INFLUENCE OF ACREMONIUM COENOPHIALUM ON FESTUCA ARUNDINACEA GROWTH, CHEMICAL COMPOSITION, DIGESTIBILITY AND TALL FESCUE TOXICOSIS

by

Sherri A. Buttrey

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APPROVED:

V.G. Allen, Chair

J.P. Fontenot

D.D. Wolf

H.E. White

R.E. Schmidt

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(ABSTRACT)

Infection of tall fescue (*Festuca arundinacea* Schreb.) with the endophyte fungus (*Acremonium coenophialum*, Morgan-Jones and Gam) has been associated with toxicity symptoms observed in cattle. The overall objective was to investigate the influence of endophyte infection on growth and chemical composition of fall fescue and the toxicity of endophyte-infected (EI) tall fescue to cattle. In a greenhouse study with pairs of genetically identical EI and non-infected (NI) 'Kenhy' tall fescue clones, concentration of N, Ca, Mg, Al, B, Mn and Zn was higher and K and S was lower in NI, compared to EI tall fescue. Insect resistance was higher in EI, compared to NI. Yield and chemical composition of high and low EI tall fescue were measured at four growth stages (stockpiled, prebloom, bloom and regrowth after harvest at bloom), two sites (Glade Spring and Blackstone) and three rates of N fertilization (0, 40 and 80 kg/ha) in a field study. Tall fescue grown at Glade Spring was higher in N, Mg, Al, Cu, Fe and Mn, compared to Blackstone. Nitrogen fertilization increased N, Mg, Ca, B, Cu, Na, Zn and decreased NDF, ADF, cellulose, P and S concentration in tall fescue. Neutral detergent fiber, ADF, cellulose, lignin, Fe and Na were higher in low, compared to high EI tall fescue. Concentrations of Cu, Na and Zn in stockpiled and Ca, Cu, Na and Zn in bloom-cut tall fescue hay were below dietary requirements for 227-kg steers. A disc meter was also evaluated for use in predicting yield of tall fescue. The meter is useful for non-destructive estimation of yield. Three feeding studies were conducted with steers (6/treatment/year). Diets were orchardgrass/alfalfa hay, spring-cut EI tall fescue hay, spring-cut EI tall fescue silage and fall-cut EI tall fescue silage. Serum prolactin and cholesterol were depressed in steers fed fescue hay and silages, compared to steers fed orchardgrass/alfalfa hay. Differences in

mineral composition of hay and silage were reflected in serum minerals in steers. Ergopeptine alkaloids in EI tall fescue may have contributed to the depression of serum prolactin. The spring-cut silage contained the highest concentration of ergopeptine alkaloids, compared to other diets. Steers fed the spring-cut tall fescue silage had the lowest basal and thyrotropin-releasing hormone stimulated prolactin compared to steers fed the other diets.

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INTRODUCTION

Poor performance of livestock grazing tall fescue (*Festuca arundinacea* Schreb.) has been associated with the presence of an endophyte fungus, *Acremonium coenophialum*, Morgan-Jones and Gams (Hoveland et al., 1980 and 1984b; Morgan-Jones and Gams, 1982). The relationship between the tall fescue host and endophyte appears to mutualistic (Siegel et al., 1985). Tall fescue provides nutrients and a means of dissemination (seed) to the endophyte (Siegel el al., 1985). Endophyte-infected tall fescue is more resistant to insect (Latch et al., 1985) and herbivor grazing (Goetsch et al, 1987b). Cattle and horses grazing endophyte-infected tall fescue may develop toxicity symptoms, including decreased gains, necrosis and sloughing off of hooves and tips of ears and tail, heat stress susceptibility, fat necrosis, and reproductive problems (failure to conceive, abortion, and agalactia) (Hemkem et al., 1984).

Researchers have attempted to identify the compound(s) responsible for toxic symptoms in animals. The diazaphenanthrene alkaloids, perloline and perlolidine (Hemken et al., 1984), the pyrrolizidine compounds, N-formyl loline and N-acetyl loline, (Bush et al., 1982), the ergopeptine alkaloids (Porter et al., 1979) and various non-alkaloid anion compounds (Williams et al., 1975; Garner et al., 1982) have been studied. It is not known if the toxic factor(s) are produced by the plant, endophyte or both. Furthermore, it is not known if the toxin(s) are activated or deactivated by ruminal microfauna catabolism.

The influence of environmental factors on plant responses to endophyte infection and fescue toxicosis in animals has largely been ignored. Plant physiology and metabolism are affected by soil fertility, soil water availability, soil type and climate. Endophyte infection, persistance, and toxic symptoms in animals may also be influenced by the environment. Certainly, a complex interaction of climate, soil, plant, fungus and animal is involved. A convenient reproducible bioassay to evaluate the toxicity of a tall fescue sample to animals is needed.

This research was designed to investigate the interaction between the environment and endophyte infection on growth and chemical composition of tall fescue and toxicity of endophyte-infected tall fescue to cattle. Specific objectives were: 1) to measure the influence of endophyte infection on the growth, chemical composition, in vitro dry matter digestibility and insect resistance of tall fescue clones; 2) to measure the influence of growth stage at harvest, endophyte infection, N fertilization and location on the dry matter yield and chemical composition of tall fescue; 3) to elucidate biological changes in cattle fed endophyte-infected tall fescue hay or silage (cut in spring or fall, after stockpiling) as compared to orchardgrass/alfalfa hay; 4) to investigate the influence of endophyte infection and seasonal effect of time of ensiling of tall fescue on basal and thyrotropin-releasing hormone stimulated prolactin in steers; and 5) to evaluate a disc meter for the estimation of the yield of tall fescue swards.

LITERATURE REVIEW

TALL FESCUE

Tall fescue, a cool season perennial forage is grown on 14 million hectares of land in the United States (Hemken, 1983) and on over 1 million hectares in Virginia, primarily, for pasture. Tall fescue grows in a wide range of soil and climatic conditions in the humid temperate areas of the United States (Buckner, 1985). Geographic distribution is limited by temperature, rainfall, soil texture and moisture. Tall fescue grows best on fertile, moist, median textured soils of neutral pH, but can tolerate thin, droughty soil conditions and pH 4.7 to 9.5 (Buckner, 1985). An extensive root system allows tall fescue to grow under drought conditions but also to survive periods of flooding and to grow on poorly drained soils (Hoveland, 1983). Tall fescue is a naturally cross-pollinated crop.

Cultivars. Tall fescue, *Festuca arundinacea,* Schreb., was most likely transported to the United States from Europe as a contaminant in imported seed (Hoveland, 1983). 'Alta' and 'Kentucky-31' were the first two varieties of tall fescue marketed in the United States and to become widely grown (Buckner and Bush, 1979). 'Alta', an early to medium maturing variety (mediterranean type), is adapted to dry summer conditions and matures about 5 days earlier than

'Kentucky-31'. 'Alta' was selected from a 4-yr old stand in 1923, was released from Oregon Agricultural Experimental Station in 1945 and is currently used predominantly in the West and Northwest areas of United States. 'Kentucky-31' was discovered growing on William O. Suiter's farm in Menifee county, Kentucky in 1931 and was released in 1943 from Kentucky Agricultural Experiment Station. This variety is a European type of medium maturity and has greater resistance to leaf diesease, compared to 'Alta', 'Goar' and 'Fawn'. Both 'Alta' and 'Kentucky-31' are similar in appearance, growth habit and productivity. Since the release of these two varieties in the early 1940's, the amount of seed produced per year and consequently, the number of ha of tall fescue have dramatically increased. Introgression has also occured between the two cultivars (Buckner and Bush, 1979), even in certified seedlots (Richard Payne, personal communication).

The increase in the popularity of tall fescue has resulted in the development of varieties with improved forage quality. Other varieties of tall fescue include: 'Goar', 'Kenmont', 'Forager', 'Fawn', 'Kenhy', 'Kenwell', 'Missouri-96', 'Johnstone', and 'AU-Triumph'. 'Goar' was released in 1964 from California (El Centro Experimental Station, Imperial Valley) and is adapted to alkaline soils and matures 1-2 weeks earlier than 'Kentucky-31'. 'Kenmont', released in 1963 jointly from Kentucky Agricultural Experimental Station and Montana Agricultural Experimental Station, has good midseason growth and is grown in the Southeast and Northern Great Plains of United States (Buckner and Bush, 1979). 'Fawn' was released in 1964 from Oregon Agricultural Experimental Station and is grown in the Northwest and Central regions of United States. In Orgeon variety trials, 'Fawn' had higher yields than 'Alta', 'Goar' or 'Kentucky-31' (Buckner and Bush, 1979). But, in Missouri and Kentucky, 'Fawn' was susceptible to crown rust. 'Kenhy', a tall fescue/annual ryegrass (*Lolium multiflorum*) hybrid, was released in 1976 from Kentucky Agricultural Experiement Station with improved quality (low alkaloid, perloline) and growth. 'Missouri 96' was released from Missouri Agricultural Experiment Station the year after the release of 'Kenhy' and was reported to have improved forage quality (Buckner and Bush, 1979). In 1983, 'Triumph' and 'Johnstone' were released from Alabama and Kentucky, respectively. 'Triumph' was established from endophyte-free seed, produces more winter growth and matures about 2 weeks earlier than 'Kentucky-31'. However, this variety may not be adapted to Virginia conditions due to poor cold tolerance.

'Johnstone', a tall fescue/annual ryegrass hybrid released in 1982, is a low-endophyte, low alkaloid variety that is more palatable than 'Kentucky-31'. 'Forager', variety marketed by Southern States Cooperative, is certified to be low in endophyte and matures two weeks earlier than 'Kentucky-31' (Buckner and Bush, 1979). The varieties 'Johnstone', 'Kenhy' and 'Kentucky-31' are thought to be better adapted to Virginia soil and climate than 'Fawn' and 'Alta'.

Growth and Chemical Composition. Growth and chemical composition of tall fescue is influenced by temperature, soil moisture, soil fertility and genetic factors. Germination of tall fescue requires temperatures of 16 to 30 C and vegetative growth is optimum at 22 to 27 C (Hoveland, 1983). Chemical composition may also be influenced by temperature (season). Buckner et al. (1967) reported that crude protein concentration in tall fescue ranged from 13.7 to 29.7% and was lowest during the hot, dry summer months and highest during cool, damp months in early spring. Total non structural carbohydrate (TNC) also varied with season and ranged from 3.6 to 30.2%.

Water is a constituent of plant tissue that functions as a reactant in photosynthesis and hydrolytic processes and maintains plant turgidity (Kramer, 1983). An excess as well as a deficiency of water can decrease plant yield and change plant chemical constituents.

Water stress may decrease plant yield due to reduction in cell division and cell enlargement (Kramer, 1983). Leaf thickness is increased, increasing weight/unit area of water stressed plants. Amylase activity in the leaves of water-stressed plants may be increased (Eaton and Ergle, 1948), decreasing the carbohydrate concentration in these plants. In water-stressed plants, there may be increased hydrolysis of protein and accumulation of amino acids. Water-stressed 'Kenhy' plants had less leaf roll, smaller stomatal pore area, and fewer stomata on the abaxial surface than 'Kentucky-31' plants (Buckner et al., 1979). Flooding decreased yield of ryegrass (Terrill, 1985). Changes in mineral composition of plants under flooded conditions vary with plant species, soil type, and initial soil condition (Kuzlowski, 1984). These changes can be related to plant availabilty and uptake of minerals. Tall fescue grown in waterlogged soil may contain lower concentrations of N, P, K, Ca, and Mg (Rogers and Davies, 1973). Under flooded condition, less N is available for plant growth due to loss of nitrate by volatilization and denitrification (Singh and Ghildyal, 1980). Also, plants take-up less of the available N in flooded soils. Foliar applications of urea to

wheat grown in waterlogged soil corrected chlorosis and increased concentration of plant N (Trought and Drew, 1980abc). Potassium and P may decrease in plants grown in flooded soils due to decreased uptake (Kozlowski, 1984). Flooding may actually increase available P in the soil by driving the pH closer to 7, resulting in release of P from insoluble, adsorbed, or bound forms (Kozlowski, 1984). Magnesium absorption by roots and transport to shoots may be inhibited by high K levels in the soil (Hannaway et al., 1982). Iron and Mn concentration in plants may increase since the reducing condition of flooded soils increases solubility and availability of these elements (Kozlowski, 1984). Soil availability of Cu and Mn increased and Zn decreased in waterlogged soil and tissue concentration of Zn, Cu, and B generally decreased in plants grown in flooded soils (Kozlowski, 1984). Flooding increased concentrations of Al, Fe, Cu, Ca, and fiber and decreased dry matter yield and concentrations of Mg, K, and Zn in annual ryegrass (*Lolium mutliflorum*, L.) (Terrill, 1985).

Nitrogen fertilization may increase dry matter production and change mineral composition. Increasing N fertilization (from 0.4 to 3.2 mM solution) of 'Kenhy' tall fescue, grown in a greenhouse in nutrient solution, increased tissue N (3.89 to 4.48%), nitrate (1.92 to 5.25%) and Ca (1.77 to 2.32%) and lowered Mg (Hannaway et al., 1982). High rates of N fertilization stimulate plant growth and as a result decrease levels of soluble carbohydrate reserves. Non-protein N compounds also accumulate with high rates of N fertilizers. Tall fescue fertilized with 0, 100, or 400 kg/ha of N as ammonium nitrate contained 2.35, 2.93, and 3.92 % total N; 90, 3325, 8310 ppm nitrate-N; and 10.2, 8.5, 7.5 % soluble carbohydrate, respectively (Belesky et al., 1984).

