO}_2$  acceptors does result in the formation of new carboxylic groups, namely oxaloacetate and malate. These newly synthesized organic anions can accumulate to higher concentrations, thus affecting the cation uptake or cation retention (Mengel, 1974).

These newly synthesized carboxylic groups possess a freely dissociable hydrogen ion, which under normal plant cell pH values is dissociated. Hydrogen ions can penetrate cell membranes at a higher rate than organic anions. This difference in diffusion potentials could be responsible for the negative electrical potential of living plant cells which has been measured (Etherton and Higinbotham, 1961; Pallaghy and Scott, 1969; Poole, 1969; Macklon and Higinbotham, 1970). This negative potential may then result in a physiological "sink" for cations.

Selectivity of cation uptake of ions is more complicated than a simple diffusion system responding to Donnan potentials; however, passive uptake of cations does depend largely upon the diffusible and indiffusible anion equivalents of the cell.

The incorporation of nitrate also greatly influences the production of organic anions. In order to maintain equilibrium between anions and cations, the uptake of larger quantities of nitrate ion must be balanced by an equivalent amount of cation or the release of anion equivalents. Anion exchange (in the form of  $\text{OH}^-$  or  $\text{HCO}_3^-$ ) is thought to account for only a small portion of the nitrate uptake (Kirkby and Mengel, 1967). The major portion of nitrate absorbed is balanced by simultaneous uptake of cations. In most cases, selective active absorption of  $\text{K}^+$  largely accounts for balancing nitrate uptake. In the reductive step from



nitrate to nitrite,  $\text{OH}^-$  is released, maintaining the cation/anion balance. This  $\text{OH}^-$  produced favors the solvation of  $\text{CO}_2$ , thus increasing the concentration of bicarbonate. Bedri, Wallace, and Rhoads (1960), and Chouteau (1963), have shown that an increase in bicarbonate concentration stimulates the formation of organic anions.

Cummings and Teel (1965) showed that nitrogen fertilization stimulates the production of organic anions. In a study with orchardgrass, the malate concentration was increased with N fertilization and decreased with K fertilization. Grunes et al. (1974) have shown that fertilization with 448 kg N/ha markedly increased the concentration of malic acid in tall fescue, and to a much lesser extent increased the concentration of citric acid. Barta (1973) has shown that N fertilization increased the concentration of citric acid in orchardgrass. Potassium fertilization was shown to decrease the concentration of malic acid. Nitrogen fertilization increased the concentration of malic acid and citric acid in bromegrass (Bromus inermis Leyss.), while K fertilization had no significant effect. Clark (1968) reported that deficiencies of K, Ca, Mg, or P increased the concentration of citric acid and malic acid. The stage of growth has also been shown by Barta (1973) to have a significant effect on the concentration of citric acid in both orchardgrass and bromegrass. A 50 percent decrease in citric acid concentration was found in orchardgrass with the advance of the growing season.

## VI. HYPOMAGNESEMIA IN ANIMALS

The incidence of hypomagnesemia or grass tetany has led to greater interest in the mineral and chemical composition of forages. Hypomagnesemia is most frequently a disease of milking cows, often shortly after calving, and in lactating ewes, but has also been reported in dry cows, steers and calves. Grass tetany usually occurs in the spring when the animal is taken from stall feeding to fresh, lush pasture which has been highly fertilized. However, tetany has also been reported on winter cereal wintertime pastures in the western states. The usual symptoms include a variety of convulsions and paralyzes, often ending in death. Treatment is an intravenous injection of 500 ml of a solution containing d-saccharate and gluconate salts of calcium with dextrose, as well as P and Mg. Each 500 ml of preparation contains Ca, 8.42 g; Mg, 1.88 g; P, 4.8 g and dextrose 83.4 g (Hall and Reynolds, 1972).

Sjollema (1932), was the first to relate the disease to low serum magnesium. Normal animals contained 1.3-2.0 mg/100 ml of Mg in the blood serum, while tetanic animals had serum Mg contents of 0.5-1.0 mg/100 ml. Meyer, quoted by Grunes et al. (1970), has classified less than 1.2 mg/100 ml of serum Mg as severely hypomagnesemic.

Plant function of Mg is of primary importance in the synthesis of chlorophyll and as an enzyme activator. In livestock, Mg functions as an important enzyme activator and cofactor in many enzyme systems. The involvement of Mg in reactions with adenosine triphosphate (ATP) both in glycolysis and oxidative phosphorylation illustrates the central role Mg plays in carbohydrate metabolism.



The majority of total body Mg (from 50-70 percent) is found in bone, with the remainder equally distributed between muscle and nonmuscle soft tissue (Todd, 1969; Wacher and Parisi, 1968). High concentrations of Mg are found in the heart, striated muscle, liver, kidney, and brain. Blood serum usually contains no more than 10 percent of that found in soft tissues (Wilson, 1960).

Urinary Mg has been described as a threshold substance by Miller, Britton, and Ansari (1972). When plasma Mg is below a specific critical level, urinary Mg loss is reduced. Storry and Rook (1963) showed that urine losses of Mg in normal animals ranged from 1 to 2 g per day and decreased to 0 when the blood serum Mg fell below 2.0 mg/100 ml. Magnesium lost in the feces dropped from a normal of 2 g per day to 1 g per day when animals were fed a diet deficient in Mg.

Maintenance of plasma Mg when intake is in excess of requirements is accomplished by increased urinary excretion. However, in the case of a rapid drop in serum Mg, no such homeostatic mechanism is available. Mobilization of bone reserves does not provide a rapid mechanism for maintaining the proper level of plasma Mg. The higher incidence of grass tetany in older cows has been partially attributed to less exchangeable Mg present in their bodies (Blaxter and McGill, 1956).

Although the Mg concentration of tetanigenic pasture forage is often as high or higher than in adjacent unaffected pastures, oral administration of Mg salts has been found to have a successful preventative effect on the occurrence of tetany. As cold weather promotes the selective absorption of K over Mg (Barnett and Reid, 1961), pastures are often

lowest in Mg concentration at the time when tetany is most common. Areas where dolomitic limestone is used appear to have higher concentrations of Mg in forage material and have a lower incidence of grass tetany. However, large between-animal variations in the efficiency of utilization of Mg and their respective endogenous fecal and urine losses further complicate the tetany problem.

It is thought that tetany is a condition brought about by a reduced absorption of magnesium. Primary absorption occurs in the first segments of small intestine (Perry et al., 1967). Several factors have been implicated in decreasing the absorption of Mg in the small intestine: (1) mineral imbalance of the herbage, (2) increased ruminal ammonia production from soluble nitrogenous components, and (3) certain Krebs cycle organic acids.

Kemp (1960) established 0.20 percent as the "safe" level for Mg in forage. A value of  $K/(Ca+Mg)$  in excess of 2.2 has been shown to increase the frequency of tetany (Kemp and 't Hart, 1957) indicating that it is the mineral balance rather than an absolute value of Mg concentration. Butler (1963) found that a very good relationship existed between the ratio of  $K:(Ca+Mg)$  and the incidence of grass tetany. However, Seekles, quoted by Grunes et al. (1970) indicated that the  $K/(Ca+Mg)$  value was not a sufficient indicant of tetany according to the data obtained over many years' samples in the Netherlands. House and Van Campen (1971) found Mg absorption to be depressed with increased dietary K in sheep, giving explanation to the antagonistic effect of K and Mg. Newton et al. (1972) reported a 46 percent depression in apparent Mg absorption when high



levels of K were fed to lambs. Fecal Mg increased while urinary Mg decreased, indicating that dietary Mg was probably not absorbed. These studies indicated that feeding a high level of K interferes with Mg absorption rather than increasing endogenous excretion into the intestine.

Fertilization of pastures with high levels of N has resulted in lowered blood serum Mg in ruminants grazing these pastures (Bartlett et al., 1954; Kemp, 1958, 1960, 1971; Kemp et al., 1961). Increased rumen ammonia production from grass feeding has been observed by Head and Rook (1955). They have suggested that hypomagnesemia of cows on grass arises from inadequate absorption of Mg associated with high rumen ammonia production.

Stillings et al. (1964) have reported decreased Mg utilization in a study with sheep fed forages fertilized with high levels of N. Increased fecal Mg and decreased urine Mg were observed. From these studies, it would be expected that pastures heavily fertilized with N and K would be associated with a high incidence of grass tetany. Kemp (1960) has shown that fertilization with a combination of N and K results in lower serum Mg than fertilization with N or K alone. Fontenot et al. (1960) reported a 38 percent increase in fecal Mg excretion by sheep fed rations containing 34.4 percent crude protein and 4.7 percent potassium compared to those fed 12.8 percent crude protein and 1.4 percent potassium on a dry weight basis. Urinary excretion of Mg was decreased, apparently a result of lowered Mg absorption.

Unpublished observations by Moore, Fontenot and Webb quoted by Grunes et al. (1970) have shown that the effect of K appears to be



independent of the dietary N level. High K levels appear to exert a sufficient depressing effect on Mg absorption to be a causative factor in inducing hypomagnesemic tetany. High dietary N increased urinary Mg excretion indicating that N intake increased Mg absorption.

Induction of tetany symptoms has been accomplished by administering KCl and either trans-aconitic acid or citric acid (Bohman et al., 1969). When one of the acids or KCl was administered alone, the symptoms of tetany were not induced. It has been suggested that citric and trans-aconitic acid were complexing Mg, thus reducing its availability to the animal.

Sodium citrate was found to decrease the concentration of Mg in blood serum of calves (Burt and Thomas, 1961). A concentration equivalent to 1 percent citric acid in the dietary dry matter was used. A relationship between tetany incidence and high levels of trans-aconitic acid in range forage species has been reported by Burau and Stout (1965) and Stout, Brownwell, and Burau (1967).

Teel (1966) found that fertilization with  $\text{NH}_4\text{NO}_3$  increased the total organic acid concentrations in tall fescue. Grunes et al. (1970) reported unpublished data of Grunes and Power; Grunes, Mayland, and Stuart; and Smith and Grunes, in which fertilization with  $\text{NH}_4\text{NO}_3$  increased the concentration of trans-aconitic acid in smooth bromegrass and crested wheatgrass (Agropyron desertorum, 'Nordan'). Potassium fertilization markedly increased trans-aconitic and total organic acids in crested wheatgrass.



Seasonal changes in trans-aconitic acid in crested wheatgrass have been reported by Stuart et al. (1973). When air temperatures increased in early May, K and trans-aconitic acid increased while Ca and Mg decreased. This relationship was moisture dependent in that when moisture stress developed, trans-aconitic acid decreased. Patterson, Grunes, and Lathwell (1972) found that the metabolism of photosynthate to the level of organic acids was not impeded by low root-zone temperatures, as concentrations of aconitate were highest when maize (Zea mays L.) was grown at 15 C compared to 25 C. An hypothesis was advanced in an attempt to explain aconitate accumulation. It was suggested that low temperature (15 C) had been shown by Brandon (1967) to lead to a dominance in activity of acid-synthesizing enzymes over the activity of acid-converting enzymes, with the synthesizing enzymes those involved in the B-carboxylase (CO<sub>2</sub>-fixation) pathway converting glycerate-3-P to malate. It was further suggested that most of the aconitate was present in the trans form, a form shown by MacLennan and Beevers (1964) to be a poor substrate for entry into the Krebs cycle, resulting in its accumulation.

Malloy and Richards (1971) have proposed that the survival of organic acids in the rumen and subsequent passage into the small intestine where Mg is absorbed is very doubtful. However, Mayland et al. (1974) have suggested that organic acids may be important in relationship to grass tetany under conditions of marginal Mg supply.

## CHAPTER III

### MATERIALS AND METHODS

Two one-year experiments were established on an Etowah silt loam at the Knoxville Plant Science Farm on an existing "Kentucky 31" tall fescue stand seeded in September 1970.

The first experiment, started October 1972, was a randomized complete block design with four replicates of two nitrogen treatments: 0 and 112 kg N/ha. The nitrogen was applied in the form of ammonium nitrate in two equal parts: on October 10, 1972 and March 23, 1973. Treatments were established on plots 2 x 6 m. No K or P fertilizer was applied. The soil test value for K was 233 kg/ha (high) and for P was 36 kg/ha (high). The pH was 6.3.

The second experiment, started October 1973 on an adjacent tall fescue stand, was a factorial arrangement of treatments in a randomized complete block design with three replicates. Treatments were three levels of nitrogen fertilization (56, 112, and 168 kg N/ha), and two levels of potassium fertilization (0, 168 kg K/ha). The nitrogen was applied in the form of ammonium nitrate on October 9, 1973 for the 56 kg treatment; on October 9, 1973, and March 22, 1974 for the 112 kg treatment; on October 9, 1973, March 22, 1974 and June 25, 1974 for the 168 kg treatment. Potassium was applied as potassium chloride on October 9, 1973. Treatments were established on plots 2 x 6 m. No P fertilizer



was applied. The soil test value for K was 256 kg/ha (high) and for P was 32 kg/ha (high). The pH was 6.3.