Tall fescue has a diverse, allopolyploid nature. Differences in the chemical composition of genotpes and varieties of tall fescue have been reported (Hill and Guss, 1976; Sleper et al., 1980). Genotypes of tall fescue differ in malic acid accumulation (Boland et al., 1977). Plant genetic character determines plant growth, development, and chemical composition. Mineral uptake and accumulation by plants in general is genetically controlled (Hill and Guss, 1976). Sleper and coworkers (1980) demonstrated that genetic control of two tall fescue genotypes was sufficiently strong to override temperature effects. Magnesium concentration of B5-62, a high Mg accumulator, was 0.38 and 0.29 % at high (25/20 C) and low (17/12 C) temperature regimes, respectively. In the

low Mg accumulator genotype, B17-42, Mg concentration was 0.22 and 0.14% at high and low temperatures, respectively. In a comparison of the chemical constituents of two ryegrass x fescue hybrids and two fescue varieties, 'Kenwell' and 'Kentucky 31', the hybrids were higher in quality than the varieties (Buckner et al., 1967). Crude protein, TNC and in vitro dry matter digestibility (range 64.9 to 81.2%) were higher and silica was lower in the hybrids compared to the varieties. The genetic character of the species was different. The chromosome number was higher in the hybrids than in the varieties. An increase in chromosome number has been associated with increased moisture content, soluble constituents and decreased structural constituents (Sullivan, 1944). Accumulation of alkaloids may also vary with the genetic character of the tall fescue. Jones and coworkers (1983a) reported differences in pyrrolizidine alkaloid concentration of four varieties. 'Kentucky 31' and 'Kenwell' (from Kentucky) had higher alkaloid values than 'Alta' and 'Fawn' (from Oregon). The genotype, V6-44, contained the highest (1934 mg/g) and I84-1 contained the lowest (3.47mg/g) malic acid concentrations of the eight genotypes tested. Free amino acid composition of leaf tissue of V6-4 and I84-1 were 6.87 and 14.82 mg/g, respectively and non-structural carbohydrate content was 11.20 and 7.09 %, respectively.

However, work by Hoveland and coworkers (1984b) indicates that drought stress may reduce the animal response to the toxic substances in tall fescue. It may be important for plants to be genetically identical when comparing the chemical constituents of endophyte-infected and endophyte-free plants.

ACREMONIUM COENOPHIALUM

The endophyte fungus in tall fescue associated with tall fescue toxicosis belongs to the family Clavicipitaceae and tribe Balansiae. Species of the Balansiae tribe produce systemic infections in leaves, stems and flower parts (Bacon and Siegal, 1988). The endophyte fungus, a clavicipitaceous systemic phytopathogen (Bacon et al., 1977; Bacon, 1983), associated with fescue toxicosis had

been identified as *Sphacelia typhina* (Pers.) Sacc., the imperfect state of *Epichloe typhina* Fr. Tol. (Siegel et al., 1984a). Recently, Morgan-Jones and Gams (1982) have reclassified the imperfect state as *Acremonium typhinum* Morgan-Jones and Gams and the endophyte in tall fescue as *Acremonium coenophialum* Morgan-Jones and Gams. The endophyte in tall fescue is ultrastructurally similar to an endophyte in perennial ryegrass (*Lolium perenne*) (Philipson and Christy, 1986) which has been associated with ryegrass staggers.

The host-fungus interaction between tall fescue and the endophyte fungus appears to be mutualistic (Siegel et al., 1985). For mutualism to occur, members of different species must both benefit by the association. The fungus is protected within the plant and is provided nutrition and a means of dissemination (the seed) and in return, the plant is provided resistance to insect attack and herbivore grazing. *Acremonium coenophialum*, cultured in vitro, inhibited the growth of three other fungi, *Alternaria alternata, Cladosporium cladosporioides, and Rhizoctonia cerealis* (White and Cole, 1985c). The fungus has been identified in many *Festuca* species; *arizonica, arundinacea, eastweedea, elatior, digulata, obtusa, pacifica, paradoxa, scabrella, subulata, and versuta* (White and Cole, 1985ab).

Siegel and coworkers (1984a) measured fungal endophyte in various parts of the tall fescue line GI-307. Concentration of endophyte in the leaf sheath, crown, stem, leaf blade, roots, and seed were 4.6, 1.1, 1.0, 0.1, 0, and 2.2 mg/g dry weight tissue, respectively. The hyphae of the fungus are found in the intercellular spaces and have not been shown to penetrate living plant cells (Bacon et al., 1977). When examined microscopically, hyphae usually appear coiled and contorted. However, in the low perloline tall fescue line GI-307, hyphae appear straight and narrow (Bacon et al., 1977). Endophyte fungus hyphae isolated from *Festuca versuta* stems sampled in April were septate, non-branching and straight with a narrow diameter (1-2um) and when sampled in June, the hyphae were convoluted, branched and had a larger diameter (4um) (White and Cole, 1986). Hyphae were also found in the intercellular spaces between the parenchyma cells of the leaf sheath, glumes, and rachilla. In the seed, hyphae were located between the aleurone layer and seed coat but none were in the starchy endosperm. Hyphae penetrated the epidermal cells of the scutellum. The endophyte in perennial ryegrass only penetrates plant tissues of the ovary and ovule after

fertilization and hyphae penetrate the embryo during early ('notched stage') embryogenesis (Philipson and Christy, 1986).

The life cycle of the endophyte is relatively simple and has been described by Bacon and Siegel (1988). The fungus in the seed invades the tissue of the seedling within 2 days after germination. Fourteen days after germination, the fungus mycelium can be detected in the meristem. When the plant is vegetative, the fungus lives in the meristem tissue of shoot apexes assimilate and storage areas and tissue of leaf sheath. When the plant flowers, the endophyte migrates into the stem and can be found in the intercellular spaces near the apex of the stem. The fungus also penetrates the ovary and ovule.

Dissimination and transmission of the fungus appears to occur only by seed. The endophyte fungus is transmitted maternally, but not paternally, by pollen (Siegel et al., 1984b). There appears to be no plant to plant transfer of fungus. Mowing within and across plots of infected and non-infected plants did not dissiminate the fungus (Siegel et al., 1984b). Attempts to infect tall fescue in culture by inoculating with fungus have been unsuccessful (Siegel et al., 1984a). Endophyte levels increased over a 3 year period in intensively grazed pastures of a 2x2 factorial experiment, high and low fungus-infected fescue and high (336 kg/ha) and low (134 kg/ha) N levels (Belesky et al., 1987). The authors cite three possible causes for the increase in endophyte-infection levels: 1) enhanced survival of infected tillers, 2) selective grazing of non-infected plants and 3) spread of the fungus by grazing animals.

Nutritional requirements of isolated fungus have been studied (Kulkarni and Nielson, 1986). Sources of carbon that were utilized by isolated fungus include hexoses (D-glucose, D-fructose, and D-mannose), disaccharides (sucrose and trehalose), mannitol and sorbitol. However, the fungus was not able to utilize pentoses (arabinose, lyxose, ribose or xylose), hexoses (galactose, sorbose or rhammose), polysaccharides (pectin, cellulose, or soluble starch), uronic acids, or carboxylic acids (acetate, citrate, propionate, or succinate). Cultured endophyte was able to use ammonium but not nitrate or urea as a source of N (Kulkarni and Nielson, 1986). Amino acids utilized by the fungus include, L-arginine, L-asparagine, L-cysteine, L-glutamine, L-proline, and L-serine but, L-

alanine, glycine, L-phenylalanine, and L-tryptophan were not utilized. In addition thiamine was the only vitamin that stimulated growth of the fungus in culture (Kulkarni and Nielson, 1986).

Ergosterol, ergosterol peroxide, and ergosta-4,6,8-(14),22-tetraen-3-one were isolated from endophyte-infected tall fescue (Davis et al., 1986). Cultures of fungus mycelium incubated in the light also contained the three steriods but cultures incubated in the dark did not contain ergosterol peroxide. Bacon and Siegel (1988) reported the following compounds were isolated from endophyte-infected tall fescue grass and also from the endophyte grown in culture: ergovaline, ergovalinine, ergosine, ergonine, chanoclavine I, agroclavine, penniclavine, ergosta-4,6,8(14),22 tetraen-3-one. Three other compounds, elymoclavine, secoagroclavine and festuclavine were isolated from the endophyte grown ***in vitro*** but not from endophyte-infected tall fescue. In addition loline, N-acetyl and N-formyl loline, N-acetyl norloline and perloline were only isolated from infected grass.

Endophyte infection of tall fescue may influence plant mineral compostion. Mineral content of 'Kentucky 31' tall fescue from two different seed lots, one endophyte-infected (94%) and one endophyte-free (< 5%) was determined by Hoveland and coworkers (1984b). The infected fescue was growing on Eutaw clay soil with pH 6.4 and Ca, Mg, P, and K levels were 0.44, 0.29, 0.35, and 2.5 %, respectively. The endophyte-free fescue was growing on Sumter clay and Houston clay soil with pH 7.6 and Ca, Mg, P, and K levels were 0.71, 0.15, 0.35, and 2.5 %, respectively. Calcium was lower in endophyte-infected plants than fungus-free plants (Hoveland et al., 1984b). Magnesium may be especially important since Mg deficiencies may increase plant susceptibility to fungus infection (Jones et al., 1983b). Odom and coworkers (1981) reported no effect of endophyte infection on tall fescue concentrations of Ca, Mg, and K. They divided one infected and one non-infected 'Kentucky-31' tall fescue plant into pots containing two types of soils, Eutaw (Entic Pelludert) and Sumter (Rendollic Eutochrept). Endophyte-infected plants contained 0.50% Ca, 0.35% Mg, and 1.6% K and the non-infected plants contained 0.56% Ca, 0.40% Mg, and 1.7% K when grown in the Eutaw soil.

It is now becoming obvious that various biotypes of the endophyte infect tall fescue. Conflicting data regarding the toxicity of endophyte-infected tall fescue to livestock and the various toxin(s) associated with the toxicity may be explained by the presence of the different biotypes.

ALKALOIDS IN TALL FESCUE

Tall fescue contains alkaloids which appear to have no role in plant growth and development (Goodwin and Mercer, 1983) and may be detrimental to animal performance (Hemken et al., 1984). Increasing N availability has been reported to increase perloline and perlolidine (Gentry et al., 1969). Typical concentrations of perloline and perlolidine in tall fescue are 2000 ug/g and 800 ug/g, respectively (Gentry et al., 1969). The diazaphenanthrene alkaloids may increase in water-stressed plants (Bush, 1983). Temperature also may affect alkaloid concentrations in tall fescue. Diazaphenanthrene was higher with a 21/15 C (day/night) temperature regime than 32/17 or 16/10 C temperature conditions (Bush, 1983).

Alkaloids represent a large, diverse group of secondary metabolites that have pharmacological activity (Goodwin and Mercer, 1983). The medicinal properties of alkaloids have been known since the time of Hippocrates (Waller and Nawacki, 1978). Alkaloids are often refered to as waste products of metabolism (Goodwin and Mercer, 1983). If this were accurate, alkaloids would be concentrated in dead leaves and not in actively growing parts or seeds (Waller and Nowacki, 1983).

Endphyte-infected tall fescue contains alkaloids that may be responsible for toxic symptoms in animals. The diazaphenanthrene (perloline and perlolidine) alkaloids have been associated with decreased digestibility of tall fescue (Bush et al., 1972) and the pyrrolizidine alkaloids (N-acetyl loline and N-formyl loline) have been associated with summer syndrome (Jackson et al., 1984). The ergopeptine alkaloids may also be associated with tall fescue toxicosis, particularly fescue foot (Yates et al., 1985). Research continues to determine the exact compound(s) responsible for toxicosis in animals and whether the toxic entity is produced by the plant, fungus or both.

The Diazaphenathrene Alkaloids

Perloline and perlolidine are diazaphenathrene alkaloids isolated from tall fescue. These compounds have a diazaphenanthrene (benzo [c] [2,7] naphthyridine) ring structure (Tookey and Yates, 1972). Perloline and perlolidine levels are highest in stem, followed by roots, leaves, panicle, and seed (Bush, 1983). Diazaphenanthrene has been associated with inhibition of fungal development.

Perloline has been reported to inhibit ruminal ***in vitro*** cellulose digestion and volatile fatty acid (VFA) production (Bush et al., 1972). Fed to lambs, perloline decreased crude protein and cellulose digestibility and increased body temperatures (Boling et al., 1975). The tall fescue strain, 'G1-307' was developed by Buckner in Kentucky for low perloline concentration. However, cattle fed 'G1-307' developed symptoms of summer syndrome (Hemken et al., 1984). Indicating that even very low levels of perloline may be enough to elicite a toxic response in the animal, or that perloline may not be related to fescue toxicosis. The tall fescue, 'G1-307' was reported to contain a high level of loline alkaloids (0.17%) (Bond et al., 1984).

The Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids include N-acetyl loline, N-formyl loline, demethyl-N-acetyl loline and loline (festucine). These compounds contain a pyrrolizidine ring, a cyclic ether bridge and a nitrogen group (Tookey and Yates, 1972). Concentration is highest in spikelet, followed by stem, leaf sheath, roots, and leaf blade (Bush, 1983).

Pyrrolizidine has been associated with insect resistance (Bush, 1983) and protection against predators (Latch et al., 1985). Endophyte-infected fescue has been reported to have greater resistance to insect feeding (Funk et al., 1985; Johnson et al., 1985). The resistance of plants to aphid feeding has been attributed to: the lack of a nutritional factor in the plant for insect growth and

development or presence of a feeding deterrent in the phloem of the plant (Dreyer and Campbell, 1987). In addition, there appears to be a relationship between the plant pectin structure, insect pectinase activity and plant resistance to insect feeding (Dreyer and Campbell, 1987).

The pyrrolizidine compounds, N-formyl loline and N-acetyl loline (FALA), have also been implicated as toxic agents and are the compounds considered to be more clearly associated with the endophyte and the toxicosis problem. Toxic fescue containing a high level of endophyte also contains FALA. Bush and coworkers (1982) reported that plants without the fungus did not contain FALA. Treating endophyte-infected fescue with a fungicide resulted in disappearance of the alkaloids. Alkaloids were not detected in fungus grown in culture but, the artifical environment of *in vitro* conditions often alter organism metabolism and may have changed alkaloid production.

The Ergopeptine Alkaloids

Smith (as cited by Porter et al., 1979) reported in 1937 the isolation and identification of ergosine and ergosinine from Claviceps purpurea. Porter and coworkers (1979) isolated three alkaloids identifed as ergosine, ergosinine and chanoclavine I from *in vitro* cultures of *Epichloe typhina* isolated from 'Kentucky-31' tall fescue. Total alkaloid content was 5.5 mg/ml in 28-d old cultures. In 1981, Porter re-identifed ergosine and ergosinine as ergovaline and ergovalinine, respectively by mass spectroscopry. In addition, Porter and coworkers (1981) also reported the production of agroclavine, elynoclavine, penniclavine and festuclavine by *Epichloe typhina*. 'Kentucky-31' endophyte-infected tall fescue contained the ergopeptine alkaloids, ergovaline and ergosine as identified by mass spectrophotometry (Yates et al., 1985). Cattle grazing 'Kentucky-31' tall fescue (95% endophyte-infected) showed signs of fescue foot (Yates et al., 1985). Total ergopeptine alkaloids of the forage averaged 0.4 ug/g. Yates and Powell (1987) separated various ergopeptide alkaloids from endophyte-infected tall fescue seed: ergovaline, ergosine, ergotamin, ergovalinine, ergosinine and ergotamine were present in the following concentrations, 2.89, 0.8,

0.21, 2.51, 0.47, 0.32 ug/g seed, respectively. Other alkaloids isolated from fescue include ergosterol, ergosterol peroxide and ergosta-4,6,8-(14),22-tetraen-3-one.