### I. SAMPLING

Samples were taken monthly, except for January 1973 and February 1974, when only trace amounts of forage were available for harvest. Sampling was with a rotary mower at a 4-cm stubble height for determination of K, Ca, Mg, P and crude protein. Samples were dried in a forced-air oven at 65 C, ground in a Wiley mill to pass a 2-mm screen, and stored in paper bags for subsequent analysis.

Hand-clipped samples were taken on each harvest date at a 4-cm stubble height and placed in plastic bags between pieces of dry ice in the field for quick freezing. Titratable ash alkalinity and organic acid analyses were performed on these samples following freeze-drying and grinding in a Thomas mill to pass a 100-mesh screen. Samples were kept at -20 C until analysis.

Dry matter yield samples were obtained by harvest with a rotary mower to a 4-cm stubble height of a strip 0.5 x 6 m in each plot. The entire experimental area was then cut to 4 cm on each sampling date.

### II. LABORATORY PROCEDURES

Simultaneous determination of K, Ca, Mg, and P was made by auto analysis on a Technicon Autoanalyzer following dry ashing at 550 C for four hours in a muffle furnace. The K and Ca were determined using a Technicon III dual-channel flame photometer. The Mg concentration was

determined colorimetrically by the magnesium blue reaction. Colorimetric P determination was made by the ammonium vanadate reaction. These procedures were described by Steckel and Flannery (1965). Results are reported as percent of each element on a dry weight basis.

Crude protein concentration was determined following digestion of the dried forage samples in concentrated sulfuric acid and in 35 percent hydrogen peroxide. The phenol-hypochlorite color reaction was utilized in the Autoanalyzer procedure described by Thomas, Sheard, and Moyer (1967). Results are reported as percent crude protein on a dry weight basis.

Titrateable ash alkalinity was determined as an indication of total organic acids by back-titration of dry ashed samples by a method modified from van Tuil, Lampe and Kykshoorn (1964). Results are reported as meq per kg.

Extracts of freeze dried samples were prepared as described by Prior et al. (1973) for subsequent malic acid and citric acid analysis. Malic acid was determined fluorometrically as described by Hummel (1949). A 0.3 ml sample size was used. Centrifugation was for 20 minutes at 2200 rpm. Fluorescence was measured on a Perkin Elmer 203 spectrofluorometer, exciter wavelength 366 nm, analyzer wavelength 444 nm.

Citric acid was determined colorimetrically on a Perkin Elmer 202 spectrophotometer using a modification of the method described by Camp and Farmer (1967). Sample size was 2.0 ml. A 1.0 ml aliquot of n-heptane was added to 3.0 ml of thiourea and sodium borate for concentration of the pale yellow color. Agitation was accomplished by use of a



vortex mixer, to avoid the need for glass-stoppered test tubes. An excess of hydrogen peroxide inhibited color formation. Maximum absorption was at 430 nm. Results are reported as meq per kg.

### III. STATISTICAL ANALYSES

Analyses of variance were calculated on an experiment basis because of different experimental designs.

In the first experiment, the effect of nitrogen level on K, Ca, Mg P, crude protein, ash alkalinity, malic acid, and citric acid concentrations and on dry matter yield was tested on each harvest date. A combined analysis was also calculated, with N level and harvest date as variables.

Similar analyses of variance for the effect of N and K level were calculated on each harvest date for the second experiment. The combined analyses of the second experiment were divided into three sections, corresponding to the N applications and material available for harvest.

In the first section, corresponding to November, December, January, and March harvest dates, treatments included two levels of K (0 and 168 kg K/ha) and one level of N (56 kg N/ha). The second section, April, May and June, included two levels of K (0 and 168 kg K/ha) and two levels of N (56 and 112 kg N/ha). The third section, July, August, September and October, included two levels of K (0 and 168 kg K/ha) and one level of N (168 kg N/ha). Only one level of N was included because of lack of forage in the other two N levels.

Statistical Analysis System (SAS), developed by Barr and Goodnight (1972) was used for the analyses of variance.



## CHAPTER IV

### RESULTS AND DISCUSSION

#### I. MINERAL RELATIONSHIPS

Analyses of variance for the first year experiment, fertilized with two levels of N, were calculated on a monthly basis with N levels as treatments (Table I and Appendix Figures 1-4). A total year analysis was also calculated with N level and date as treatments.

The K concentration was significantly ( $P \leq .05$  throughout unless otherwise specified) increased by N fertilization on the November, December, March, and April harvest dates and reached the highest concentration for the year in April with a value of 2.48 percent K. By the August and September harvests, a significant difference was noted with the 0 N rate higher in concentration. Seasonal variation in the concentration of K was observed, with N fertilized grass significantly higher, and date of harvest highly significant ( $P \leq .0001$ ).

Calcium concentration was significantly increased by N fertilization in March and June. A slightly higher concentration of Ca was noted for the 112 kg N/ha rate in November, February, April, May, and September. Seasonal differences were highly significant ( $P \leq .0001$ ) for Ca concentration. The lowest Ca concentration was found in March, with depressed values in February and May. The lower percentage of Ca in May was associated with a higher percentage of stems at harvest time.

TABLE I  
 CHEMICAL COMPOSITION AND YIELD OF TALL FESCUE FORAGE  
 FERTILIZED WITH TWO LEVELS OF NITROGEN AND  
 HARVESTED 11 TIMES DURING 1972-73

Harvest Date	Concentration in Forage				Protein	Forage DM kg/ha
	K	Ca	Mg	P		
	Percent					
November 1972 <sup>a</sup>						
0 N	2.16	0.29	0.28	0.31*	11.73	322
112 N	2.31*	0.30	0.31	0.24	16.50*	582*
December 1972						
0 N	1.44	0.28	0.25	0.23	11.50	73
112 N	2.30*	0.28	0.32*	0.29*	15.35*	119*
February 1973						
0 N	1.90	0.28	0.32	0.29*	13.50	22
112 N	1.91	0.34	0.30	0.24	14.05	58*
March 1973						
0 N	1.65	0.23	0.26	0.28	12.85	252
112 N	2.07*	0.25*	0.30	0.27	14.73	392*
April 1973 <sup>a</sup>						
0 N	2.24	0.34	0.31	0.30	14.10	138
112N	2.48*	0.36	0.32	0.30	17.65*	768*
May 1973						
0 N	2.02	0.25	0.37	0.31*	9.63	556
112 N	1.98	0.27	0.35	0.27	9.55	1520*
June 1973						
0 N	2.00	0.31	0.54*	0.45	12.23	168
112 N	2.02	0.35*	0.46	0.40	12.63	387*



TABLE I (continued)

Harvest Date	Concentration in Forage				Protein	Forage DM kg/ha
	K	Ca	Mg	P		
July 1973						
0 N	2.01	0.30	0.51	0.50	12.13	252
112 N	1.99	0.30	0.51	0.50	12.18	365*
August 1973						
0 N	2.21*	0.34	0.49	0.47	10.80	421
112 N	2.14	0.33	0.47	0.49	11.10	465*
September 1973						
0 N	2.21*	0.33	0.42	0.44	10.60	---
112 N	2.03	0.41	0.42	0.44	10.70	---
October 1973						
0 N	2.13	0.28	0.36	0.37	11.38	65
112 N	2.09	0.24	0.32	0.36	10.75	73

<sup>a</sup>56 kg N/ha was applied on October 10, 1972 and after the March 23, 1973 harvest date.

\*Indicates significant difference ( $P \leq .05$ ) within a pair in the same column.

Magnesium concentration was significantly increased in December by N fertilization. Small increases associated with N fertilization were noted in November, March, and April. The Mg concentration was decreased by N fertilization during February, May, June, and October. The marked seasonal response in the Mg concentration was a lower percentage of Mg during the cooler months and an increased Mg concentration during the summer months. The lower concentration of Mg during May was associated with the stemmier material. In no case did the percentage of Mg drop below the suggested "safe" value of 0.20 percent.

Calculation of the ratio of  $K:(Ca + Mg)$  resulted in values not expected to cause adverse effects to animals grazing this forage. Values in excess of 2.2 have been shown to increase the frequency of tetany (Kemp and 't Hart, 1957). Only three values were greater than 1.6, with many values less than 1.0. Phosphorus concentration showed variable response to fertilization but a very distinct seasonal response, with the highest concentrations in June, July, August, and September.

Increases in the concentration of Ca and Mg due to N fertilization were not as pronounced as those found by Grunes et al. (1974) with a 448 kg N/ha treatment. This is probably due to the difference in fertilization rate, with more pronounced effects resulting from higher fertility rates.

Analyses of variance for the effect of N and K level were calculated on each harvest date for the second experiment (Tables II, III, IV, and Appendix Figures 10-13). The combined analyses of the second experiment



TABLE II

CHEMICAL COMPOSITION AND YIELD OF TALL FESCUE FERTILIZED  
WITH ONE LEVEL OF NITROGEN AND TWO LEVELS OF POTASSIUM  
AND HARVESTED NOVEMBER 1973, DECEMBER 1973,  
JANUARY 1974, AND MARCH 1974

Harvest Date	Concentration in Forage				Protein	Forage DM kg/ha
	K	Ca	Mg	P		
November 1973						
N <sub>1</sub> K <sub>1</sub> <sup>a</sup>	1.71	0.36	0.22*	0.24*	15.94	170
N <sub>1</sub> K <sub>2</sub>	1.77	0.36	0.20	0.23	15.76	163
December 1973						
N <sub>1</sub> K <sub>1</sub>	1.60	0.39	0.21*	0.22	14.14	49*
N <sub>1</sub> K <sub>2</sub>	1.78*	0.39	0.18	0.22	13.74	45
January 1974						
N <sub>1</sub> K <sub>1</sub>	1.88	0.37	0.20*	0.26	16.49	31
N <sub>1</sub> K <sub>2</sub>	2.00*	0.35	0.17	0.26	17.03	35
March 1974						
N <sub>1</sub> K <sub>1</sub>	2.22	0.43	0.24*	0.28	16.78	116
N <sub>1</sub> K <sub>2</sub>	2.33*	0.43	0.22	0.28	16.60	115

<sup>a</sup>Fertilization for the two treatments was:

N<sub>1</sub>K<sub>1</sub> 56 kg N/ha, 0 kg K/ha  
N<sub>1</sub>K<sub>2</sub> 56 kg N/ha, 168 kg K/ha

\*Indicates significant difference ( $P < .05$ ) within a pair in the same column.

TABLE III

CHEMICAL COMPOSITION AND YIELD OF TALL FESCUE FERTILIZED  
WITH TWO LEVELS OF NITROGEN AND TWO LEVELS OF POTASSIUM  
AND HARVESTED APRIL 1974, MAY 1974, AND JUNE 1974

Harvest Date	Concentration in Forage				Protein	Forage DM kg/ha
	K	Ca	Mg	P		
April 1974						
N <sub>1</sub> K <sub>1</sub> <sup>1</sup>	1.96 c <sup>2</sup>	0.29 a	0.16 a	0.22 a	12.07 b	274 b
N <sub>1</sub> K <sub>1</sub>	2.07 b	0.32 a	0.13 b	0.22 a	11.93 b	243 b
N <sub>1</sub> K <sub>2</sub>	2.03 b	0.31 a	0.17 a	0.23 a	14.33 a	687 a
N <sub>2</sub> K <sub>1</sub>	2.27 a	0.31 a	0.15 b	0.23 a	13.67 a	751 a
N <sub>2</sub> K <sub>2</sub>						
May 1974						
N <sub>1</sub> K <sub>1</sub>	1.86 b	0.20 b	0.17 a	0.24 a	6.33 a	975 b
N <sub>1</sub> K <sub>1</sub>	1.90 b	0.18 b	0.16 a	0.24 a	6.30 a	1033 b
N <sub>1</sub> K <sub>2</sub>	1.92 b	0.20 a	0.16 a	0.22 a	6.63 a	1540 a
N <sub>2</sub> K <sub>1</sub>	2.06 a	0.26 a	0.17 a	0.22 a	6.90 a	1389 a
N <sub>2</sub> K <sub>2</sub>						
June 1974						
N <sub>1</sub> K <sub>1</sub>	1.69 a	0.38 a	0.30 a	0.31 a	9.5 a	251 b
N <sub>1</sub> K <sub>1</sub>	1.85 a	0.47 a	0.31 a	0.35 a	10.5 a	287 b
N <sub>1</sub> K <sub>2</sub>	1.81 a	0.39 a	0.32 a	0.33 a	9.5 a	339 a
N <sub>2</sub> K <sub>1</sub>	1.80 a	0.40 a	0.30 a	0.31 a	9.5 a	359 a
N <sub>2</sub> K <sub>2</sub>						

<sup>1</sup>Fertilization for the four treatments was:

N<sub>1</sub>K<sub>1</sub> 56 kg N/ha, 0 kg K/ha  
 N<sub>1</sub>K<sub>1</sub> 56 kg N/ha, 168 kg K/ha  
 N<sub>1</sub>K<sub>2</sub> 112 kg N/ha, 0 kg K/ha  
 N<sub>2</sub>K<sub>1</sub> 112 kg N/ha, 168 kg K/ha  
 N<sub>2</sub>K<sub>2</sub>

<sup>2</sup>Values in the same column within each month followed by the same letter are not significantly different ( $P \leq .05$ ).