Other Compounds

Attempts have been made to associate other minor constituents of tall fescue to the toxicosis problem. The non-alkaloid anion fraction of an 80% ethanol extract of fescue produced clinical signs of fescue foot when tested in a bovine intraperitoneal assay (Williams et al., 1975; Garner et al., 1982). Yates and coworkers (1983) dosed (intraperitoneal) calves with plant extracts (80% ethanol) separated into cation (alkaloid and non-alkaloid), anion, and neutral fractions. The two calves dosed with the anion fraction showed symptoms of lameness, tail necrosis, and had a red line at the coronary band. One of the two calves dosed with the non-alkaloid cation fraction became lame and had a red line at the coronary band. Neither calf injected with the alkaloid cation fraction showed signs of fescue foot or tail necrosis. Coronary band temperature, measured by videothermometry (infrared-sensitive camera), was lower in calves administrated subfractions of the anion fraction as compared to the control (isotonic saline). Dihydroxybutyric acid, a minor component of the anion fraction, caused tail necrsois when administered intraperitoneally but not when administered orally or intraruminally.

The Inheritance Pattern

The one-gene-one enzyme hypothesis was proposed by Beadle and Tatum in 1941 (as cited by Strickberger, 1976) to explain gene function. By this hypothesis, genes control the reproduction of a specific enzyme. Lack of a gene results in disruption of some biochemical process due to the

absence of a required enzyme. Plants that do not contain measurable amounts of alkaloids could lack genes to code for enzymes required for biosynthesis or catabolism of specific alkaloids.

The heritability in the broad sense for alkaloid production has been documented (Buckner et al., 1973; Bush et al., 1982). However, the types and concentrations of alkaloids appear to vary among species and among tall fescue varieties. The total plant alkaloid content and the relative percentage of specific alkaloids have been reported to differ. Gentry (1968) reported the total alkaloids in 'Kentucky-31' and 'Alta' to be 0.096 and 0.075%, respectively. Perloline concentration was higher in 'Kenwell' than 'Kentucky-31' and lower in 'Alta' and 'Goar' tall fesuce varieties (Gentry, 1968). Jones and coworkers (1983a) reported differences in pyrrolizidine alkaloid concentration in four varieties of tall fescue. 'Kentucky-31' and 'Kenwell' had hgher alkaloid values than 'Alta' and 'Fawn'.

A positive relationship between chromosome number and perloline concentration was reported by Buckner and coworkers (1973). Tall fescue (6x) and perennial ryegrass (2x) contained 716 and 209 ug perloline/g dry weight tissue, respectively. The F1 populations of ryegrass x fescue cross had a ploidy number of 4x and perloline content was approximately half of the fescue parent (368 ug/g). Sullivan (1944) also reported a positive association between chromosome number and soluble constituents.

There appears to be significant variations in N-formyl plus N-acetyl loline alkaloid (FALA) due to genotype (Jones et al., 1985). Concentration of FALA in 11 parental clones of 'Kenhy' tall fescue were significantly ($P < 0.05$) different. Total loline concentrations ranged from 2998 to 5257 mg/g with a mean of 4120 mg/kg. Some of the plants were later discovered to be infected with *Acremonium coenophialum* and genetic variation could not be separated from endophytic fungus differences. Jones and coworkers (1983b), reported that non-infected progeny of 'Kentucky-31' fescue had lower FALA than parent plants. They also suggest a significant parental entry effect for FALA variability due to genetic differences.

Biosynthesis of Alkaloids

Alkaliods contain both carbon and nitrogen and as such have a potential function as a C and N sink. Alkaloid biosynthesis removes fixed carbon from the formation of soluble carbohydrates and other primary metabolites. Also, N in the form of amino acids are diverted from the synthesis of proteins, nucleic acids and other N-containing compounds. Carbon fixation in tall fescue occurs via the Calvin-Benson cycle. Fructose-6-phosphate from carbon dioxide fixation leads to the formation of pyruvate by glycolysis. By the Krebs cycle, pyruvic acid yields oxaloacetic acid which is converted to alpha-ketogluteric acid. Ketogluteric acid is converted to ornithine. Dimerization of two molecules of ornithine forms retronecine, norloline and loline alkaloid production. Perloline may be synthesized from tryptamine, a three carbon compound and an aromatic ring.

Environmental Factors

Although biosynthesis is genetically controlled, the concentration and types of alkaloids produced in plants may vary under different environmental conditions. Environmental factors such as light, soil moisture availability, temperature and pasture management may influence the relative levels of alkaloids in plants.

The synthesis of alkaloids may only be indirectly controlled by the amount and quality of light. Light-dependent activities such as carbon dioxide fixation and growth may influence the production of secondary metabolites. Seasonal variations in alkaloid production may be related to temperature and water availability but light could also be a factor. Perloline content of tall fescue was low in winter, when photosynthesis and the number of hours of light per day are lower and increased to 3800 ug/g in late July when the number of hours of light per day are greater (Gentry et al., 1969). Alkaloids in shaded (up to 73% of incident light) reed canarygrass, *Phalaris arundinacea*, L. increased 25 to 50% compared to controls in full sunlight (Frelich and Marten,

1972). Nowacki (1973, as cited by Waller and Nowacki, 1978) reported that the percentage of alkaloids in plant tissue (dry matter basis) decreased as the number of hours light/day and intensity of light increased. The total accumulation of alkaloids in plants exposed to high light was greater (yield of these plants were higher) than plants exposed to low light. The percentage of free alkaloids decreased and the hydroxylupanine ester fraction of the total alkaloids increased. The growth of the high-light exposed plants was better and the total amount of alkaloids was higher than those receiving low amounts of light.

Kennedy and Bush (1983) measured FALA of the tall fescue experimental line, G1-307, under three levels of moisure availability. Concentration of FALA in moderate and severely water stressed plants were greater than in adequately watered controls by the sixth week of treatment. By the twelfth week, N-formyl loline in the severely stressed plants increased 200% and N-acetyl loline increased 300% as compared to the control. After a three-week recovery period of optimum soil moisture availability, the alkaloid concentration in the stressed plants decreased. The authors suggest that the increased synthesis of alkaloids in water stressed plants may be due to non-specific effects of moisture stress or to increased availability of substrate (amino acids) for alkaloid production.

The percentage of perloline and FALA in tall fescue fluctuates with changes in temperature. Seasonal variation in alkaloid concentration in tall fescue was reported by Gentry and coworkers (1969). Perloline content of 'Kenwell' tall fescue was low in winter, increased in early spring and summer and peaked in late July and early August. In field studies, Kennedy and Bush (1983) reported increased FALA in midsummer when temperatures were high. Pyrrolizidine concentration varied with season in a 3-yr study, but, the peak alkaloid concentration occured at different time periods (Belesky et al., 1987). In controlled greenhouse experiments, the percentage change in alkaloids was higher in plants grown under moderate (21/15 C) than high (32/27 C) or low (16/10 C) temperature regimes. Fluctuations in loline concentrations in plants over the grazing season (Belesky et al., 1987), may be more useful to indicate time periods when the pasture is toxic rather than as an indicator of animal performance over the entire grazing season.

Mineral deficiencies may disturb normal metabolic functions in plants and result in accumulation of certain compounds. Nitrogen availability to plants is important in the biosynthesis and relative amounts of certain alkaloids. Marten et al. (1974) reported that N fertilization (up to 200 kg/ha) would increase alkaloids in plants naturally high in alkaloids but not in plants inherently low in alkaloids. Higher amounts of alkaloids were found in plants fertilized with an ammonia source than with a nitrate source.

Yoshida (1973, as cited by Waller and Nowacki, 1978) reported a decrease in enzyme activity in N, P, K, Ca, Mg, S, and B deficient plants resulted in decreased nicotine production in tobacco roots. Potassium deficiency in some species of *Hordium, Lupine, Trifolium and Melanthus* has been shown to increase the percentage of alkaolids/plant, but decrease the total amount of alkaloids/plant (Waller and Nowacki, 1978).

The effect of N fertilization on alkaloid accumulation in fescue appears to vary depending on the particular alkaloids considered. Increasing N availability has been reported to increase perloline and perlolidine (Gentry et al., 1969). Bush and Buckner (1973) reported a 10-fold increase in N availability to fescue resulted in an increase in perloline from 700 to 3000 ug/g. Gentry (1968) reported that perloline in fescue increased 75% by the addition of K and P and 186% with N, P, and K as compared to non-fertilized controls. However, high N fertilization may inhibit pyrrolizidine production in fescue. Over a 12-wk period, the largest increase in pyrrolizidine occured in plants receiving no nutrients and the least in plants recieving 30 meq N/liter (Kennedy and Bush, 1983). Nitrogen fertilization (112 to 336 kg/ha) of pearl millet (*Pennisetum americanum*) increased alkaloid concentration linearly from 20 to 50 mg/kg (Krejsa et al., 1987). Fertilization of tall fescue with 22 metric tons of broiler litter per hectare (775 kg of N) increased total alkaloid content of the plant material (Burns, 1978). Total alkaloid content was 0.34 %, N-acetyl loline plus N-formyl loline concentration was 0.18 % and perloline was 0.16 %. The form of N fertilizer may be important. Reed canarygrass fertilized with N in the form of nitrate contained lower alkaloid levels than plants fertilized with ammonium-N (Marten et al., 1974).

Gentry (1968) studied the interaction of N fertilization, disease, and production of alkaloids. Fescue infected with *Rhizoctonia solani*, Kuch, fertilized with 0 or 112 kg N/ha produced 50% and

67% less perloline, respectively as compared to non-infected plants. The diseased plants also had lower loline and the percentage reduction in alkaloid production also varied with the variety of fescue.

After cutting, reserves for alkaloid production may be mobilized. Kennedy and Bush (1983) reported increased FALA concentration in regrowth tissue after harvesting forage accumulated for six or eight weeks as compared to two or four weeks. Fribourg and Loveland (1978) reported an increase in perloline in tall fescue after stockpiling. Summer regrowth after stockpiled fescue was removed contained 0.46 mg perloline/g dry weight. The control, not stockpiled or allowed to accumulate, contained lower perloline (0.16 mg/g) levels. Field curing influences the percentage perloline relative to FALA. Tookey and Yates (1972) reported destruction of 15 to 72 % of perloline in field-cured tall fescue and as a result, loline increased to 50% of the total alkaloid content. Perloline levels in fescue peak during July and August (Gentry et al., 1969), a time when summer syndrome is observed.

TALL FESCUE TOXICOSIS

Although the nutritive value of tall fescue is high and comparable to orchardgrass (Jacobson, 1957), animal performance on fescue pasture is not consistent with forage quality as determined by chemical analysis (Buckner et al., 1967; Daniels et al., 1983). Animal performance on tall fescue is variable. Some producers claim excellent animal production on fescue pastures, while others experience problems with animal performance.

Cunningham (1949) reported the symptoms of fescue foot of cattle grazed on tall fescue in New Zealand. Other early reports that descibed the tall fescue toxicosis occurrence and symptoms include Pulsford (1950) and Goodman (1952). Jacobson and coworkers (1963) tried to fractionate 'toxic' fescue and measured the change in skin temperature in cattle administered the fractions. No conclusive results were obtained. J. D. Robbins, a chemist, W. C. Bacon, a fungal physiologist

and J. K. Porter, an organic chemist, speculated on the relationship between tall fescue toxicosis and plant infection with an endophytic fungus. In 1973, Bacon (as cited by Stuedemann and Hoveland, 1988) tested two pastures on the farm of A. E. Hays for the presence of a fungus. Cattle grazing one pasture showed signs of tall fescue toxicosis and plants were infected with an endophytic fungus. Cattle grazing a second pasture showed no symptoms and the plants were not infected with the endophyte. This information led to the testing of many tall fescue pastures and the search to elucidate the etiology of the tall fescue problem.

Tall fescue pastures have been associated with low animal gains. Average daily gain of steers on tall fescue was reported by Daniels et al. (1983) to be about 0.4 kg/day. Depression in livestock gain and/or intake may be due to the presence of an endophyte fungus and toxin(s) in the plant. Average daily gain of steers has been reported to be lower on pastures with a high percentage of endophyte infection as compared to pastures with a low percentage of infection (Pedersen et al, 1986; Read and Camp, 1986 and Stuedemann et al., 1986). Intake of tall fescue seeds by rats was depressed from 16.8 (non-infected seeds) to 7.7 (infected seeds) g/day by the presence of the endophyte in the seeds (Neal and Schmidt, 1985). Average daily gains decreased from 6.7 to 1.8 g/day in rats fed non-infected and infected seed diets, respectively. Addition of cultured fungus to the non-infected seeds at feeding did not decrease average daily gains or intake by rats. Infected seeds contained 5134 ug/g of N-acetyl loline and N formyl loline whereas non-infected seeds contained only 652 ug/g. Dry matter intake expressed as a percent of body weight of Holstein steers decreased ($P < 0.05$) by 0.0055% for each 1% increase of endophyte-infected fescue in the diet (Goetsch et al., 1987a). Increasing the percentage of infected fescue in the diet (tall fescue hay) from 0 to 100% increased ($P < 0.05$) dry matter, NDF, and N digestibility.

Other disorders have been associated with tall fescue. It is not known if these disorders are caused by the same toxic factors (Hemken et al., 1984). Fescue toxicosis, a term used to describe problems associated with tall fescue include fescue foot, summer syndrom, fat necrosis, and agalactia. Fescue foot is generally observed in late fall and winter when temperatures are cooler and is characterized by rough hair coat, hyperemia of coronary band and soreness of rear limbs (Jacobson et al., 1963). Eventually, the swelling advances to such a severe degree that the hooves

may slough off (Hemken et al., 1984). These symptoms are similar to those produced by ergot (*Claviceps purpurea*, Fries).