TABLE IV  
 CHEMICAL COMPOSITION AND YIELD OF TALL FESCUE FERTILIZED  
 WITH ONE LEVEL OF NITROGEN AND TWO LEVELS OF  
 POTASSIUM AND HARVESTED JULY 1974,  
 AUGUST 1974, SEPTEMBER 1974,  
 AND OCTOBER 1974

Harvest Date	Concentration in Forage				Protein	Forage DM kg/ha
	K	Ca	Mg	P		
July 1974						
$N_3K_1^a$	1.84	0.46	0.31	0.32	13.03	261
$N_3K_2$	1.91	0.46	0.32	0.32	13.90*	320
August 1974						
$N_3K_1$	2.06	0.38	0.31	0.37	12.17	144
$N_3K_2$	2.31	0.40	0.32	0.40	12.30	173
September 1974						
$N_3K_1$	1.88	0.30	0.30	0.35	12.37	150
$N_3K_2$	1.98	0.29	0.32	0.36	12.90	165
October 1974						
$N_3K_1$	1.69	0.47	0.31	0.25	10.90	93
$N_3K_2$	1.73	0.44	0.29	0.26	11.13	96

<sup>a</sup>Fertilization for the two treatments was:

$N_3K_1$  168 kg N/ha, 0 kg K/ha  
 $N_3K_2$  168 kg N/ha, 168 kg K/ha

\*Indicates significant difference ( $P \leq .05$ ) within a pair in the same column.

were divided into three sections, corresponding to the N applications and material available for harvest.

In the first section, corresponding to November, December, January, and March harvest dates, treatments included two levels of K (0 and 168 kg K/ha) and one level of N (56 kg N/ha). The second section, April, May, and June, included two levels of K (0 and 168 kg K/ha) and two levels of N (56 and 112 kg N/ha). The third section, July, August, September, and October, included two levels of K (0 and 168 kg K/ha) and one level of N (168 kg N/ha).

Potassium fertilization increased the concentration of K in all months (Appendix Figure 10). Significant increases in K concentration were observed in December 1973, January 1974, and March 1974. Highest concentrations of K were found in the March and April harvests (2.33 percent and 2.27 percent respectively). Nitrogen fertilization also significantly increased the K concentration in the April and May harvests.

Calcium concentration was not significantly affected by K fertilization. Nitrogen fertilization did result in a significant increase in Ca on the May harvest date. The April and May harvests contained the lowest concentration of Ca, with highest concentrations noted in June and July.

The Mg concentration in November, December, January, March, and April was significantly depressed by K fertilization. Nitrogen fertilization had no significant effect on the Mg concentration. The concentration of Mg was lowest during April with values at or below the suggested 0.20 percent observed in the 168 kg K/ha treatment in November, December,



January, April, and May. A considerable increase in the Mg concentration was noted by the June harvest, with adequate levels of Mg maintained during the summer months.

During April and May, several of the 168 kg K/ha treatments exceeded the suggested value of 2.2 for the K:(Ca + Mg) ratio, the maximum value attained was 2.6. Phosphorus concentration was not significantly affected by fertilizer treatment. The increase in concentration of P during June is comparable to the seasonal effect on the Mg and Ca concentrations. The highest value of P for the experiment was attained during August.

Of primary interest in the mineral relationships was the effect of N and K fertilization on the Mg concentration of forage material in early spring. Nitrogen fertilization resulted in variable responses of forage Mg. Increases associated with N fertilization were noted during March and April. Potassium fertilization significantly decreased the Mg concentration during March and April, when the stress is greatest on lactating cows following spring calving. The Mg level dropped below the suggested 0.20 percent value in April and May, and resulted in values in excess of 2.2 for the K:(Ca + Mg) ratio.

## II. FERTILIZATION AND SEASONAL EFFECTS ON CRUDE PROTEIN AND YIELD

In the first experiment, crude protein values were significantly increased by N fertilization in November, December, and April (Table I, page 21, and Appendix Figure 5). Increases associated with N fertilization were also noted in February, March, June, July, August, and

September. The lowest values for percent crude protein were observed in May, associated with the stemmy material. Even at this late stage of maturity the forage contained 9.6 percent crude protein. The highest concentration of crude protein was observed in April (17.65 percent) with the 112 kg N/ha treatment.

Forage dry matter yields were significantly increased by N fertilization in all harvests except October (Table I, page 21, and Appendix Figure 6). Only a trace of forage was available for the September harvest date, necessitating the use of freeze dried samples for mineral and crude protein analyses. Marked seasonal response in production of dry matter was observed. Very little forage was available for the December and January harvests. Increased dry matter was produced by the March harvest date, with a peak in May and continued growth through the early part of August.

In the second experiment, the percent crude protein was significantly increased in April by N fertilization (Table III, page 25, and Appendix Figure 14). Potassium fertilization also significantly increased the crude protein during July (Table IV, page 26). Slightly increased values associated with K fertilization were observed from the June through the October harvest dates. The lowest crude protein value was obtained in May (6.3 percent), when stemmy material was harvested. Depressed values were also observed for the June and October harvest dates, when N fertilization was depleted.

Forage dry matter yields were significantly increased by N fertilization during April, May, and June (Table III, page 25). Potassium



fertilization did not result in significant increases in dry matter yields, but slight increases were observed in the July, August, September, and October harvests. The seasonal increase in production was observed by the March harvest and continued through the November harvest date with very little forage available for harvest during December, January, and February (Appendix Figure 15).

### III. ORGANIC ACID RELATIONSHIPS

Total organic acid concentration, as estimated by the ash alkalinity procedure, was significantly increased by N fertilization in many of the harvests with increases noted in all months (Table V and Appendix Figure 7). Highest values in the first experiment occurred in April and June with the 112 kg N/ha treatment. Lowest values for ash alkalinity were found in February, but increased in the March harvest.

Malic acid concentration was significantly increased by N fertilization in many of the harvest dates (Table V and Appendix Figure 8). Highest concentrations were found in March and April, during which months malic acid accounted for greater than 67 percent of the total acidity in the 112 kg N/ha treatment.

Citric acid concentration was decreased by nitrogen fertilization during many of the harvest months (Table V and Appendix Figure 9). Citric acid contributed a much lower percentage of the total acidity than did malic acid. Highest concentrations were noted in April and June.

The increased concentration of malic acid due to N fertilization agrees with data of Grunes et al. (1974). However, increases in citric

TABLE V

MALIC ACID, CITRIC ACID, AND TOTAL ASH ALKALINITY OF TALL  
FESCUE FERTILIZED WITH TWO NITROGEN TREATMENTS AND  
HARVESTED 11 TIMES DURING 1972-1973

Harvest Date	Concentration in Forage		Ash Alkalinity
	Malic	Citric meq/kg	
November 1972 <sup>a</sup>			
0 N	118	45	455
112 N	161	41	626*
December 1972			
0 N	108	39*	500
112 N	159	24	565
February 1973			
0 N	174	38*	483
112 N	240	22	485
March 1973			
0 N	274	63	515
112 N	420*	65	565*
April 1973			
0 N	197	88*	525
112 N	455*	77	673*
May 1973			
0 N	244	54	504
112 N	389*	47	581
June 1973			
0 N	211	71	619
112 N	353*	63	686



TABLE V (continued)

Harvest Date	Concentration in Forage		
	Malic	Citric	Ash Alkalinity
	meq/kg		
July 1973			
0 N	144	41	644
112 N	183*	37	663*
August 1973			
0 N	168	60	619
112 N	215	50	655*
September 1973			
0 N	277	24	615
112 N	332	24	648
October 1973			
0 N	89	28	535
112 N	100	28	535

<sup>a</sup>56 kg N ha was applied on October 10, 1972 and after the March 23, 1973 harvest date.

\*Indicates significant difference ( $P \leq .05$ ) within a pair in the same column.



acid concentration due to N fertilization were not observed, possibly due to the lower rates of N fertilization in this study.

In the second experiment, N fertilization significantly increased total organic acid concentration in April and May (Appendix Figure 16). Potassium fertilization significantly decreased ash alkalinity values in November, January, May, and September (Tables VI, VII, and VIII). Highest values were obtained in March, April, June, July, and August, with lowest values in December.

Malic acid was increased by N fertilization during April, May, and June (Table VII). Potassium fertilization resulted in significant reductions in malic acid in December, March, and July.

Changes in the citric acid concentration were mainly associated with K fertilization (Tables VI, VII, and VIII). A significant increase in the citric acid concentration due to K fertilization was observed in April. The highest concentrations of citric acid were found on the November, April, July, and October harvests.

Malic acid concentration was decreased with K fertilization. When forage was analyzed from plots receiving both N and K fertilization, the main effect observed on the concentrations of malic acid and citric acid was that of the K fertilization (Table VII). The concentration of malic acid decreased, while the concentration of citric acid increased, although the response of citric acid was less pronounced.

The response of malic acid to N and K fertilization agrees with the work by Cummings and Teel (1965) on orchardgrass in which malic acid was increased with N fertilization and decreased with K fertilization. It



TABLE VI

MALIC ACID, CITRIC ACID, AND TOTAL ASH ALKALINITY OF TALL  
FESCUE FERTILIZED WITH ONE LEVEL OF NITROGEN AND TWO  
LEVELS OF POTASSIUM AND HARVESTED NOVEMBER 1973,  
DECEMBER 1973, JANUARY 1974, AND MARCH 1974

Harvest Date	Concentration in Forage		
	Malic	Citric	Ash Alkalinity
	meq/kg		
November 1973 <sup>a</sup>			
N <sub>1</sub> K <sub>1</sub>	175	51	587*
N <sub>1</sub> K <sub>2</sub>	94	55	477
December 1973			
N <sub>1</sub> K <sub>1</sub>	142*	39	547
N <sub>1</sub> K <sub>2</sub>	60	39	440
January 1974			
N <sub>1</sub> K <sub>1</sub>	139	3	572*
N <sub>1</sub> K <sub>2</sub>	106	3	513
March 1974			
N <sub>1</sub> K <sub>1</sub>	128*	12	708
N <sub>1</sub> K <sub>2</sub>	85	25*	643

<sup>a</sup>Fertilization for the two treatments was

N<sub>1</sub>K<sub>1</sub> 56 kg N/ha, 0 kg K/ha  
N<sub>1</sub>K<sub>2</sub> 56 kg N/ha, 168 kg K/ha

\*Indicates significant difference ( $P \leq .05$ ) within a pair in the same column.

TABLE VII

MALIC ACID, CITRIC ACID, AND TOTAL ASH ALKALINITY OF TALL  
FESCUE FORAGE FERTILIZED WITH TWO LEVELS OF NITROGEN  
AND TWO LEVELS OF POTASSIUM AND HARVESTED  
APRIL 1974, MAY 1974, AND JUNE 1974

Harvest Date	Concentration in Forage		
	Malic	Citric	Ash Alkalinity
	meq/kg		
April 1974			
N <sub>1</sub> K <sub>1</sub> <sup>1</sup>	140 a <sup>2</sup>	64 b	552 b
N <sub>1</sub> K <sub>2</sub>	104 b	71 a	512 b
N <sub>2</sub> K <sub>1</sub>	128 a	56 b	610 a
N <sub>2</sub> K <sub>2</sub>	111 b	69 a	586 a
May 1974			
N <sub>1</sub> K <sub>1</sub>	126 a	28 a	463 b
N <sub>1</sub> K <sub>2</sub>	62 b	23 a	408 c
N <sub>2</sub> K <sub>1</sub>	172 a	31 a	562 a
N <sub>2</sub> K <sub>2</sub>	87 b	30 a	482 b
June 1974			
N <sub>1</sub> K <sub>1</sub>	35 a	43 a	550 a
N <sub>1</sub> K <sub>2</sub>	20 b	48 a	503 a
N <sub>2</sub> K <sub>1</sub>	74 a	52 a	585 a
N <sub>2</sub> K <sub>2</sub>	27 b	50 a	514 a

<sup>1</sup>Fertilization for the four treatments was:

N<sub>1</sub>K<sub>1</sub> 56 kg N/ha, 0 kg K/ha  
 N<sub>1</sub>K<sub>2</sub> 56 kg N/ha, 168 kg K/ha  
 N<sub>2</sub>K<sub>1</sub> 112 kg N/ha, 0 kg K/ha  
 N<sub>2</sub>K<sub>2</sub> 112 kg N/ha, 168 kg K/ha

<sup>2</sup>Values in the same column within each month followed by the same letter are not significantly different ( $P \leq .05$ ).