Summer syndrome (summer toxicosis) is associated with reduced performance of cattle at high environmental temperatures. Symptoms of summer syndrome include: reduced growth or milk production, decreased feed intake, rough hair coat, elevated body temperature, increased respiration rate, excessive salivation, reduced reproductive performance, and cattle seek wet spots or shade (Jacobson et al., 1970; Hemken et al., 1979; Steen et al., 1979). Simmental cows grazing tall fescue pastures (67-100% endophyte-infected) were thin, had a rough hair coat, diarrhea and loss of tail switch. The cattle had normal rectal temperature (37.3 C) and were acidotic (blood pH = 7.1) (Niederman et al., 1987). A great deal of research has concentrated on summer toxicosis because of the large economic losses suffered by some producers. It has been estimated that fescue toxicosis costs livestock producers in United States, $50 to 200 million annually (Siegel et al., 1984a).

There appears to be an interaction between environmental temperature and toxic symptoms. Cattle fed infected and non-infected tall fescue hay showed no signs of toxicosis at temperatures of 10 to 13 C (Hemken et al., 1981). When environmental temperature was increased to 34 to 35 C, cattle fed the toxic fescue had decreased intake, increased rectal temperatures, increased respiration rates and decreased average gains. Higher environmental temperatures may increase the sensitivity of the animal to the toxin or in a grazing situation, increase the level of toxin in the forage.

Fat necrosis (lipomatosis) has also been associated with cattle grazing tall fescue (Hemken et al., 1984). Fat necrosis is characterized by hard fat masses located in adipose tissue of abdominal cavity and is associated with heavy rates of poultry litter (Hemken et al., 1984) or N applications to the forage (Stuedemann, 1984).

Agalactia has also been associated with tall fescue. Low milk production, thickened placenta and weak foals have been observed in horses grazing tall fescue (Hemken et al., 1984). Selenium supplementation has been suggested to reduce the incidence of retained placenta in cattle (Heimann et al., 1981ab; Julien et al., 1976).

Serum cholesterol and prolactin changes in animals consuming tall fescue may also be symptomatic of fescue toxicity. Stuedemann (1984) reported that cattle grazing tall fescue, fertilized

with 660 kg N/ha, had lower plasma cholesterol levels than cattle grazing tall fescue not fertilized with N. The tall fescue line, GI-307 had the highest endophyte infection, the lowest perololine concentration (0.39 mg/g DM) and highest FALA concentration (0.665 mg/g DM) compared to the other line and cultivars examined. Serum cholesterol levels were lower in steers grazing the tall fescue line GI-307 than GI-306 or varieties, 'Kentucky 31' and 'Kenhy'. Steers grazing the tall fescue line GI-307 were the only animals to show signs of summer toxicosis. Results of Stuedemann's research indicate lipid metabolism in cattle may be influenced by grazing endophyte-infected tall fescue. Plants can adjust cellular lipid composition in relation to the environment. High or low temperatures influence the percentage of saturated as compared to unsaturated fatty acids (Goodwin and Mercer, 1983). Lipids in plants and animals need further evaluation concerning their relationship to infected tall fescue.

The toxic factor(s) in tall fescue may also depress serum prolactin levels. Prolactin functions in reproduction, growth promotion, water and electrolyte balance and in synergistic reactions with steroid hormones (Church aand Pond, 1978). Serum prolactin decreased when endophyte-infected fescue was fed to rats (Porter et al., 1985). Cows grazing tall fescue gained less weight and had lower serum prolactin levels than those grazing orchardgrass (Daniels et al., 1983). Low serum prolactin decreases milk production possibly resulting in decreased growth of calves. Supplementation of cows with Se may enhance prolactin production or release. Serum prolactin of Holstein steers was increased ($P < 0.05$) by supplementing infected fescue with clover hay (Goetsch et al., 1987b). Steers fed non-infected fescue had higher serum prolactin levels then those fed infected fescue (15.0 vs 0.0 ng/ml). N-acetyl loline and N-formyl loline concentrations in the infected fescue were 231 and 1.38 mg/kg, respectively. The non-infected fescue did not contain detectable levels of alkaloids. Serum prolactin in rats fed Yohimbine hydrochloride (YOH) in concentrations from 0 to 250 mg/kg decreased from 56.7 to 20.5 ng/ml (Jernigan et al., 1986). Yohimbine hydrochloride (YOH) is an indole alkaloid that blocks α_2-adrenergic and dopamine receptors and stimulates serotonergic recepters. However, injecting YOH intraperitonaly in doses of 0 to 25 mg/kg, increased serum prolactin from 184.3 to 1430.0 ng/ml. Factors that may inhibit prolactin release include γ-aminobutyric acid (Schally et al., 1977), dopamine and prolactin by negative feedback (Jernigan

et al., 1986). Factors that may promote prolactin release are thyrotropin-releasing hormone (Chen and Meites, 1975), serotonin and a hypothalamic vasoactive intestinal polypeptide (Clemens and Shaar, 1980). Administration of ergocryptine (80 mg) depressed serum prolactin in lactating Holstein cows (Smith et al., 1974). Milking the control animal increased serum prolactin but had no effect on treated cows. Prolactin in serum taken 5 min. before and 10 min. after milking averaged 20 and 35 ng/ml ($P < 0.01$) in control animal and remained at 1.4 ng/ml in cows administered ergocryptine. Incubation of bovine pituitary cells with ergocryptine depressed prolactin excretion by 60% ($P < 0.001$) compared control (no ergocryptine) (Smith et al., 1974). Ectors et al. (1972) documented by electron microscopic techniques that prolactin granules were formed but not exocytosed from cells exposed to ergot compounds.

LABORATORY ASSAYS FOR ENDOPHYTE FUNGUS

The endophyte fungus associated with tall fescue toxicosis produces no visible changes in the external appearance of tall fescue. Five laboratory techniques are available to screen plants for the presence of the endophyte: a staining test, grow-out, ELISA, plating and callus culture.

1. Staining Test. In this method, one layer of epidermis is peeled away from the lower sheath tissue with a fine forceps, placed on a slide, and stained with 1.0% aniline blue dye (Bacon et al., 1977; Hall et al., 1984). The slide is examined under the microscope. Hyphae will be stained dark blue. This method accurately determines if there is fungus in the plant, it is simple and requires only a microscope. However, the procedure is tedius and impractical for large numbers of samples. The staining test should not be used for old seed because it cannot differentiate live from dead fungus (Conger et al., 1986).

2. Grow-out Test. This test is used to determine the live fungus status of seed. Seeds are planted and allowed to grow for two months. Plants are then tested by the staining procedure for fungus infection.

3. Plating. In this method, sections of flowering plant stems are surface sterlized and plated on corn meal-malt agar. Fungus will be observed growing on the stems in four to six weeks (Bacon, 1983).

4. Enzyme-Linked Immunosorbent Assay (ELISA). In this procedure an antiserum to the fungus is used to detect antibody reactions to fungus antigens in plant samples (Johnson et al., 1982). This is a rapid test that allows many samples to be processed daily. However, it is expensive and only determines if the fungus is present but does not distinguish live from dead fungus.

5. Callus culture. Mature embroys are plated on dichloro-o-anisic acid medium and stored in the dark for 28 days (Conger and McDaniel, 1983). The presence or absence of the fungus in the culture is noted. This technique is faster than the grow-out test to determine viability of fungus in seeds and can be used for old or fresh seeds (Conger et al., 1986).

BIOASSAYS FOR FESCUE TOXICOSIS

Yates et al. (1983) used an intraperitoneal assay to study the influence of various extracts of tall fescue on the development of fescue foot as detected by a videothermometer. Difficulties in using large animals to evaluate fescue toxicity such as the requirement of large amounts of test feed and problems with reproducibility point to the need for more convenient assays. Porter et al.

(1985) used rats as the test animal. Extracts of tall fescue seeds were administered to rats for three days. Rats fed fungus infected seeds had lower serum prolactin, and lower dihydroxyphenylacetic acid (DOPAC) and homovanillic acid levels in the brain.

Chicken embryo's are a convenient test subject (Verrett et al., 1964). The eggs are injected and then by candling daily, percentage survival can be determined and deformities noted in the hatched chicks. Bacon et al. (1977) injected isolated fungi into eggs, incubated them for five days and determined the percentage survival. Results from this study indicated that one biotype of the fungus is toxic to chicken embryos. However, variations in the constituents of the culture media influenced production of toxic metabolites by the fungus. Bacon et al. (1977) suggested that the nature of nutrients provided by different host plants might also affect the metabolism of the fungus and the toxicity similar to effects in artifical environments.

Tetraenone (Ergosta, 4,6,8(14)22-tetraen-3-one) is a fungal steroid (Bacon et al., 1977). The measurement of this compound in tall fescue may also be correlated with the occurrence of fescue foot in cattle (Porter et al., 1975). This is an area that deserves further attention. Plants were sampled from tall fescue pastures where grazing cattle showed symptoms of toxicity and from pastures of healthy cattle. Percentage endophyte infection was measured and greater than 50% infection was required before toxic symptoms were observed. All plants that were infected with fungus produced tetraenone (Bacon et al., 1977). Results from this study point out a criticism of assays that detect fungus but are not correlated with toxic symptoms in animals.

It was noted by Wilkinson (1984) that armyworm survival is lower on endophyte-infected fescue. Fall armyworms *Spodoptera frugiperda* reared on perennial ryegrass (*Lolium perenne*, L.) infected with the endophyte fungus had reduced larval weights and delayed development compared to larvae reared on non-infected ryegrass (Hardy et al., 1985). Neonate larvae consumed more non-infected ryegrass than infected ryegrass (Hardy et al., 1985). Fall armyworm survival growth and development were also reduced by feeding endophyte-infected perennial ryegrass and tall fescue as compared to non-infected grasses (Clay et al., 1985). Latch et al. (1985) reported that aphids fed on non-infected fescue and not on infected fescue when confined in petri dishes. If an objective

method to measure feeding of armyworms or aphids on infected and non-infected plants can be developed, this assay may be effective.

Toxicity of brine shrimp to extracts of tall fescue have also been used successfully (Davis et al., 1986). Toxic symptoms in brine shrimp range from stunning to death.

RUMEN MICROORGANISMS

Some rumen microorganisms act to detoxify compounds potentially poisonous to the host animal (Hungate, 1966). The use of rumen microbes as a bioassay for fescue toxicosis may be feasible. Bush et al. (1972) reported that perloline (9.4×10^{-5} M) inhibited VFA production and cellulose digestion in invitro studies. At less then 9.4×10^{-5} M perloline inhibited cellulose but increased VFA and at greater than 1.13×10^{-3} M, peroline killed protozoa.

If the toxic factor changes rumen microorganism survival or is modified by rumen microorganisms to produce a substance toxic to the animal, it would be possible to supplement the animal with a compound that would counteract the toxicity. However, thiabendazole (oral paste) had no effect on ADG, rectal temperature, serum prolactin, or hair coat scores of weaned heifer calves fed broiler-litter-based diets after grazing of endophyte-infected tall fescue (Wahberg et al., 1987). Results of this work does not indicate whether such a product would be helpful while cattle are actually grazing endophyte-infected tall fescue. Compounds could be screened by in vitro incubation studies to determine usefulness as inhibitors of tall fescue toxicosis.

GROWTH, CHEMICAL COMPOSITION, IVDMD AND INSECT RESISTANCE OF ENDOPHYTE-INFECTED AND NON-INFECTED TALL FESCUE CLONES

INTRODUCTION

Infection of tall fescue (***Festuca arundinacea*** Schreb.) with an endophyte fungus, ***Acremonium coenophialum.*** Morgan-Jones and Gams has been associated with toxicity symptoms observed in cattle (Hoveland et al., 1980). Alkaloids produced by the endophyte and/or plant are currently considered to be the agents responsible for toxicosis in cattle (Porter et al., 1979; Bush et al., 1982; Hemken et al., 1984). The influence of endophyte infection on tall fescue yield is not clear. Endophyte infection has been reported to increase yield (Read and Camp, 1984; Bush et al., 1986; Belesky et al., 1987; Arechavaleta et al., 1989). Others have reported no influence of endophyte

infection on yield of tall fescue (Pederson et al., 1982; Hill et al., 1987; Bush and Burrus, 1988). Reports in the literature also differ concerning the influence of endophyte infection on tall fescue chemical composition. Odom and coworkers (1981) reported concentrations of Ca and Mg of endophyte-infected 'Kentucky-31' were numerically but not significantly lower than for non-infected plants. Hoveland and coworkers (1984) reported Ca concentration in endophyte-infected (94%) 'Kentucky-31' tall fescue was numerically but not significantly lower as compared to endophtye-free (< 5%) tall fescue. Wilkinson (1987) reported higher K and lower B in endophyte-infected as compared to non-infected tall fescue. Micronutrients were not measured.

Early reports on tall fescue toxicosis dismissed the relationship of plant nutrients with the problem. Jacobson et al. (1963) eliminated boron and selenium as causitive agents in tall fescue toxicity. Although a mineral imbalance is not known to be a causitive agent for tall fescue toxicosis, endophyte infection may affect the mineral composition of tall fescue.

Tall fescue has a diverse allopolyploidal nature that may influence chemical composition of individual plants. Plant yield and chemical composition (Buckner et al., 1967; Brown and Sleper, 1980; Sleper et al., 1980; Jones et al., 1983; and Soh et al., 1984) have been reported to be influenced by tall fescue variety. The influence of the tall fescue variety on growth and chemical composition must be separated from the influence of the endophyte. Controlled experiments using pairs of genetically identical infected and non-infected plants are needed.

Total alkaloid concentration and various alkaloid compounds also differ in tall fescue varieties (Gentry, 1968). Tall fescue contains alkaloids that may be involved in the symptoms of toxicity in animals. Alkaloids may also decrease digestibility of tall fescue by inhibiting rumen microorganisms. Perloline has been reported to inhibit cellulose digestion ***in vitro*** (Bush et al., 1970 and 1972). In **in vivo** studies with lambs perloline decreased digestibility of cellulose (Boling et al., 1975).

The influence of endophyte-infection on tall fescue growth may be related to insect resistance. Endophyte-infected tall fescue plants have been reported to be more insect resistant than non-infected plants (Clay et al., 1985; Latch et al., 1985). Endophyte-infection provides protection to the plant from insect (Latch et al., 1985) and herbivore (Goetsch et al., 1987) feeding. However,

the endophyte also receives nutrients from the plant. The use of plant photosynthates and minerals by the endophyte could suppress the growth and tillering of infected plants.