TABLE VIII

MALIC ACID, CITRIC ACID, AND TOTAL ASH ALKALINITY OF TALL  
FESCUE FERTILIZED WITH ONE LEVEL OF NITROGEN AND TWO  
LEVELS OF POTASSIUM AND HARVESTED JULY 1974,  
AUGUST 1974, SEPTEMBER 1974,  
AND OCTOBER 1974

Harvest Date	Concentration in Forage		
	Malic	Citric	Ash Alkalinity
	meq/kg		
July 1974			
N <sub>3</sub> K <sub>1</sub> <sup>a</sup>	263*	58	734
N <sub>3</sub> K <sub>2</sub>	103	70	626
August 1974			
N <sub>3</sub> K <sub>1</sub>	232	41	700
N <sub>3</sub> K <sub>2</sub>	122	47*	614
September 1974			
N <sub>3</sub> K <sub>1</sub>	211	52	604*
N <sub>3</sub> K <sub>2</sub>	126	47	538
October 1974			
N <sub>3</sub> K <sub>1</sub>	127	57	568
N <sub>3</sub> K <sub>2</sub>	120	55	518

<sup>a</sup>Fertilization for the two treatments was

N<sub>3</sub>K<sub>1</sub> 168 kg N/ha, 0 kg K/ha  
N<sub>3</sub>K<sub>2</sub> 168 kg N/ha, 168 kg K/ha

\* Indicates significant difference ( $P \leq .05$ ) with a pair in the same column.

was suggested that the marked increase in malic acid under conditions of low K was due to the transformation of phosphoenolpyruvate (PEP) to oxaloacetate (OAA) under the influence of PEP carboxylase.

The formation of OAA and its subsequent conversion to malate is considered to be one of the two important mechanisms by which carbon enters the TCA cycle. The other reaction is via decarboxylation of pyruvate to acetyl-CoA which condenses with OAA to form citrate. Thus, those conditions which favor the formation of malate (high N, low K) result in a lower concentration of citrate, while those conditions favoring citrate formation (low N, high K) result in a lower concentration of malate.

While the concentrations of organic acids were markedly influenced by fertilization and by season it is not expected that the forage produced would result in adverse effects to animals grazing this forage. Concentrations of citric acid corresponding to 1 percent of the diet in combination with KCl have been used in inducing grass tetany (Burt and Thomas, 1961). The citric acid concentration of this forage was considerably less than 1 percent. The highest values for citric acid were obtained in April of both experiments, with concentrations less than 0.6 percent of dry matter.

Malic acid has not been implicated as important in inducing grass tetany. The highest concentration of malic acid was calculated to be 3.05 percent of dry matter in April of the first experiment. Should malic acid be shown to be capable of inducing tetany, such a high concentration could be important. However, induction of tetany has required the presence of both KCl and one of two organic acids (citric or



t-aconitic). This fact greatly reduces the possibility that malic acid would be found to be a tetany inducing TCA intermediate, since K fertilization reduced the concentration of malic acid.

Concentrations of organic acids were found to increase in concentration in March and April in the first experiment and in April of the second experiment. These increases correspond to the increase in K concentration and decrease in Ca and Mg concentrations. Thus, malic acid and citric acid concentrations are highest at a time when the K:(Ca + Mg) ratio is highest. The stimulatory effect of N and K fertilization on the K concentration of forage material and the decrease in concentration of both Ca and Mg following K fertilization indicates that fertilization practices need to be compatible with animal health concerns. It is suggested from these experiments that N fertilization be applied as split applications rather than in one early spring application. This will not only reduce the stimulatory effect on K uptake during early spring but also provide for continued dry matter production throughout the year. Potassium fertilization should follow the grass tetany season and may in some cases be omitted if high rates of N fertilization are not applied and soil test values indicate high levels of exchangeable K.

## CHAPTER V

### SUMMARY AND CONCLUSIONS

The chemical composition and yield of an existing Kentucky 31 tall fescue stand was determined in two one-year fertility experiments. In the first experiment two levels of N fertilization were used: 0 and 112 kg N/ha. Nitrogen fertilization was found to increase the concentration of K from November to April. Calcium and Mg concentrations were also increased with N fertilization on several harvest dates. Phosphorus concentration was not significantly affected by N fertilization. Seasonal variation in the mineral concentrations was observed. Potassium concentration was highest during March and April. Calcium and Mg concentrations were lowest during the cooler months and increased during the summer months. Phosphorus concentration was markedly affected by season with highest concentrations in the warmer summer months.

The second experiment consisted of three N levels: 0, 112, and 168 kg N/ha and 2 K levels: 0 and 168 kg K/ha. Potassium fertilization increased the K concentration of forage material on all harvest dates. Calcium concentration was not significantly affected by K fertilization. The Mg concentration was significantly depressed by K fertilization from the November through the April harvest date. Phosphorus was not significantly affected by K fertilization. Seasonal variation was noted in the mineral composition. Highest values of K were found in the cooler months with highest values for Ca, Mg, and P during the warmer months.



Values at or below the suggested 0.20 percent Mg were observed in the 168 kg K/ha treatment from November through the May harvest date. During April and May several of the 168 kg K/ha treatments exceeded the suggested value of 2.2 for the K:(Ca + Mg) ratio. The maximum value attained was 2.6.

Crude protein and yield were significantly increased by N fertilization in both experiments. Potassium fertilization increased the crude protein concentration in several months. Dry matter yields were not significantly increased by K fertilization.

Total organic acid and malic acid concentrations were significantly increased by N fertilization. Decreases in citric acid were associated with N fertilization. Potassium fertilization decreased total organic acid and malic acid concentration but increased the citric acid concentration.

Concentrations obtained for citric acid were below 0.6 percent on all dates, with highest concentrations obtained in April of each year. This is significantly below the 1.0 percent level used in producing tetany in conjunction with KCl. A value of 3.05 percent dry matter was obtained for malic acid on the April 1973 harvest date; however, malic acid has not been implicated as a tetanigenic TCA cycle intermediate.

It is suggested that N fertilization be divided into three applications to reduce the negative effects of N fertilization on the K:(Ca + Mg) ratio in early spring and to provide for a more uniform distribution of dry matter. Potassium fertilization, if required, should

follow the grass tetany period to reduce the possible danger to animals grazing the forage produced.



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APPENDIX

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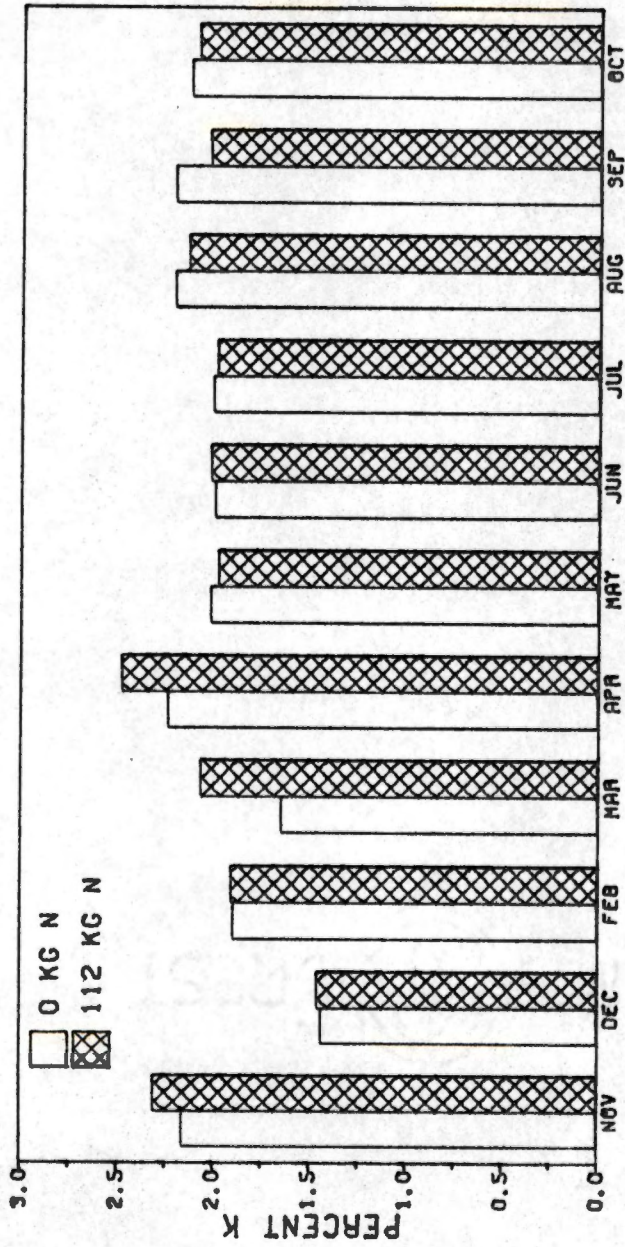


Figure 1. Seasonal changes in the potassium concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



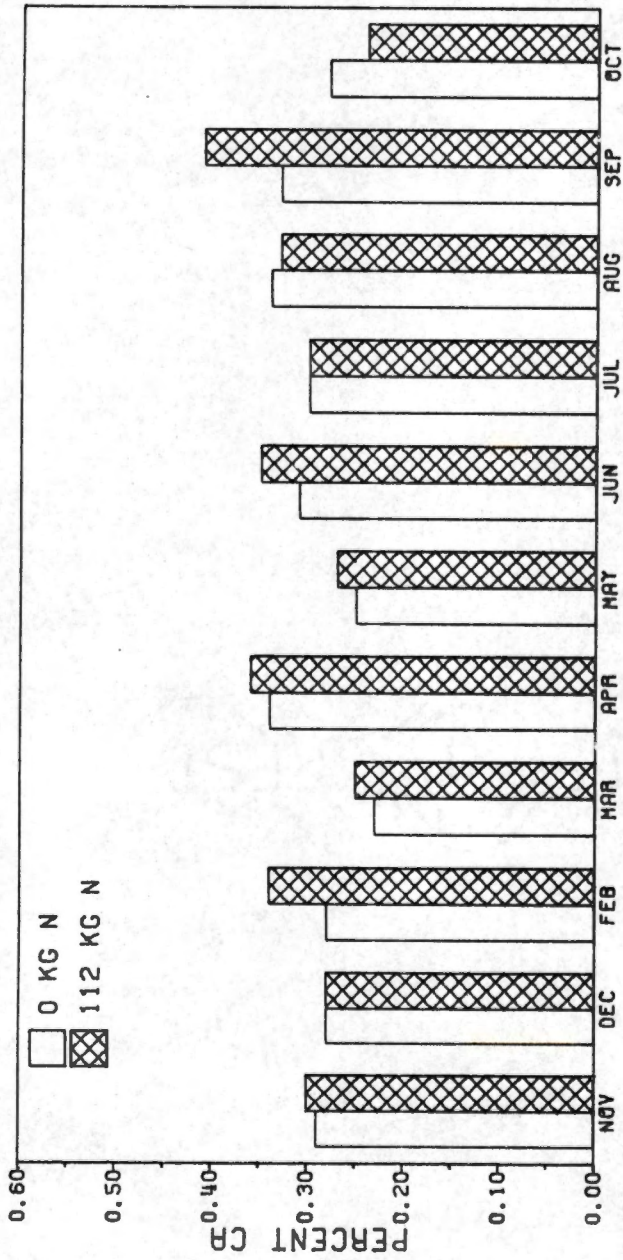


Figure 2. Seasonal changes in the calcium concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



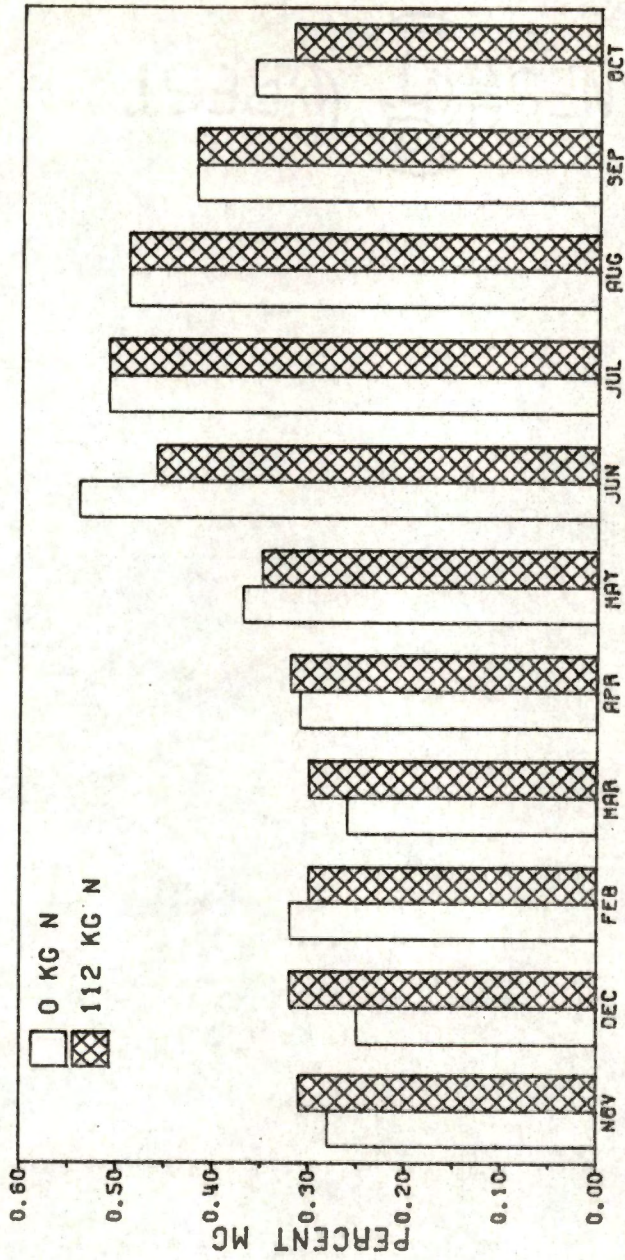


Figure 3. Seasonal changes in the magnesium concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



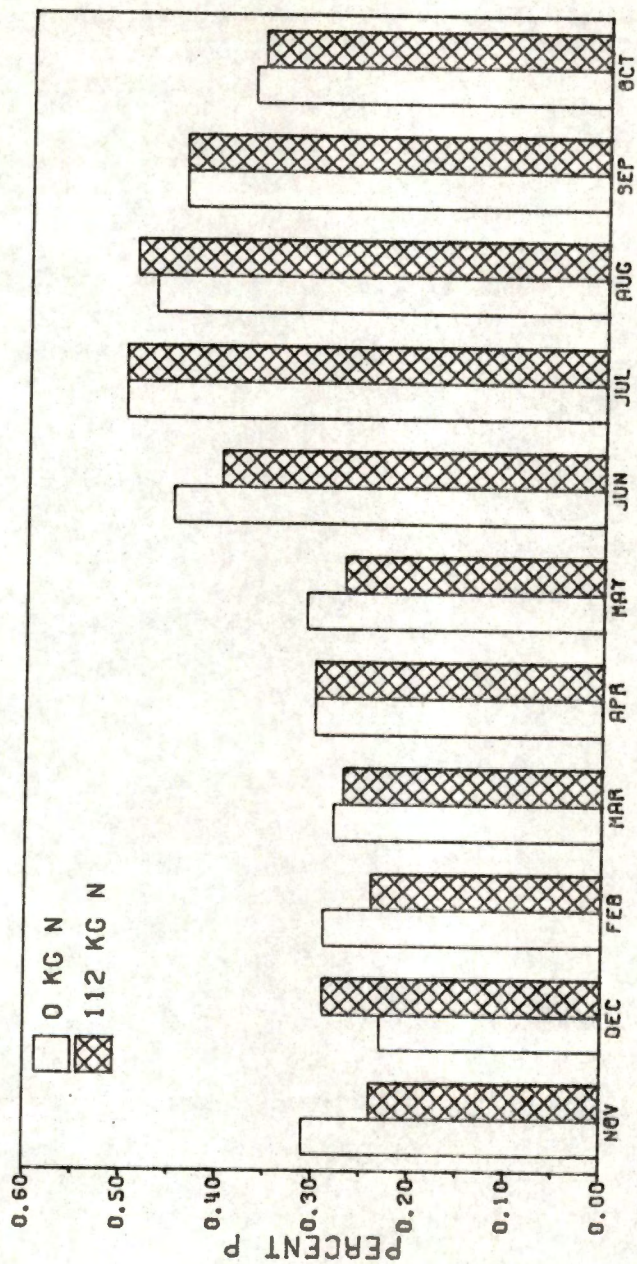


Figure 4. Seasonal changes in the phosphorus concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



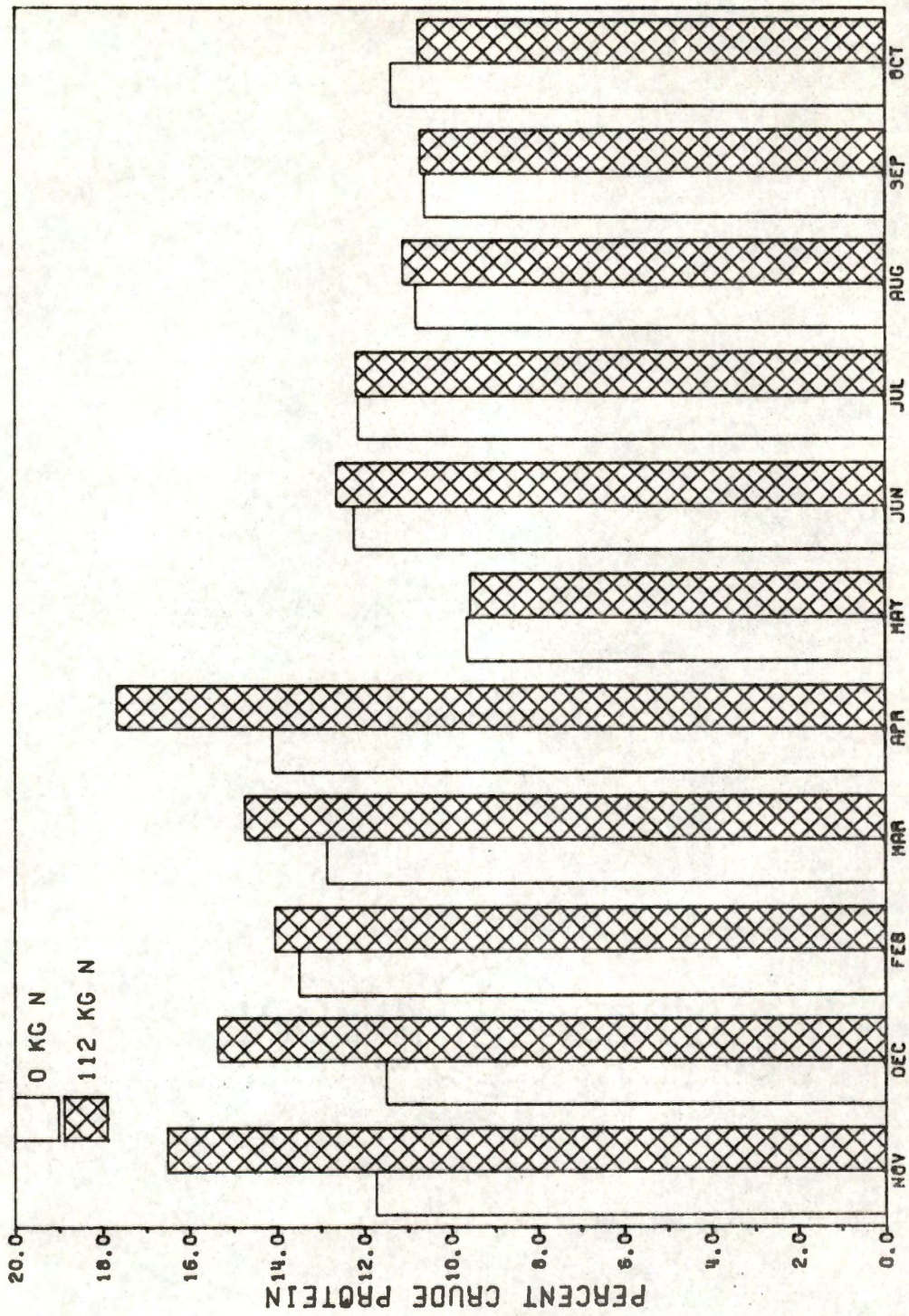


Figure 5. Seasonal changes in the crude protein concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



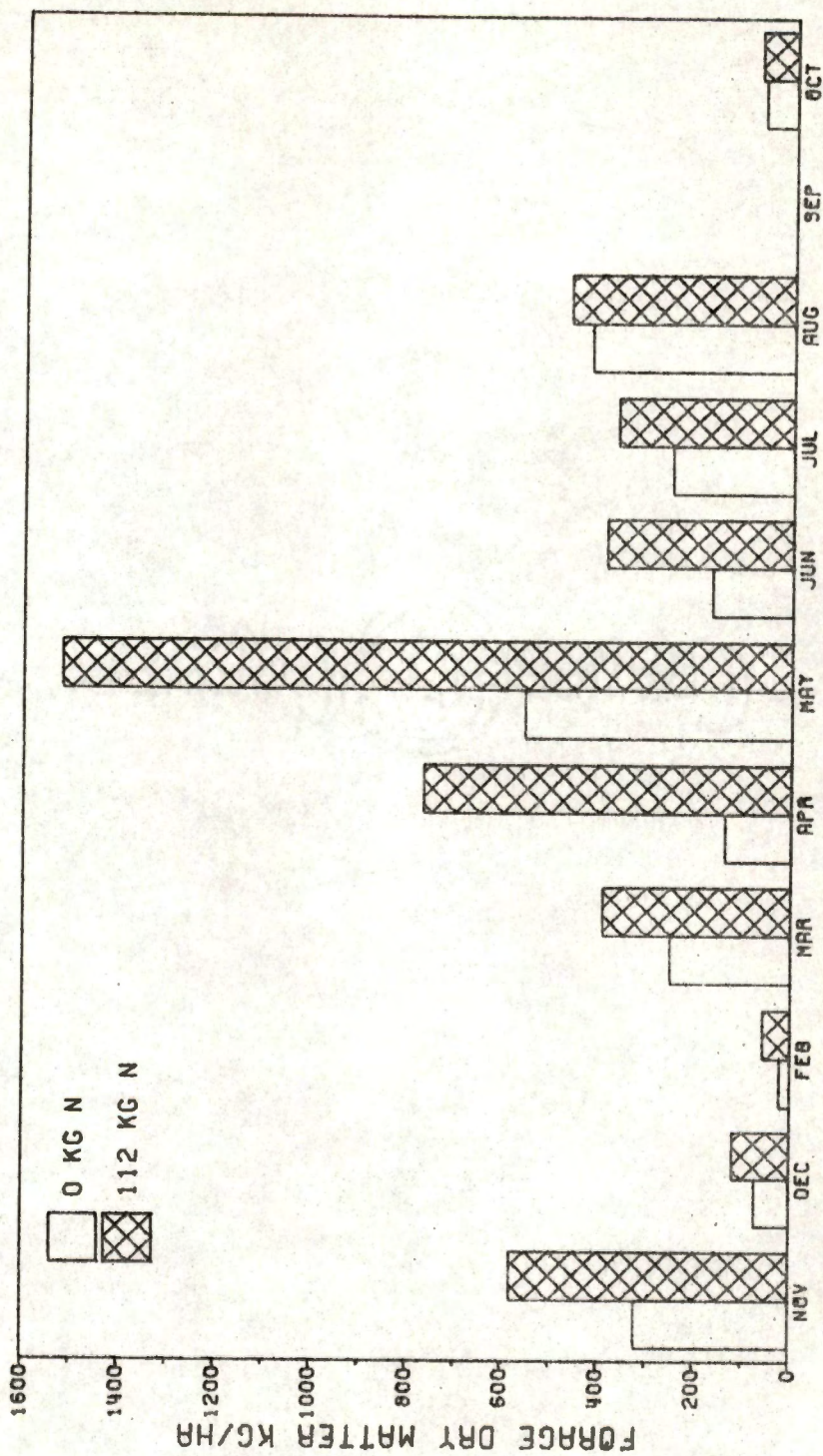


Figure 6. Seasonal changes in the dry matter production of tall fescue fertilized with two levels of nitrogen during 1972-1973.