The objectives of this research were: 1) to evaluate the influence of endophyte infection on tall fescue yield, growth, tillering, chemical composition and IVDMD; and 2) to measure the influence of endophyte infection on insect resistance in tall fescue.

MATERIALS AND METHODS

***Phase 1*: Nursery Establishment.** 'Kenhy' tall fescue (*Festuca arundinacea*, Schreb.) plants were randomly selected from a 'toxic' tall fescue pasture at the Virginia Forage Research Station, Middleburg on 24 March 1986. Cattle grazed in this pasture during previous studies developed symptoms of summer syndrome (Byrant and Hammes, 1981). Seed from these plants were submitted to the Livestock and Seed Division (USDA, Beltsville, MD) to verify cultivar. One tiller was isolated from each of 33 endophyte (*Acremonium coenophialum*, Morgan-Jones and Gams). infected tall fescue plants obtained from the pasture. Each tiller was planted into an individual pot that was filled with perlite, for a total of 33 pots. All pots were watered with nutrient solution (Hoagland and Arnon, 1950) and deionized water. The nutrient solution was prepared as follows: 1 ml of 1-*M* KH_2PO_4, 5 ml of 1-*M* KNO_3, 5 ml of 1-*M* $Ca(NO_3)_2 \bullet 4\ H_2O$, 2 ml of 1-*M* $MgSO_4 \bullet 7\ H_2O$, 1-ml of microelement solution (2.5 g H_3BO_3, 1.54 g $MnSO_4$, 2.2 g $ZnSO_4 \bullet 7\ H_2O$, 0.05 g $CuCl_2 \bullet 2\ H_2O$ and 0.08 g MoO_3 per liter distilled H_2O) and 5-ml Fe-tartartic acid solution (5 g $FeCl_3$ and 5 g tartartic acid per 500 ml H_2O) diluted to 1-l with distilled H_2O. Plants were maintained in a greenhouse.

Plants grew rapidly and tillered to fill available space in the pots. On 17 April, 1986, two tillers from each pot were removed and microscopically tested (Fescue Diagnostic Center, Auburn) to verify the presence of the endophyte. All plants were infected with the endophyte. Each pot of tillers was divided in half on 6 May, 1986 to form genetically identical plant pairs (clones). All plants were replanted into perlite and watered with nutrient solution. One plant from each pair of clones was treated with Benomyl (0.5 g a.i./pot) on 21 May, 1 July and 28 Sept. 1986. Two tillers from each plant were submitted to the Fescue Diagnostic Center at Auburn on 9 Dec. 1986 to verify endophyte status.

Phase 2: Chemical composition and in vitro dry matter digestibility of plants composited by endophyte level. Plant-clones (26 pairs) from the nursery, were harvested and composited by level of endophyte infection on 8 Nov. 1986, 4 Jan. 1987, 16 Jan. 1987, 2 Feb. 1987, 18 Feb. 1987, 9 Mar. 1987 and 26 Mar. 1987. Harvested plants were dried at 60 C and ground (1mm) in a stainless steel Wiley Mill[1]. Minerals (P, K, Ca, Mg, S, Na, Al, B, Cu, Fe, Mn and Zn) were determined by inductively-coupled plasma optical emission spectrophotometry (ICP-OES) on plant tissue (0.500 g) digested with nitric and perchloric acid (3:1 ratio) (Muchovej et al., 1986). Digests were filtered through ashless Whatman paper (number 42) prior to analysis. Total non-structural carbohydrates (TNC) were hydrolyzed (Smith, 1969) and assayed as glucose equivalents (Davis, 1976). In vitro dry matter digestibility (IVDMD) was determined by the two-stage method of Tilley and Terry (1963). Total N was determined colorimetrically as ammonia-salicylate with a Technicon Autoanalyzer[2] (Technicon Industrial Systems, 1976) on plant material (200 mg) digested in a salt/catalyst mixture (94% K_2SO_4, 4% HgO, 1% $CuSO_4$ and 1% pumice) with 2.5 ml H_2SO_4 at 400 C for approximately 40 minutes (McKenzie and Wallace, 1954). Since Phase 2 was a preliminary study and not replicated, statistical analyzes were not applied.

Phase 3: **Yield and chemical composition of individually harvested endophyte-infected and non-infected tall fescue plants.** The influence of individual clones on yield and chemical composi-

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[1] Thomas-Wiley Mill. Model ED-5. Authur H. Thomas Company, Philadelphia, PA

[2] Technicon Industrial Systems, Tarrytown, NY 10591

tion of plants was measured. The experimental design was a replicated, completely randomized design. Individual plant-clones (17 pairs) were harvested separately for dry matter yield, chemical composition and IVDMD on: 11 April, 1 May, 19 May, and 7 June 1987. Yield was measured by harvesting (hand-powered sheep shears) the plant tissue to a height of 3-cm. Plant material from each pot was placed in a separate cloth bag, quick-frozen in liquid N and placed on dry ice for transport to the laboratory. Plant material was dried in a forced-air oven at 60 C and weighed for dry matter yield. Dried plant material was ground and analyzed for chemical composition and IVDMD as previously stated in Phase 2.

Statistical analysis. Effects of endophyte level and date of harvest on yield, IVDMD and chemical composition of tall fescue were examined by analysis of variance (SAS, 1985). The main effects of endophyte level and date of harvest were tested by the residual error term. Difference in the means of harvest date were separated by the Tukey pairwise comparison procedure. Differences between individual endophyte-infected and non-infected clones were analyzed by paired comparisons (TTEST) procedure (SAS, 1985).

Phase 4. **Growth and tillering of endophyte-infected and non-infected tall fescue.** The top 10-cm of soil (Chester-Brandywine loam, 2-7% slope) was obtained from the 'toxic' tall fescue pasture at the Middleburg Research Station on 11 October, 1986. Soil was air-dried and seived. Three kg of soil was weighed into each of 36 pots. A starter fertilizer, 33.6 kg N/ha as ammonium nitrate, 67.2 kg P_2O_5/ha as potassium phosphate, and 168.0 kg K_2O/ha as potassium phosphate and potassium chloride was applied before transplanting the tillers. Six plant-clones were randomly selected from the nursery of plants grown in nutrient solution. Each pair of clones consisted of one endophyte-infected and one non-infected tall fescue plant from the same genetic base. Each plant was divided into five sections. Three tillers were isolated from each section for a total of 15 tillers from each plant. Five tillers from each individual plant were transplanted into a pot containing soil. There were three pots of each plant for a total of 36 pots. Tillers (two per pot) were submitted to the Fescue Diagnostic Center, Auburn for endophyte testing to verify the presence or absence of the endophyte. On 21 April, 1987 and 19 May, 1987 all plants were cut to a 10-cm height with hand-held sheep shears. Plant heights were measured and the number of tillers were counted on

28 April, 5 May, 10 May, and 30 May 1987. Plants were discarded June 1987 due to death from undetermined causes. Death may have been caused by the stress of transplanting and invasion of insects in the greenhouse. Data were analyzed by the general linear method (SAS, 1985) for completely randomized design with replications.

Phase 5: **Insect resistance of endophyte-infected tall fescue.** Twenty-six pairs of tall fescue plant-clones that were either infected or non-infected with the endophyte were placed in a greenhouse infested with aphids, white flies and mealybugs. The number of aphids and white flies present on each plant was visually determined on 16 Jan. 1987. The presence of mealybugs (order: Pseudococcidae; *Trionymus clandestimus*) was determined on infected and non-infected fescue on 7 July, 1987. Data were analyzed as described previously (Phase 3).

RESULTS

Phase 1. Results of testing of seed by electrophoresis for protein banding patterns verified that fescue plants were 'Kenhy' tall fescue. Endophyte testing verified that all plants treated with Benomyl were endophyte-free and non-treated plants were infected with the endophyte, ***Acremonium coenophialum***, Morgan-Jones and Gams. These clones were used to measure the influence of endophyte infection on plant growth, chemical composition, IVDMD and resistance to insect feeding.

Phase 2. Total non-structural carbohydrate, N and P concentration of endophyte-infected tall fescue were numerically higher as compared to non-infected tall fescue (Table 1). Potassium concentration was numerically higher in endophyte-infected as compared to non-infected tall fescue. Concentrations of Ca and Mg were numerically higher in non-infected as compared to endophyte-infected tall fescue. Sulfur concentration was numerically higher in endophtye-infected as compared to non-infected tall fescue. Microminerals were higher in non-infected as compared to endophyte-

infected tall fescue. Concentration of B, Mn and Zn were higher in non-infected as compared to endophyte-infected tall fescue. Aluminium, Cu, Fe and Na were numerically higher in non-infected as compared to endophyte-infected tall fescue. Endophyte infection did not appear to influence IVDMD.

Phase 3. Yield of endophyte-infected tall fescue was higher ($P < 0.05$) as compared to non-infected tall fescue (Table 2). The highest ($P < 0.01$) yield occured on 1 May 1987 (4.8 g/kg) and lowest (3.7 g/kg) on the final harvest date (7 June, 1987). Endophyte infection did not influence IVDMD. Total non-structural carbohydrate concentration was numerically but not significantly higher in endophyte-infected as compared to non-infected tall fescue. Concentration of TNC in the leaf tissue was higher ($P < 0.01$) on 19 May 1987 (15.2 g/kg) as compared to the other harvest dates. Nitrogen concentration was higher ($P < 0.05$) in non-infected as compared to endophyte-infected tall fescue. The highest ($P < 0.01$) concentration of N occured on 1 May, 1987 (44.7 g/kg) and the lowest (33.4 g/kg) on the final harvest date (7 June, 1987). Phosphorus and potassium concentrations were similar in infected and non-infected tall fescue. The lowest ($P < 0.01$) concentration of P (3.3 g/kg) and K (38.6 g/kg) in tall fescue occurred on the final harvest date. Magnesium concentration was higher ($P < 0.01$) in non-infected as compared to infected tall fescue. The highest ($P < 0.01$) concentration of Mg in tall fescue occured in the 1 May 1987 (4.4 g/kg) harvest and the lowest in the 19 May 1987 (3.6 g/kg) harvest date. Aluminium, B, Cu, Mn and Zn concentration was higher ($P < 0.01$) in non-infected as compared to endophtye-infected tall fescue. Concentration of Fe and Na were numerically but not significantly higher in non-infected as compared to endophtye-infected tall fescue. Concentration of Al was highest ($P < 0.01$) on 11 April and 1 May 1987 and lowest on 19 May and 7 June 1987. Boron concentration was higher ($P < 0.01$) on 19 May, 1987 (15.2 ug/g) than B concentration at the other three harvest dates. Concentrations of Cu and Fe were highest ($P < 0.01$) on 11 April and lowest on 19 May 1987.

Accumulation of P, K and S was higher ($P < 0.05$) in endophyte-infected as compared to non-infected tall fescue (Table 3). Magnesium accumulation was higher ($P < 0.01$) in non-infected as compared to infected tall fescue. Concentration of N, P, K, S and Mg was highest ($P < 0.01$) at on 1 May 1987 as compared to the other harvest dates. Concentration of Al and Mn was higher

Table 1. Chemical composition and IVDMD of tall fescue as influenced by endophyte infection.

Item[1]	Endophyte level	
	Infected	Non-infected
	- - g/kg, dry basis - -	
TNC	55.8	49.6
Nitrogen	42.0	40.9
Phosphorus	3.5	3.3
Potassium	42.4	40.4
Calcium	4.2	5.6
Magnesium	3.9	4.6
Sulfur	5.8	5.3
	- - ug/g, dry basis - -	
Aluminum	21.5	24.2
Boron	39.8	46.2
Copper	4.0	4.6
Iron	84.6	88.8
Manganese	31.7	45.4
Sodium	463.4	564.4
Zinc	35.3	39.2
	- - - - - % - - - - -	
IVDMD	83.1	82.3

[1]Mean of 7 harvests, 11-8-86 to 3-27-87.

($P < 0.05$) in non-infected as compared to endophyte-infected tall fescue. Accumulation of Al and Mn was highest ($P < 0.01$) on 1 May (91.8 and 198.1 g/plant, respectively) and lowest on 7 June 1987 (41.1 and 126.8 g/plant, respectively). Boron accumulation was highest ($P < 0.01$) on 19 May 1987 (186.3 g/plant) and lowest (108.3 g/plant) on 7 June 1987. Total non-structural carbohydrate accumulation was higher ($P < 0.05$) in infected as compared to non-infected tall fescue. Accumulation of TNC in tall fescue was higher ($P < 0.01$) on 19 May 1987 than on the other three dates.

Phase 4. Tiller numbers were numerically but not significantly higher in non-infected as compared to endophyte-infected tall fescue (Table 4). Height of tillers was higher ($P < 0.05$) in non-infected as compared to endophyte-infected on 28 April, 1987 and also higher but not significantly on 5 May, 10 May and 30 May, 1987.

Phase 5. Presence of the endophyte decreased ($P < 0.01$) the number of aphids, whiteflies and mealybugs observed feeding on tall fescue (Table 5).

DISCUSSION

In the present study, dry matter yield was influenced by endophyte infection of tall fescue. In another study, differences in yield of 'Kenhy' tall fescue planted from seed obtained from five different sources were not related to level of endophyte in the plants (Bush and Burrus , 1988). West and coworkers (1987) also reported no difference in yield of endophyte-infected and non-infected 'Kentucky-31' tall fescue grown with optimum moisture. However, they reported endophyte-infected tall fescue had higher ($P < 0.05$) yield and greater ($P < 0.05$) leaf area per tiller as compared to non-infected plants when grown in droughty conditions. These researchers attribute the lower drought tolerance of non-infected plants to the substantially higher ($P < 0.05$) numbers of nematodes on the roots of non-infected as compared to endophyte-infected plants. In the present study, plants were grown in a nutrient solution and did not experience moisture stress.

Table 2. Yield, chemical composition and IVDMD of individually harvested tall fescue plants.

Item	Endophyte level		
	Infected	Non-infected	SE[1]
Yield[2,3], g/pot	4.4	4.1	0.1
IVDMD, %	84.0	84.4	0.6
	- - g/kg, dry basis - -		
TNC[3]	104.0	101.0	3.0
Nitrogen[2,3]	38.4	39.6	0.5
Phosphorus[3]	3.8	3.7	0.1
Potassium[3]	40.1	39.5	0.4
Calcium	4.4	5.2	0.3
Magnesium[3,4]	3.6	4.3	0.1
Sulfur[3]	5.2	4.9	0.2
	- - ug/g, dry basis - -		
Aluminum[3,4]	12.4	16.1	0.5
Boron[3,4]	31.2	35.5	1.1
Copper[3,4]	2.8	3.4	0.1
Iron[3]	71.5	74.3	1.6
Manganese[4]	34.6	43.3	1.4
Sodium	462.7	542.9	42.4
Zinc[4]	36.2	41.6	1.2

[1]Standard error of mean.
[2]Indicates difference between treatments ($P < 0.05$).
[3]Indicates date effect ($P < 0.01$).
[4]Indicates difference between treatments ($P < 0.01$).

Table 3. **Accumulation of minerals and total non-structural carbohydrates in tall fescue plants.**

Item	Endophyte level		SE[1]
	Infected	Non-infected	
	- - kg/plant, dry basis - -		
Nitrogen[2]	1.7	1.6	0.1
Phosphorus[23]	16.8	15.1	0.6
Potassium[24]	179.1	158.6	5.7
Calcium	20.6	21.1	2.1
Magnesium[24]	15.9	17.6	0.7
Sulfur[23]	23.7	20.2	1.1
	- - g/plant, dry basis - -		
Aluminum[23]	56.6	66.0	3.1
Boron[2]	142.3	144.4	7.3
Copper	12.3	13.7	0.6
Iron[2]	316.3	297.7	11.6
Manganese[23]	152.8	180.9	8.8
Sodium	2.1	2.1	0.2
Zinc[2]	106.3	166.3	6.5
TNC[23]	50.3	44.4	2.0

[1]Standard error of mean.
[2]Indicates date effect ($P < 0.01$).
[3]Indicates difference between treatments ($P < 0.05$).
[4]Indicates difference between treatments ($P < 0.01$).

Table 4. Tall fescue tiller development and height.

Item	Date	Endophyte level: Infected	Non-infected	SE[1]
		- - number/plant - -		
Tiller				
	4-28-87	1.4	1.7	0.3
	5-05-87	4.1	5.4	0.7
	5-10-87	6.3	7.8	0.7
	5-30-87	22.3	23.2	0.9
		- - - - - cm - - - - -		
Height				
	4-28-87[2]	14.7	15.9	0.4
	5-05-87	17.5	18.2	0.4
	5-10-87[3]	18.3	19.3	0.4
	5-30-87	20.6	20.6	0.3

[1]Standard error of mean.
[2]Difference between treatments ($P < 0.05$).
[3]Plants harvested to 10-cm height on 5-19-87.

Table 5. Insect resistance of endophyte-infected tall fescue.

Insect	Endophyte level		SE[1]
	Infected	Non-infected	
	- - number/plant - -		
Aphid[2]	0.1	12.9	1.2
Whitefly[2]	0.3	4.6	0.4
Mealybug[2]	0.0	11.0	1.0

[1]Standard error of mean
[2]Difference between treatments ($P < 0.01$).

Results of the present study, do not agree with those of Bush and coworkers (1986), who reported higher tiller numbers for infected as compared to non-infected tall fescue plants grown in pots in the greenhouse.

Changes in the growth of infected plants could be related to altered metabolism of plant growth regulators. Cultured ***in vitro, Balansia epichloe***, produces indole derivatives including indole acetic acid, indole ethanol and indole acetamide (Bacon et al., 1986). Indole-3-acetic (IAA) functions as an auxin in plants (Wareing and Phillips, 1981). Other indoles that are chemically related to IAA also occur in plants. Indole-3-acetic acid is synthesized in the leaves located in the apical region of the shoot and is transported down the stem to target sites. The amino acid, L-tryptophan is a precurser for IAA synthesis (Goodwin and Mercer, 1983). Auxins are involved in plant internode elongation, root initiation and vascular tissue development (Goodwin and Mercer, 1983). Auxin also appears to be involved in apical dominance by supressing lateral bud development (Wareing and Phillips, 1981). Hormonal control in plants is complex and involves interactions of compounds in complicated positive and negative feedback mechanisms.

Few reports in the literature have measured any difference in the chemical composition of endophyte-infected and non-infected tall fescue. In an early report, Jacobson et al. (1963), reported values for P, K, Ca, Mg and Mn of 100 samples of 'toxic' tall fescue hay were within 'normal' values. Hoveland and coworkers (1984) reported lower concentrations of Ca, higher Mg and similar P and K in endophyte-infected as compared to non-infected 'Kentucky-31' tall fescue, but their studies contained a soil type bias that could have influenced the results. Results from the present study agree with Hoveland and coworkers (1984) that endophyte-infection did not appear to alter the concentration of P in tall fescue but do not agree in regard to Mg and K.

Nitrogen concentration was numerically higher in composited and significantly higher in individually harvested non-infected as compared to endophyte-infected tall fescue. Bush and Burrus (1988) reported no difference in crude protein content of endophyte-infected and non-infected tall fescue plants averaged over varieties, years, time of year of sampling and drying treatments. The 'biological-value' (based on amino acid composition) was not influenced by endophyte-infection (Neal and Schmidt, 1985).

Plants (composited by endophyte level) infected with the endophyte contained higher ($P < 0.05$) concentrations of K as compared to non-infected plants. Potassium increases the osmotic potential, can influence stomatal closure (Gardner et al., 1985) and is required for water uptake (Epstein, 1972). Without irrigation, non-infected plant swards had higher canopy temperatures, an indication of drought stress, as compared to endophyte-infected tall fescue swards (West et al., 1987). With drought stress, leaf stomata close, reducing the evaporative cooling by leaves and the canopy temperature increases. Water stress causes stomatal closure, reducing carbon dioxide uptake and as a result will decrease dry matter production. In the present experiment, when clones were individually harvested, the increase in K concentration of infected plants was not observed. Odom and coworkers (1981) reported no influence of endophyte infection on tall fescue K concentration.

Plants (composited by endophyte level) infected with the endophyte contained lower ($P < 0.05$) concentrations of Ca than non-infected plants. Calcium has an important function in the maintenance of cell wall integrity (Gardner et al., 1985) and in the stabilization of pectin (Jarvis, 1984). A similar response to Mg concentration was measured in both composited and individually harvested plants. Magnesium may be especially important since Mg deficiencies may increase plant susceptibility to fungus infection (Jones et al., 1983). The present study does not agree with the results of Odom and coworkers (1981) who reported no statistically significant influence of endophyte infection on tall fescue concentration of Ca or Mg.

Accumulation of S was higher in endophyte-infected as compared to non-infected tall fescue. Sulfur has been correlated with drought tolerance in plants (Bixby and Beaton, 1970) and sulfhydryl groups are involved in hardening the protoplasm to drought (Gardner et al., 1985). The accumulation of minerals, calculated as concentration times the dry matter yield, is useful if a dilution effect on concentration from increased yield is suspected. Differences in mineral accumulation may indicate changes in growth rates of infected and non-infected plants in response to changes in environmental temperature.

Aluminium concentration in individually harvested plants was higher in non-infected as compared to endophyte-infected tall fescue. Jacobson and coworkers (1963) reported Al concen-

tration in 'nontoxic' fescue was 0.64 % ash and in 'toxic' fescue was 0.29 % of ash. Muchovej and coworkers (1986) reported flooding and chelating agents (citric acid and nitrilotriacetate) increased ($P < 0.01$) Al concentration in annual ryegrass (***Lolium multiflorum*** Lam.).

In both composited and individually harvested plants, concentration of B was higher in non-infected as compared to endophyte-infected tall fescue. In the present study, B was also lower in endophyte-infected as compared to non-infected tall fescue. Results do not agree with Jacobson and coworkers (1963) who reported lower B in 'non-toxic' (3.27 ppm) as compared to 'toxic' 'Kentucky-31' tall fescue (5.24 ppm). The level of endophyte in the tall fescue hay samples in their research was not reported, since endophyte-infection was not associated with tall fescue toxicosis until the 1970's. The biochemical role of B in plants is uncertain but B is known to be involved in carbohydrate metabolism, cell division and development (Mortvedt et al., 1972).

Non-infected tall fescue contained higher concentrations of Mn and Zn as compared to endophyte-infected tall fescue in both composited and individually harvested plants. Manganese activates IAA oxidase, decreasing IAA concentration in plant tissues (Gardner et al., 1985). Zinc is essential for the activation of enzymes involved in the synthesis of tryptophan (the precursor to IAA). Plants deficient in Zn are low in tryptophan and IAA, have smaller leaves and early abscission (Gardner et al., 1985). Tryptophan is also a precursor for alkaloids (Goodwin and Mercer, 1983) and if not utilized for IAA synthesis would be available for alkaloid synthesis.

Endophyte infection did not influence IVDMD in the present study, these results do not support the findings of Goetsch and coworkers (1987), that increasing the percentage of endophyte-infected tall fescue hay in the diet of Holstein steers increased ($P < 0.05$) dry matter digestibility. Intake was decreased as the percentage of endophyte-infected tall fescue hay in the diet increased. The change in intake may have influenced the measured digestibility.

In the present study, insect resistance was higher in endophyte-infected as compared to non-infected tall fescue. Fall armyworm, ***Spodoptera frugiperda*** larvae prefered non-infected perennial ryegrass (***Lolium perenne*** L.) and consumed greater amounts of endophyte-free leaves (Hardy et al., 1985). Also, the weight of fall armyworm larval fed non-infected tall fescue was 80% higher than insects fed endophyte-infected tall fescue (Clay et al., 1985). Latch and coworkers (1985) re-

ported bird-cherry oat aphids, ***Rhopalosiphum padi***, L. consumed ($P < 0.01$) non-infected but not endophyte-infected tall fescue.

The inhibition of insect feeding on endophyte-infected tall fescue could be related to metabolites in the plant. A methanol extract of endophyte-infected tall fescue detered feeding of oat bird cherry aphid, ***Rhopalosiphum padi*** and greenbug, ***Schizaphis graminum*** (Johnson et al., 1985). The methanol extract of tall fescue contained 15,578 ug/ml of N-acetyl and N-formyl loline. Host-plant resistance to aphid feeding may also be attributed to structural and chemical differences in plant tissues that limit aphid feeding. Aphids feed by probing with a stylet into the middle lamella that is located between cells (Dreyer and Campbell, 1987). The middle lamella is composed mainly of pectin. Pectin is a branched polysaccharide, the main galacturonan chain is bent by alternating units of rhamnose (which also has sidechains of arabinan and galactin chains) and unbranched units (does not contain rhamnose) (Jarvis, 1984). As aphids feed, they inject salivary pectinase into the middle lamella (Dreyer and Campbell, 1987). Pectinase catalyzes the depolymerization of the intercellular pectin, which allows the aphid access to the phloem. In aphid resistant plants, the rate of depolymerization of the intercellular pectin is slow, compared to susceptible plants (Dreyer and Campbell, 1987). Also, pectin may function as a binding site for bacteria (Slusarenko and Wood, 1983) and fungal spores (Dreyer and Campbell, 1987). Pathogenic fungi may also be able to break-down pectins (Dreyer and Campbell, 1987).

It should be noted that in turf species and breeding programs, endophyte infection is considered beneficial. In a turfgrass trial of 400 single-plant progenies of tall fescue, the 32 plants which performed the best were endophyte-infected (Funk et al., 1985). In contrast, the plants with low levels of endophyte exibited poor persistence after 3-yrs, poor resistance to crabgrass invasion and poor recovery from summer stress. Funk and coworkers (1985) suggest insect resistance was a major factor influencing the improved persistance of endophtye-infected as compared to non-infected tall fescue.

Results of this research are unique in that any influence of tall fescue variety on yield, chemical composition or growth were eliminated by use of endophyte-infected and non-infected clones. Also, determinations were measured on numerous plants over a longer period of time than in other

reports in the literature. However, it must be noted that the response of plants grown in pots in a greenhouse may not reflect the response of plants grown in a more natural environment, in the field. These plants were grown under optimum light, fertility and temperature conditions in a greenhouse and may not reflect plant reponse in the field. Plants (composited by endophyte level) infected with the endophyte contained lower ($P < 0.05$) concentrations of Ca, Mg, B, Mn and Zn than non-infected plants. Nitrogen, Mg, Al, B, Cu, Mn, and Zn concentrations in individually harvested plants were higher in non-infected as compared to endophyte-infected tall fescue. Overall, non-infected tall fescue contained higher Mg, B, Mn and Zn as compared to endophyte-infected plants. The influence of changes in environment on the chemical compositon of endophyte-infected and non-infected plants should be studied.

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TALL FESCUE GROWTH AND CHEMICAL COMPOSITION AS AFFECTED BY GROWTH STAGE, SITE, ENDOPHYTE INFECTON AND NITROGEN FERTILIZATION

INTRODUCTION

Tall fescue (***Festuca arundincea*** Schreb.) is a cool season forage grown on 14 million ha of land in the United States (Hemken, 1983) and on over 1 million ha in Virginia, primarily as a pasture species. Productivity and quality of tall fescue are influenced by many factors including soil temperature, moisture and fertility (Heath et al., 1985). Infection with an endophyte fungus (***Acremonium coenophialum***, Morgan-Jones and Gams) may also alter tall fescue growth and chemical composition. Preliminary studies indicate wide-spread endophyte-infection of tall fescue pastures in Virginia. Hall and coworkers (1984) reported that 75% of the pastures tested in Virginia

were at least 50% infected with the endophyte. The percent infection of the tall fescue pastures ranged from 5 to 100%. The influence of endophyte infection on tall fescue growth and chemical composition under Virginia conditions needs clarification.

Growth and chemical composition of tall fescue are influenced by soil temperature, moisture and fertility. Germination of tall fescue requires temperatures of 16 to 30 C and vegetative growth is optimum at 22 to 27 C (Hoveland, 1983). Chemical composition is also influenced by temperature (season). Buckner and coworkers (1967) reported that crude protein concentration in tall fescue ranged from 13.7 to 29.7% and was lowest during hot dry summer months and highest during cool damp months in early spring. Total non-structural carbohydrate also varied with season and ranged from 3.6 to 30.2%. Temperature may also affect alkaloid concentration in tall fescue. Alkaloid concentration in tall fescue varies with season (Gentry 1968; Gentry et al., 1969). Diazaphenathrene concentration in tall fescue grown in a greenhouse was higher at 21/15 C (day/night), compared to 32/17 or 16/10 C temperature regimes (Bush, 1983). Perloline is the major alkaloid in the diazaphenathrene group. Concentration of perloline in 'Kenwell' tall fescue was low in winter, increased in early spring and summer and peaked in late July. Alkaloids of the pyrrolizidine group, N-acetyl loline and N-formyl loline (FALA) were reported to increase in midsummer when temperatures were high (Kennedy and Bush, 1983). Belesky et al. (1987) reported pyrrolizidine concentration varied with season.