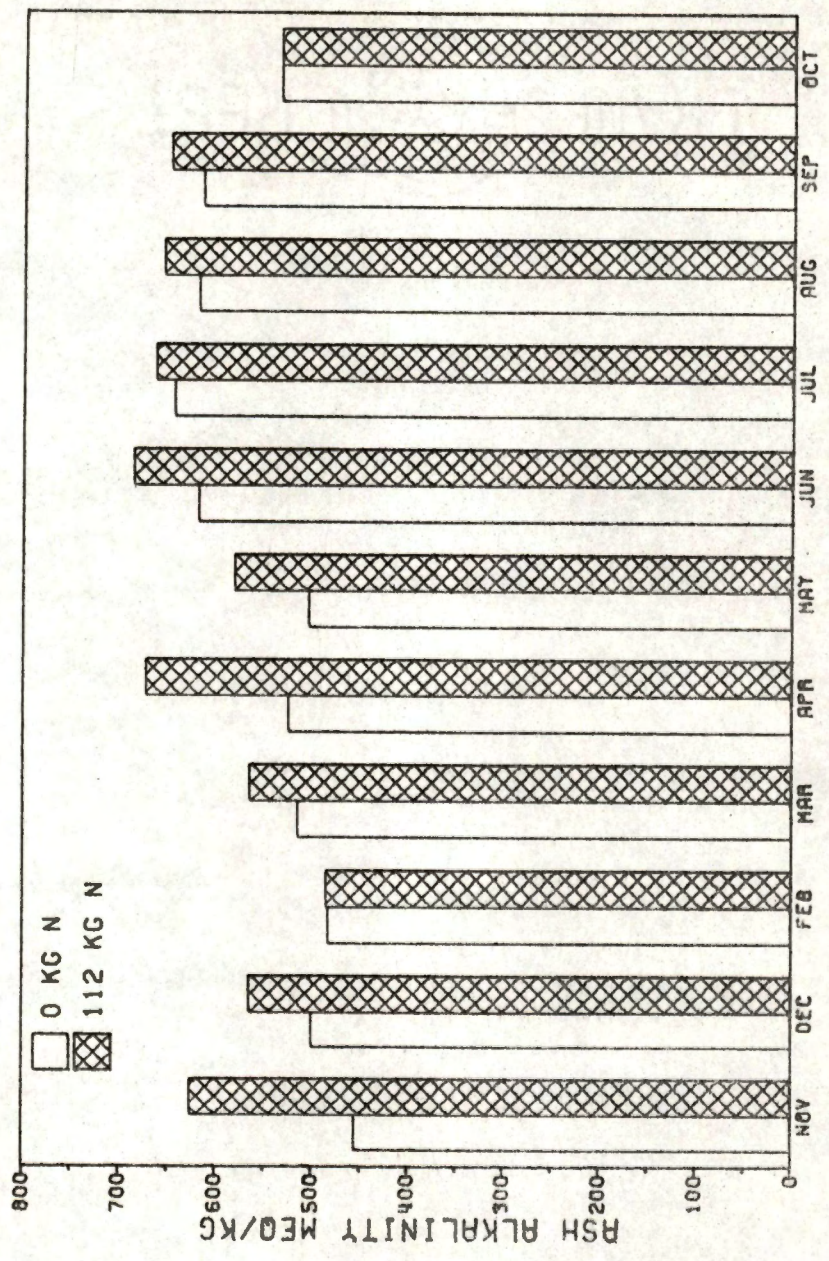


Figure 7. Seasonal changes in the ash alkalinity concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



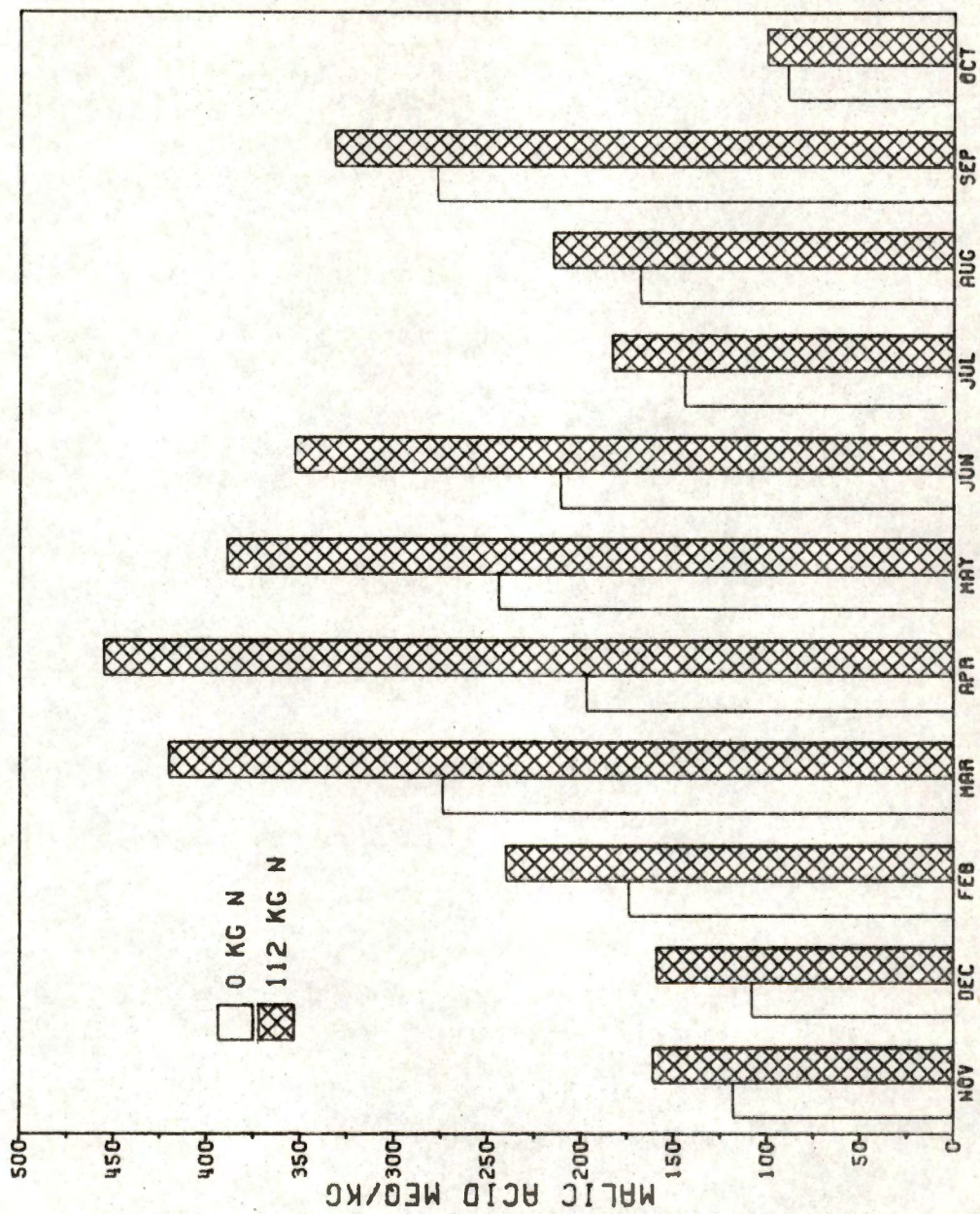


Figure 8. Seasonal changes in the malic acid concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



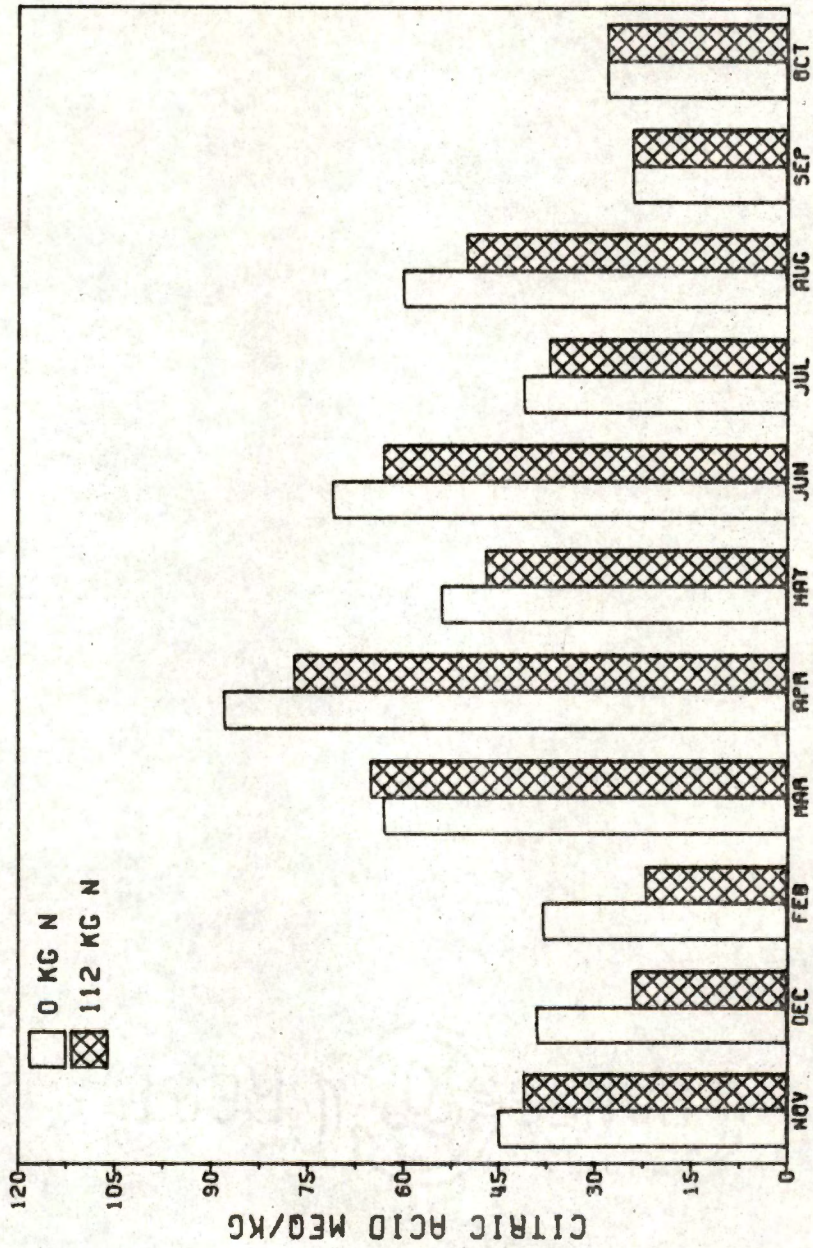


Figure 9. Seasonal changes in the citric acid concentration of tall fescue fertilized with two levels of nitrogen during 1972-1973.



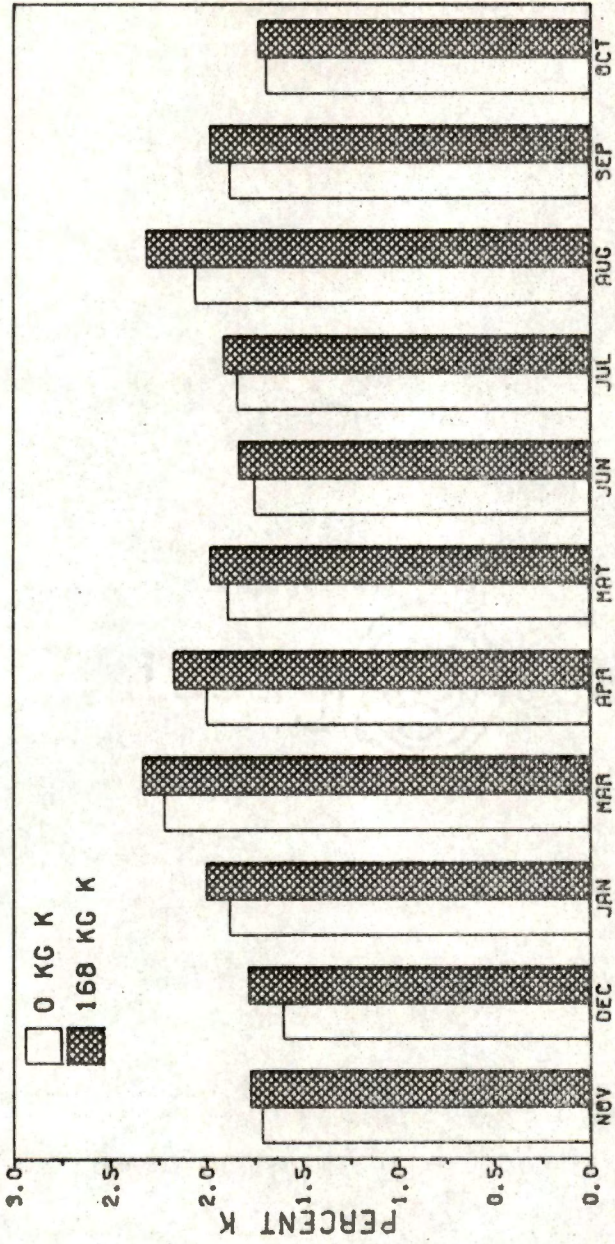


Figure 10. Seasonal changes in the potassium concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



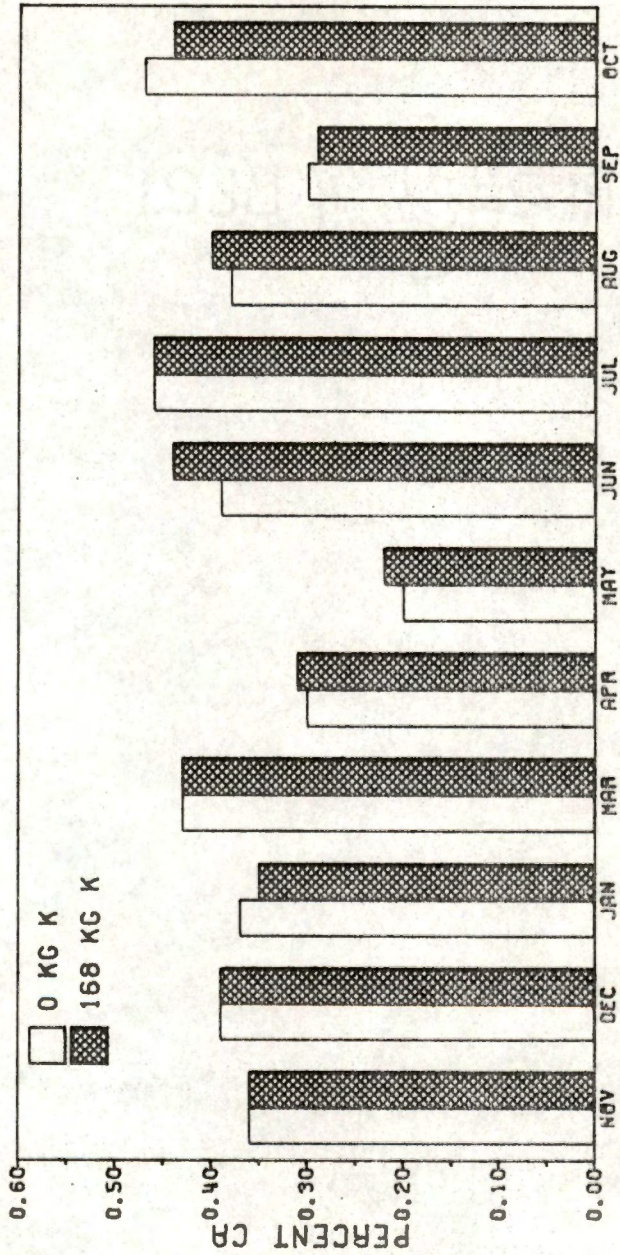


Figure 11. Seasonal changes in the calcium concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



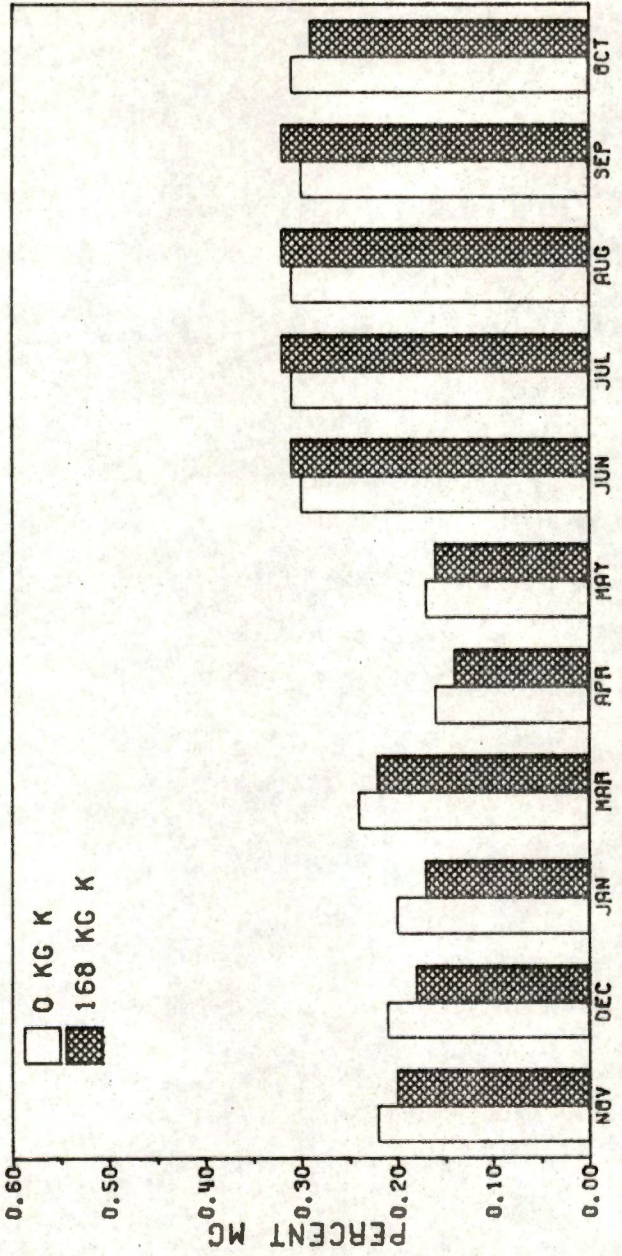


Figure 12. Seasonal changes in the magnesium concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



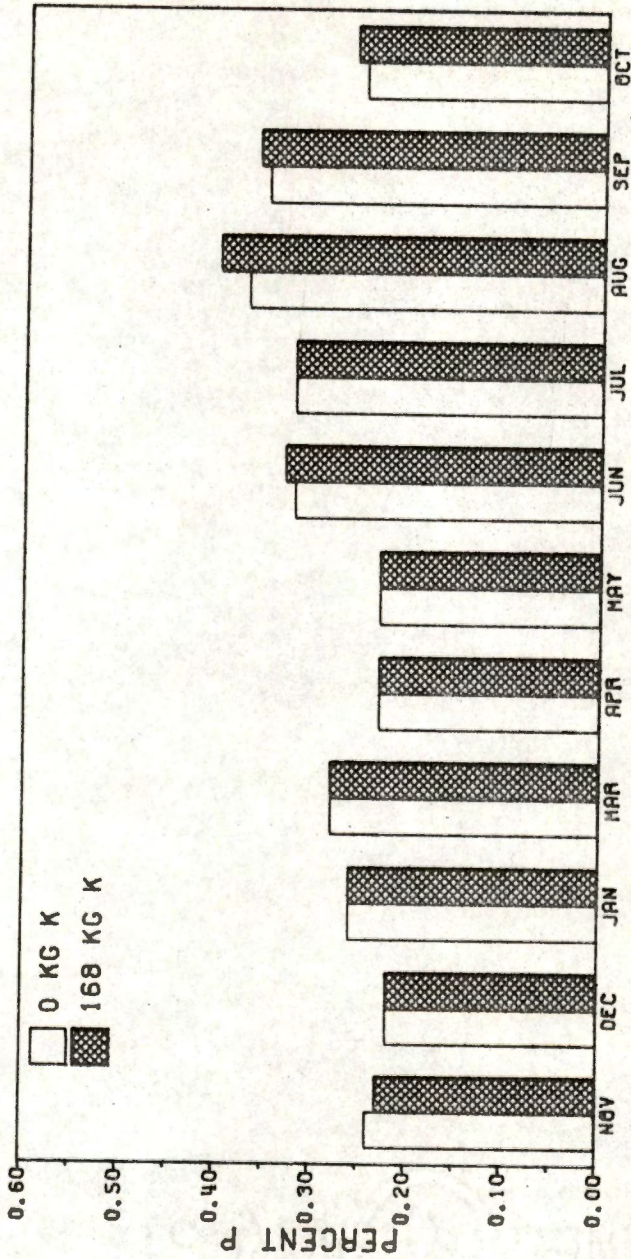


Figure 13. Seasonal changes in the phosphorus concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



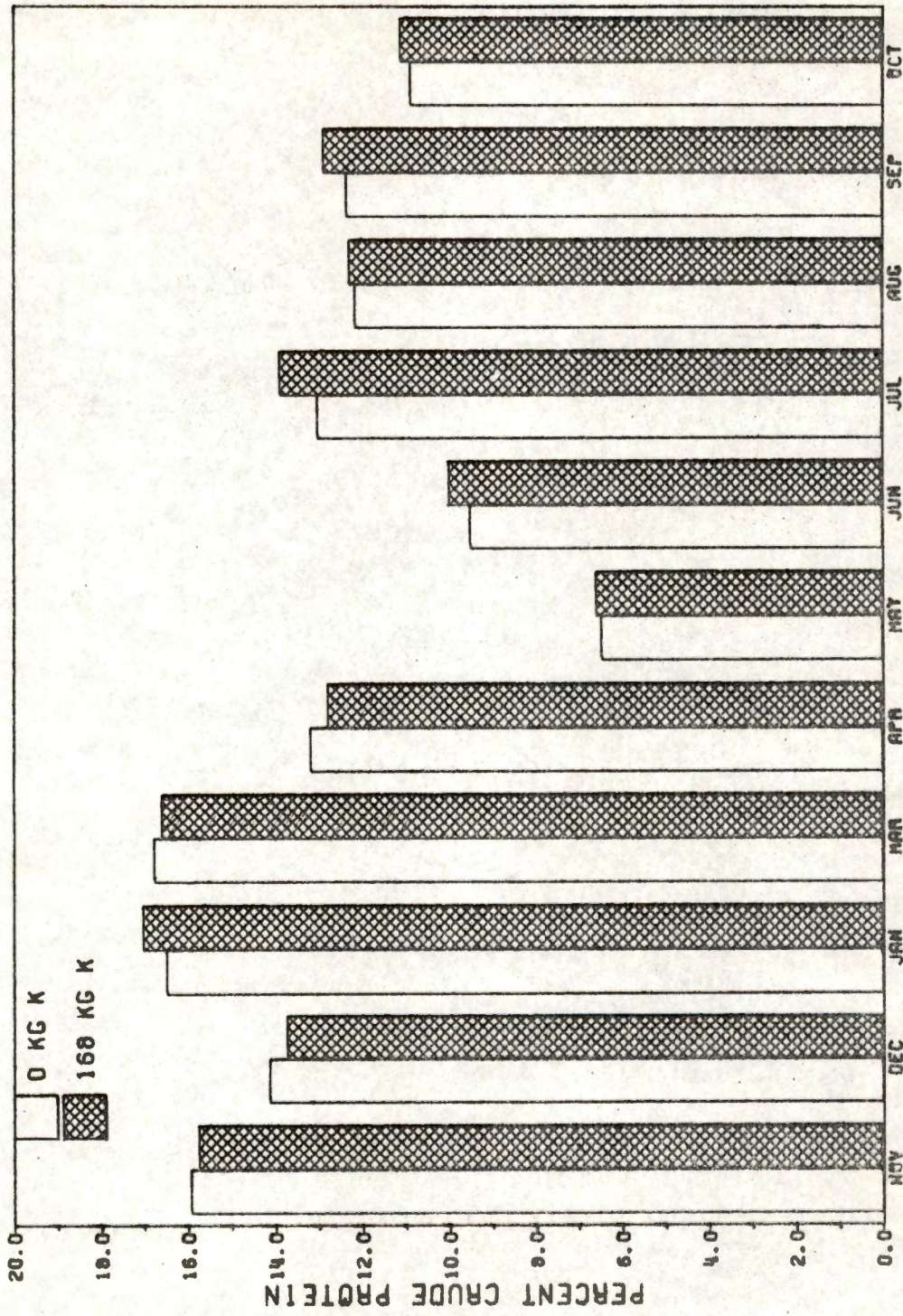


Figure 14. Seasonal changes in the crude protein concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