Tall fescue is a hardy plant that can grow under droughty conditions (Buckner, 1985) but can also survive periods of flooding (Hoveland, 1983). A deficiency or an excess of water can decrease the yield and change the chemical composition of tall fescue. Water stress may decrease plant yield by reducing cell division and enlargement (Kramer, 1983). In water-stressed plants, proteins are hydrolyzed and amino acids accumulate (Eaton and Ergle, 1948). Amylase activity in leaves may also be increased, decreasing carbohydrate content of these plants. Concentration of FALA in water-stressed tall fescue plants were higher than in tall fescue controls which received adequate moisture (Kennedy and Bush, 1983). The authors suggested that the increased synthesis of alkaloids in water-stressed plants may be due to increased substrate (amino acid) availability for alkaloid production. Flooding has been shown to decrease dry matter yield (Terrill, 1985).

Changes in mineral composition of plants grown under flooded conditions vary with plant species, soil type and initial soil conditions (Kuzlowski, 1984). These changes can be related to changes in plant availability and uptake of minerals. For instance, Mg and K decreased in tall fescue grown in flooded conditions (Rodgers and Davies, 1973) whereas Cu and Fe were increased in plants grown in flooded conditions (Kuzlowski, 1984).

Nitrogen fertilization increases dry matter production and may change chemical compositon (Eck et al.,1981). Yield is increased in N-fertilized plants by increasing cell volume and decreasing cell walls (Heath et al., 1985). Belesky and coworkers (1984) reported N fertilization of tall fescue increased yield by increasing leaf area, number of tillers and decreasing number of senescing leaves. Nitrogen also stimulates meristematic activity (Heath et al., 1985).

Nitrogen fertilization influences tall fescue chemical composition. Water soluble carbohydrates were decreased in tall fescue fertilized with N (Belesky et al., 1984; Heath et al., 1985). Reid and Jung (1974) reported N fertilization of tall fescue increased K by 50% but had little effect on P, Ca, Mg, Mn and Zn. Belesky and coworkers (1984) reported N fertilization of tall fescue increased ($P < 0.05$) total N, nitrate-N, K, Ca, Mg, Zn, and Cu but decreased P. Eck et al. (1981) reported N fertilization of tall fescue increased N and K content of tall fescue but decreased P, Ca and Mg. Reid and Jung (1965) reported N fertilization decreased cellulose and acid detergent fiber in tall fescue. The effect of N fertilization on alkaloid accumulation in tall fescue varies with type of alkaloid. Increasing N availablity has been reported to increase perloline and perlolidine (Gentry 1968; Buckner and Bush, 1979). However, high N fertilization may inhibit pyrrolizidine production (Kennedy and Bush, 1983). Over a 12-wk period, the largest increase in pyrrolizidine occurred in plants that received no N and the lowest accumulation occurred in plants that were fertilized with 30 meq N/l. Fertilization of tall fescue with 775 kg N/ha (as broiler litter) increased total alkaloid concentration (Burns, 1978). Fribourg and Loveland (1978) reported an increase in perloline after stockpiling tall fescue. Perloline was reported to inhibite cellulose digestion both **in vitro** (Bush et al.,1970 and 1972) and ***in vivo*** (Boling et al, 1975). The endophyte has been shown to produce ergopeptine alkaloids both ***in vitro*** (Porter et al., 1981) and ***in vivo*** (Yates et al., 1985). The influ-

ence of N fertilization on ergopeptine alkaloid accumulation in endophyte-infected tall fescue needs further evaluation.

The influence of endophyte, *Acremonium coenophilium* infection on tall fescue growth and chemical composition needs claricifation. Yield of endophyte-infected plants has been reported to be higher than non-infected plants (Bush et al., 1986; Belesky et al., 1987; Arechavaleta et al, 1989). However, no difference in yield of endophyte-infected and non-infected plants has also been reported (Pedersen, 1982; Hill et al., 1987; Siegel et al., 1987; Bush and Burrus, 1988). Yield of infected tall fescue was higher than non-infected tall fescue when plants were drought-stressed (Read and Camp, 1986). West and coworkers (1987) reported no influence of endophyte infection on yield when moisture was adequate. But in droughty conditions endophyte-infected plants had higher yield as compared to non-infected plants. These researchers attributed the difference to substantially higher ($P < 0.05$) numbers of nematodes on roots of non-infected plants, compared to endophyte-infected plants. Arechavaleta and coworkers (1989) suggested drought tolerance was related to differences in morphology of endophyte-infected plants. Endophyte-infected plants had thicker and more narrow leaf blades and had increased leaf rolling.

Tillering has been reported to be higher in endophyte-infected as compared to non-infected plants fertilized with high rates of N (Belesky et al., 1987; Clay 1987; Hill et al., 1987). However, low N fertiliation application did not influence tillering of endophyte-infected, compared to non-infected plants (Arechavaleta et al., 1989).

Endophyte infection may alter the chemical compostion of tall fescue. In a recent review, Bush and Burrus (1988) reported that no relationship was apparent between endophyte infection and fiber content of forages or seeds. Wilkinson (1987) reported higher K and lower B in endophyte-infected as compared to non-infected tall fescue. Odom and coworkers (1981) reported concentrations of Ca and Mg of endophyte-infected 'Kentucky-31' were numerically but not significantly lower than for non-infected plants. Calcium concentration of endophyte-infected 'Kentucky-31' tall fescue was numerically but not significantly lower as compared to endophtye-free tall fescue (Hoveland et al., 1984a). Micronutrients were not measured.

Yield response of endophyte-infected and non-infected tall fescue to N fertilization may be different (Arechaveleta et al., 1989). The influence of N fertilization on yield and chemical composition of endophyte-infected tall fescue needs to be studied under field conditions.

Few studies have compared the same variety or have compared plants that are 0 and 100% endophyte-infected. Confounding factors such as variety, harvest date, percentage infection, environmental condition, or soil fertility may obscure a direct relationship between chemical composition and endophyte-infection. More controlled studies under field conditions are needed. The effects of endophyte-infection on macro- and micro-mineral concentration in tall fescue needs further research particularly in relation to animal nutritional requirements.

The objectives of this research were 1) to elucidate the influence of growth stage, endophyte infection and N fertilization on dry matter yield and chemical composition of tall fescue swards grown at two locations in Virginia and 2) to relate the nutritional value of high and low endophyte-infected tall fescue to nutrient requirements of steers.

MATERIALS AND METHODS

The influence of N fertilization on high and low endophyte-infected tall fescue was studied at two locations, Glade Spring (Ridge and Valley physiographic region) and Blackstone (Southern Piedmont region), Virginia. The experimental design was a randomized complete block design with split-plot arrangement of treatments with endophyte infection as the main plot and N fertilization as subplots. Endophyte treatments were randomized within the four blocks for a total of eight pastures at each site. Each pasture was 1.5 ha at Glade Spring and 2.4 ha at Blackstone. In each pasture, an area (3 x 6 m) was selected (Aug. 1987) and divided into three equal plots (2 x 3 m). Nitrogen treatments (0, 40 and 80 kg N/ha as ammonium nitrate) were randomly allotted to the three plots within each pasture. Nitrogen treatments were applied at Glade Spring on 13 Aug. 1987,

22 Mar. 1988 and 16 May 1988 and at Blackstone on 20 Aug. 1987, 14 Mar. 1988 and 13 May 1988. Plots were mowed (Gravely tractor[3]) to remove excess and mature forage prior to each N application.

At the Glade Spring location, the existing sod was mowed and sprayed with Glyphosate [N-(phosphonomethyl)glycine] (7 l/ha) on 19 Sept. 1985. A thatch cover was burned on 7 Oct. 1985. Tall fescue was no-till seeded into the burned field on 8 and 9 Oct. 1985. Winter heaving caused a complete loss of the stand. The research area was sprayed with paraquat (1,1′-dimethyl-4,4′-bipyridinium ion as dichloride salt) at 2.3 l/ha on 19 Aug. 1986 and the area was no-till seeded on 22 and 23 Sept. 1986. On 15 July 1987, the entire area was sprayed with 2,4-D [(2,4 Dichlorophenoxy) acetic acid] at 3.5 l/ha to control thistles.

The soils at Glade Spring are deep and well-drained with slopes of 15 to 25% (Danny Hatch, personal communication). In block 1 and 2, the soil was Frederick (silt loam mixed, mesic, Typic Paleudult). The soil in blocks 3 and 4 was Hagerstown (fine, mixed, mesic, Typic Hapludalf).

At Blackstone, the existing sod was sprayed with Glyphosate (7 l/ha) on 13 and 14 Sept. 1985 and with paraquat (2.3 l/ha) on 7 and 8 Oct. 1985. Tall fescue was no-till seeded on 9, 10 and 11 Oct. 1985. A poor stand of the low endophyte-infected fescue was obtained. On 26 Mar. 1986, the low endophyte-infected fescue was reseeded. In Aug. 1986, the low endophyte-infected fescue pastures were mowed and sprayed with paraquat (2.3 l/ha) and the areas were reseeded Sept. 1986.

The soils at the Blackstone site are sandy loams and were formed from granite and granite gneiss (Soil Conservation Service, 1960). In block 1, the soil was Appling (fine sandy loam, mixed, mesic, Typic Hapludult). Block 2 consisted of 45% Appling, 45% Colfax (sandy loam, mixed, mesic, Typic Hapludult), 5% Durham (fine sandy loam, mixed, mesic, Typic Hapludult) and 5% Worsham (sandy loam, mixed, mesic, Typic Hapludult). Ninety percent of block 3 was Cecil (fine sandy loam, mixed, mesic, Typic Hapludult) and 10% was Appling series. Block 4 consisted of 98% Appling series and about 2% Cecil soil series. In the Blackstone area, the Appling series frequently occurs in association with Cecil, Burhan, Colfax and Worsham. The subsoil of the Appling

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[3] Gravely tractor, 500 Series. One Gravely Lane, Clemmons, NC 27012

is more coarse in texture as compared to the Cecil, does not have the compacted zone as found in Durham, and is better drained than the Colfax and Worsham. Worsham is the most poorly-drained of the soils at this site.

Seed was sampled and tested for variety by the method of electrophoresis for protein banding patterns (Livestock and Seed Division, USDA, Beltsville, MD). The variety test was necessary since the seeds for the high and low endophyte-infected fescue were obtained from two different sources. The level of endophyte infection was determined on all plots in June 1988. In each plot, 15 tillers were randomly sampled, wrapped in a wet paper towel, placed in a plastic bag and submitted to the Fescue Diagnostic Center at Auburn for endophyte testing.

Environmental data, temperature and precipation, were provided by The Department of Environmental Science, University of Virginia. Data were obtained from weather stations close to the experimental sites. The Marion station was used to estimate Glade Spring and for Blackstone, the Farmville station was used to estimate temperature and precipation.

Soil samples were taken in November 1987 (before) November 1988 (after) application of N fertilizer. For each soil sample, six cores were taken to a 15-cm depth and composited. Each plot was sampled separately. Soil was analyzed for pH, P, K, Ca and Mg by the VPI and SU Soil Testing Laboratory (Donohue and Freidericks, 1984).

Yield was determined by harvesting the sward at a 3.0-cm height with hand-powered sheep-shears from within a 0.5 m^2 quadrant. A subsample of the fresh forage was taken for chemical composition and was packed in dry-ice for transport to the lab. Forages were sampled: stockpiled (19 Nov. 1987, Glade Spring and 20 Nov. 1987, Blackstone), prebloom (9 May 1988, Glade Spring and 4 May 1988, Blackstone), full bloom, hay-cut stage (16 May 1988, Glade Spring and 13 May 1988, Blackstone), and re-growth after hay-cut (24 June 1988, Glade Spring and 22 June 1988, Blackstone).

Yield and quality samples were dried in a forced-air oven at 60 C. After drying, yield samples were weighed and discarded. Dried samples for quality analysis were ground through a screen (1

mm) in a stainless steel Wiley mill[4]. Minerals (P, K, Ca, Mg, S, Na, Al, B, Cu, Fe, Mn and Zn) were determined with a inductively-coupled plasma optical emission spectrophotometer (ICP-OES) on plant material (0.5 g) digested with nitric and perchloric acid (3:1 ratio) (Muchovej et al., 1986). Total non-structural carbohydrates were hydrolyzed (Smith, 1969) and reported as glucose equivalents (Davies, 1976). Neutral detergent fiber (Van Soest and Wine, 1967 and Goering and Van Soest, 1970), acid detergent fiber (Van Soest, 1963; Goering and Van Soest, 1970), lignin and cellulose (Van Soest and Wine, 1968; Goering and Van Soest , 1970) were determined. Total N was determined colorimetrically as ammonia-salicylate with a Technicon Autoanalyzer[5] (Technicon Industrial Systems, 1976), on plant material (200 mg) digested in a salt/catalyst mixture (94% K_2SO_4, 4% HgO, 1% $CuSO_4$ and 1% pumice) with 2.5 ml H_2SO_4 at 400 C for approximately 40 minutes (McKenzie and Wallace, 1954).

Statistical analysis. Possible influence of growth stage at harvest, site, endophyte level and N fertilization on dry matter yield and chemical composition of tall fescue were examined using analysis of varience (SAS, 1985) for a randomized block design with split-plot arrangement of treatments. The main effect of site was tested by block (nested within site). The main effect of endophyte level and the two-way interaction of site and endophyte level were tested by the interaction of endophyte and block (nested within site). The main effect of N fertilization and two-way interaction of N fertilization and site were tested by the interaction of N fertilization and block (nested within site). The two-way interaction of endophyte level and N fertilization and the three-way interaction of endophyte level and N fertilization and site were tested by the interaction of endophyte level, N fertilization, and block (nested within site). The main effect of growth stage at harvest (date) and the two-way interactions of date and site, date and endophyte level, date and N fertilization and the three-way interactions of date and site with endophyte level and N fertilization were tested by the residual error term. Tukey pairwise comparison procedure was used to separate mean of growth stage at harvest (SAS, 1985).