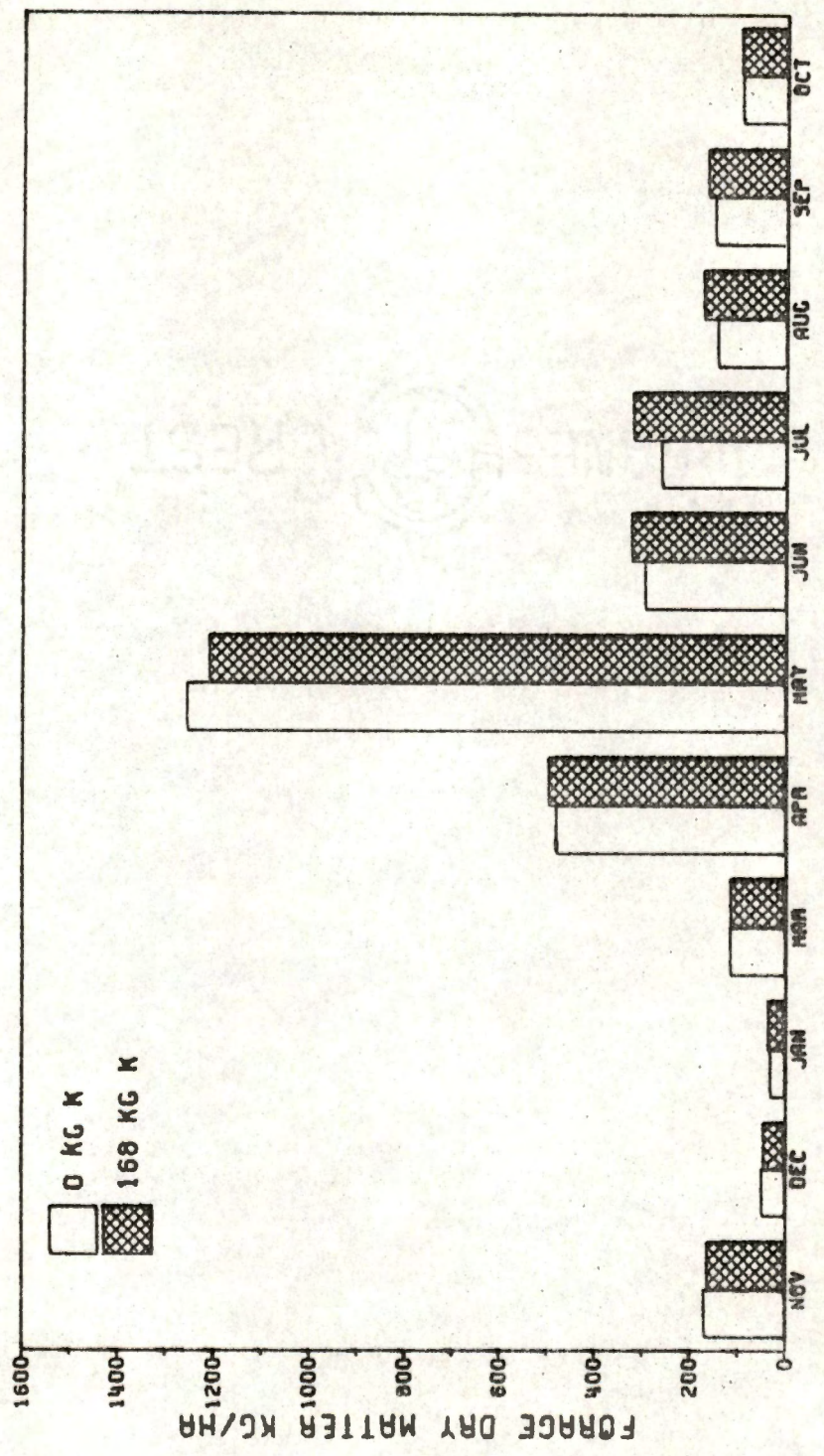


Figure 15. Seasonal changes in the dry matter production of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



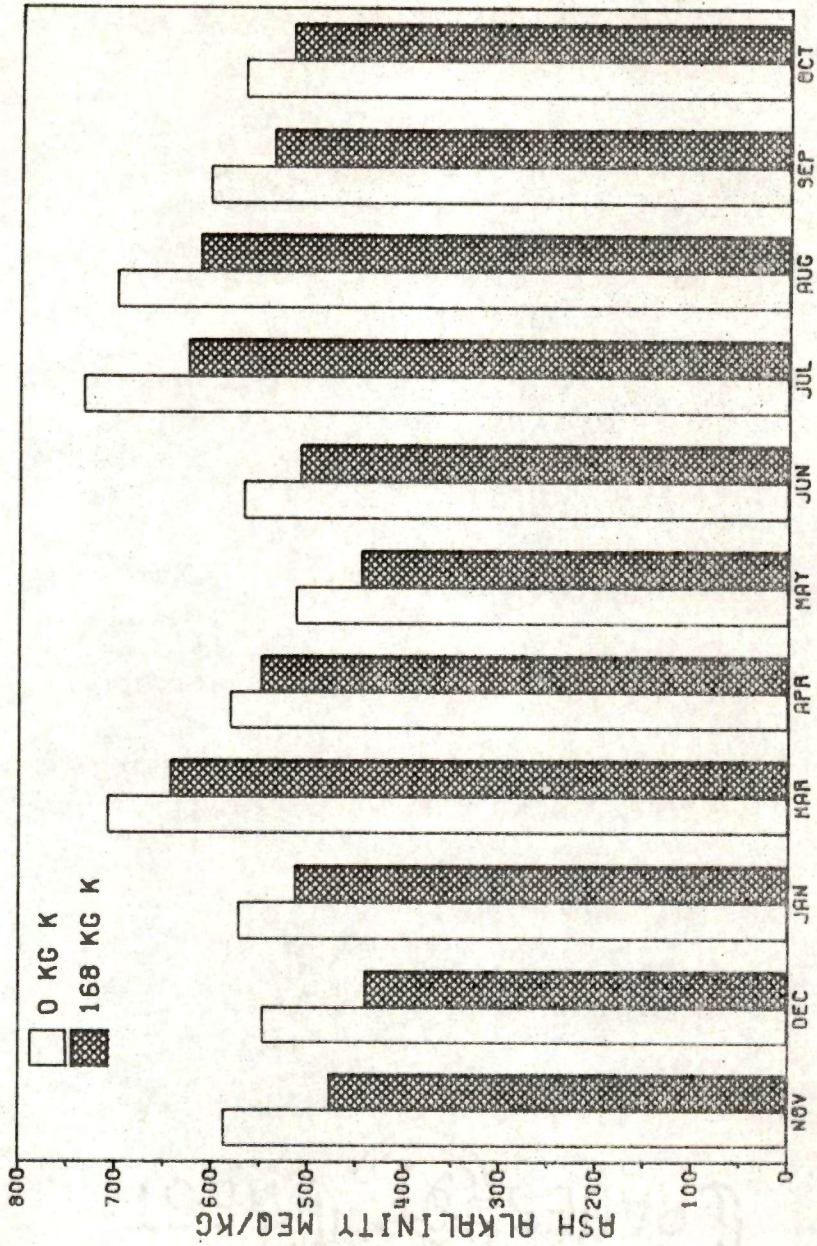


Figure 16. Seasonal changes in the ash alkalinity concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



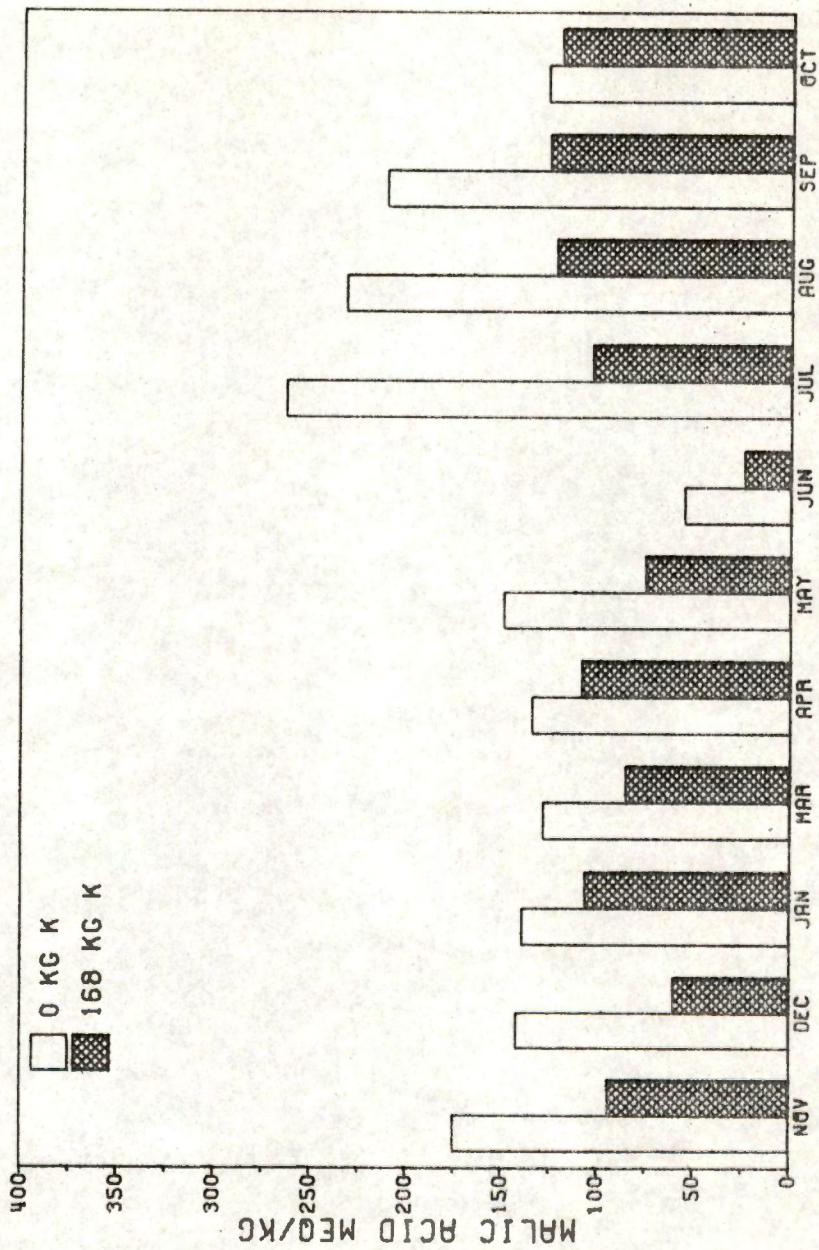


Figure 17. Seasonal changes in the malic acid concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



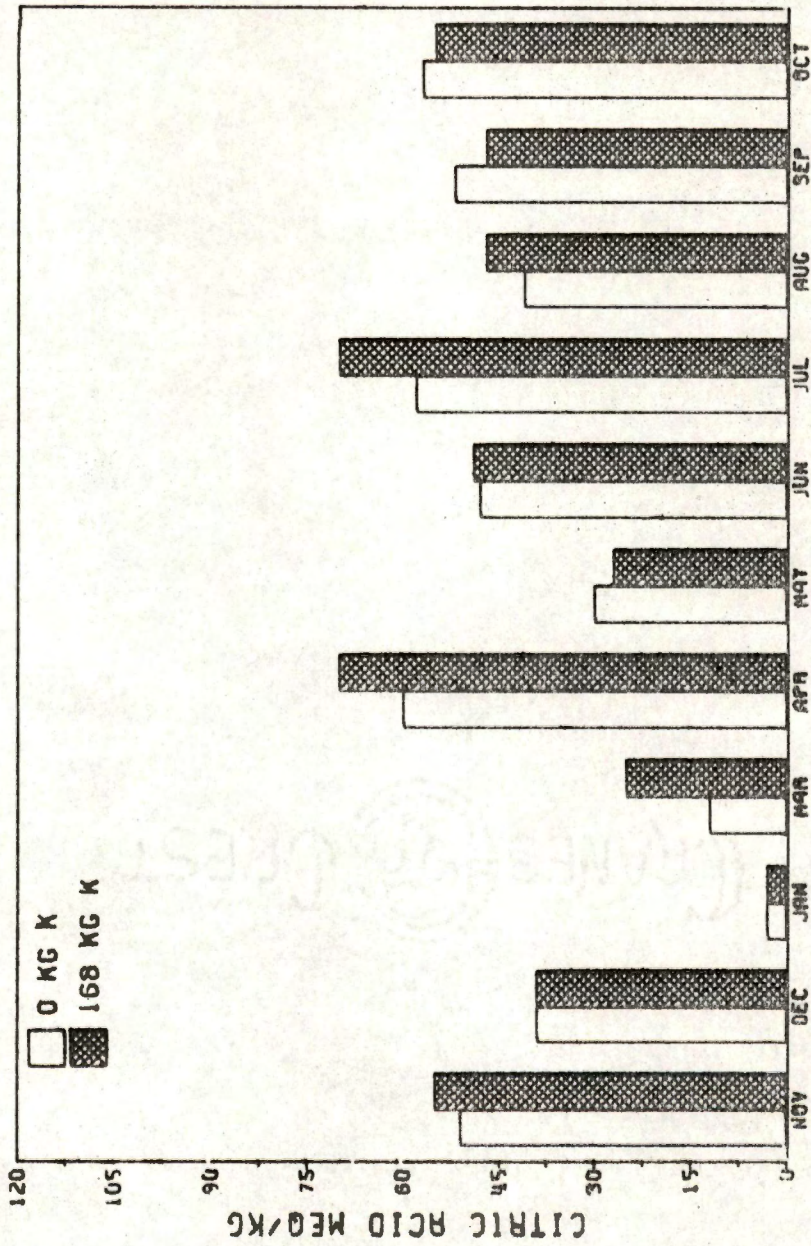


Figure 18. Seasonal changes in the citric acid concentration of tall fescue fertilized with nitrogen and potassium averaged across all nitrogen treatments during 1973-1974.



## VITA

David Byron Hannaway was born in Philadelphia, Pennsylvania, September 14, 1951. His parents are W. Gordon and Elizabeth Hunsburger Hannaway. In 1969 he graduated from Sterling Regional High School, Somerdale, New Jersey. In 1973 he received the Bachelor of Science degree with distinction in Plant Science from the University of Delaware. In 1975 he received the Master of Science degree from The University of Tennessee with a major in Plant and Soil Science. He is a member of the Delaware Academy of Science and of the Gamma Sigma Delta honor society.