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[4] Thomas-Wiley mill. Model ED-5 Arthur H. Thomas Company, Philadelphia, PA 19105

[5] Technicon Industrial Systems, Tarrytown, NY 10591

Data were also analyzed with regard to growth stage at harvest. The main effect of site was tested by block (nested within site). The main effect of endophyte level and the two-way interaction of site and endophyte level were tested by the interaction of endophyte and block (nested within site). The main effect of N fertilization and two-way interaction of N fertilization and site were tested by the interaction of N fertilization and block (nested within site). The two-way interaction of endophyte level and N fertilization were tested by the residual error term.

RESULTS AND DISCUSSION

Cultivar and endophyte level. Seeds planted at both sites were of different varieties. The low endophyte-infected tall fescue was 'Kentucky-31' as indicated on the seed label. The high endophtye-infected was not 'Kentucky-31' as indicated on the seed label but was 'Alta' or possibly 'Falcon'. Based on the morphology and growth characteristics, the high endophyte-infected tall fescue was probably 'Alta'. At Glade Spring, the high and low endophyte plots were 73 and 2 % infected, respectively and at Blackstone, the high and low endophyte plots were 81 and 0 % infected, respectively.

Environmental data. Temperature flucations were similar at both locations, but the overall temperature was warmer at Blackstone as compared to Glade Spring (Fig. 1). Precipation was higher at Blackstone as compared to Glade Spring (Fig. 2). Over the 2-yr period, total precipation was 198 cm and 254 cm at Glade Spring and Blackstone, respectively. Precipation was 87 cm and 108 cm for Glade Spring and Blackstone, respectively from Nov. 1987 to Aug. 1988 (the period of time experimental plots were harvested for yield and chemical composition).

The climate in the Piedmont region is continental, having mild winters with short cold periods (Soil Conservation Service, 1960). The majority of the rainfall occurs in the spring and summer. The climate in the Piedmont tends to be warmer than in the Ridge and Valley region. The

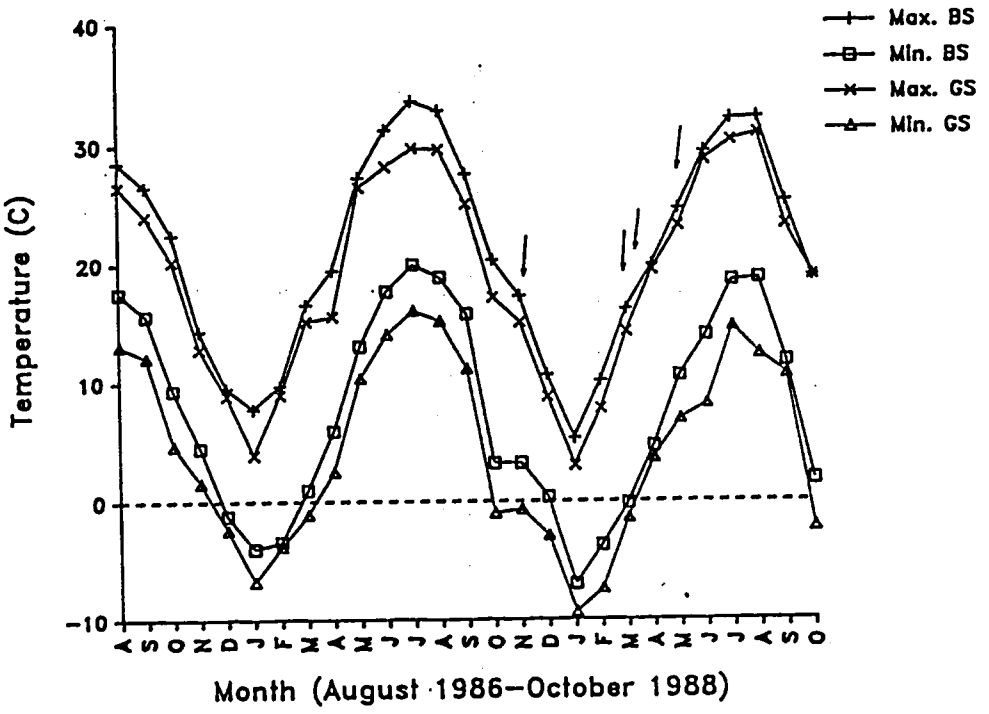

Figure 1. Maximum (max.) and minimum (min.) temperature flucations at Blackstone (BS) and Glade Spring (GS): Arrows indicate sampling dates.

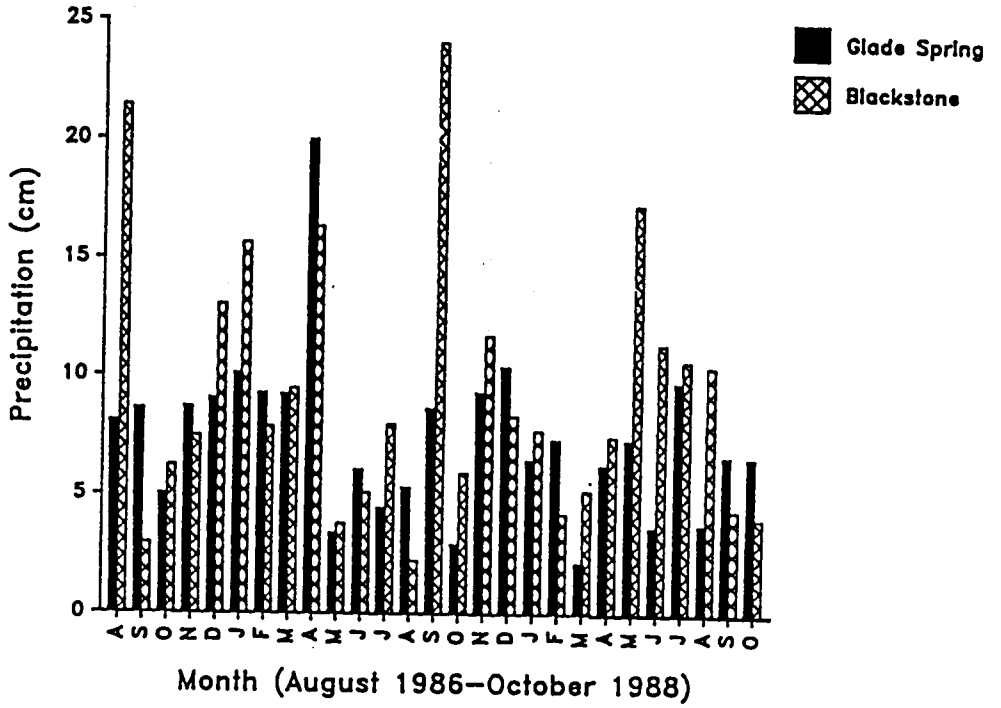

Figure 2. Precipitation (cm) at Blackstone and Glade Spring.

Piedmont province topography is that of gentle slopes which have undefined valleys and hilltops (Buol, 1973).

In general, the Ridge and Valley province topography is determined by the composition and angle at which the bedrock lies (Kroontje et al., 1985). The bedrock in this region is sedimentary. Differential weathering and erosion of the bedrock, resulted in the varied topography in the region. The ridges are the more weather resistant sandstone or conglomerate outcrops and the valleys are the more easily weathered limestones, dolomites and shales. The limestone valley soils in this region are generally deep and well-drained.

Soil composition. Soil fertility was high at both sites (Table 6). The availability of Mg in the soil was higher ($P < 0.01$) at Glade Spring as compared to Blackstone. In 1988, after N fertilization, soil Ca and P availability were increased ($P < 0.01$) as compared to 1987, before N fertilization at Glade Spring. At Blackstone, Ca and K were increased ($P < 0.01$) in 1988 as compared to 1987. Soil pH at Blackstone was increased ($P < 0.01$) by N fertilization.

Yield. Yield of tall fescue was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 7). The highest ($P < 0.01$) yield occurred at the bloom stage (4367.7 kg/ha) and the lowest at the 5-wk regrowth (754.1 kg/ha) after harvest (at bloom). Yield of tall fescue at bloom was higher ($P < 0.01$) than stockpiled, prebloom and 5-wk regrowth yield. The yield of tall fescue at prebloom and stockpiled were not significantly different but were superior to yield at 5-wk regrowth ($P < 0.01$). Two-way interactions of growth stage at harvest with site ($P < 0.01$) and N fertilization ($P < 0.01$) were present. Yield of stockpiled tall fescue was 81% higher ($P < 0.01$) at Blackstone, compared to Glade Spring. Tall fescue yield, harvested at prebloom was 72% higher ($P < 0.05$) at Glade Spring as compared to Blackstone. Nitrogen fertilization increased ($P < 0.01$) the yield of tall fescue at Blackstone and numerically but not significantly increased yield at Glade Spring. At the prebloom and bloom harvests, a linear increase ($P < 0.01$) in yield with increasing N fertilization was measured at both sites. Yield of regrowth (5-wk after cutting at bloom) was also linearly increased ($P < 0.01$) by N fertilization at both sites. At Blackstone, yield of the regrowth of the low endophyte-infected tall fescue was higher ($P < 0.05$) than high endophyte-infected tall fescue.

Table 6. Soil pH and mineral availability before (1987) and after (1988) N fertilization at two sites.

Year	Item	Nitrogen fertilization, kg/ha 0 HE[1]	0 LE[2]	40 HE	40 LE	80 HE	80 LE	SE[3]
		------- Glade Spring ----------						
1987								
	pH	6.3	5.9	6.2	6.2	6.4	6.2	0.2
	Phosphorus[4], mg/kg	12	13	11	11	13	14	1
	Potassium, mg/kg	41	46	47	47	47	50	4
	Calcium[4], mg/kg	765	567	654	651	714	684	39
	Magnesium[5], mg/kg	262	160	251	209	268	224	20
1988								
	pH	6.6	6.8	6.6	6.7	6.3	6.5	0.2
	Phosphorus[4], mg/kg	19	22	19	18	20	15	2
	Potassium, mg/kg	61	55	43	52	40	44	6
	Calcium[4], mg/kg	927	894	945	888	865	810	76
	Magnesium[5], mg/kg	297	265	311	269	269	241	28
		------- Blackstone ----------						
1987								
	pH	6.4	6.4	6.1	6.4	6.1	6.2	0.2
	Phosphorus, mg/kg	18	22	19	22	14	24	3
	Potassium[4], mg/kg	39	41	37	34	39	34	5
	Calcium[4], mg/kg	648	606	540	630	537	603	49
	Magnesium[5], mg/kg	161	120	105	124	106	120	21
1988								
	pH[6]	6.5	6.6	6.5	6.6	6.1	6.0	0.2
	Phosphorus, mg/kg	19	27	25	26	18	27	4
	Potassium[4], mg/kg	50	56	52	40	42	42	8
	Calcium[4], mg/kg	699	780	828	780	639	666	82
	Magnesium[5], mg/kg	122	144	137	128	103	108	13

[1]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[2]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[3]Standard error of mean.
[4]Indicates difference between years, within site ($P < 0.01$).
[5]Indicates difference between sites ($P < 0.01$).
[6]Indicates effect of N fertilization ($P < 0.01$), within year and site.

Table 7. Tall fescue yield as affected by four growth stages, endophyte infection, N fertilization and location.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - kg/ha (dry basis) - - - - - - - - - -						
Stockpiled[5]								
	Glade Spring	1892	1666	2265	2177	2750	2353	302
	Blackstone[6,7]	2257	2666	3087	3321	4131	4421	234
Prebloom[8]								
	Glade Spring[6,7]	2102	3138	3747	3752	4694	3835	429
	Blackstone[6,7]	813	2298	2250	3331	3169	3501	408
Bloom								
	Glade Spring[6,7]	3172	3735	4696	4784	5343	5560	456
	Blackstone[6,7]	1651	3322	4580	4354	5676	5539	500
Regrowth								
	Glade Spring[6,7]	340	569	491	1045	740	1173	103
	Blackstone[6,7,9]	220	375	790	1073	1033	1198	106
Pooled[10]		1556	2221	2738	2979	3455	3447	132

[1]Means of bloom > stockpiled = prebloom > regrowth (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[6]Indicates effect of N fertilization ($P < 0.01$).
[7]Indicates a linear response to N fertilization ($P < 0.01$).
[8]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[9]Indicates effect of endophyte level ($P < 0.05$).
[10]Pooled over site and growth stage. Indicates differences due to growth stage ($P < 0.01$), effect of N fertilization ($P < 0.01$), linear effect of N fertilization ($P < 0.01$), two-way interaction of site x N fertilization ($P < 0.05$), growth stage x site ($P < 0.01$), and growth stage x fertilization ($P < 0.01$).

Little information is available comparing 'Alta' and 'Kentucky-31' in Virginia conditions. In a 3-yr variety trial at the Piedmont Research Station in Orange, Virginia, the yield of 'Alta' was numerically but not significantly higher as compared to 'Kentucky-31' (Jones, 1961). 'Alta' is better adapted to the West and Northwest areas of the United States and 'Kentucky-31' is grown more in the transition zone in the United States (Heath et al., 1985).

Yield of stockpiled tall fescue was higher at Blackstone than at Glade Spring. Blackstone received a greater amount of precipitation and had warmer temperatures during the stockpiling period, prior to sampling. However, yield at prebloom was higher at Glade Spring, even though the precipation was 2 two times higher at Blackstone than at Glade Spring. Yield was increased with N fertilization. These results agree with those of Belesky and coworkers (1984). The influence of endophyte on yield was not consistent. Yield of high endophyte-infected plants was lower as compared to low endophyte-infected plants at the 5-wk regrowth. Others have reported yield of endophyte-infected plants to be higher than non-endophyte infected plants grown in the greenhouse (Belesky et al., 1987; Arecharaleta et al., 1989). However, in field studies, no difference between endophyte-infected and non-infected tall fescue yield was measured (Pederson, 1982; Siegel et al., 1987). Endophyte-infected plants appear to tolerate water-stress conditions better than non-infected plants (Read and Camp, 1986; Arechavaleta et al., 1989). In the present study, the difference in yield at the one harvest may reflect a better adaption of the low endophyte-infected variety, 'Kentucky-31' as compared to the high endophyte-infected variety, 'Alta' to Virginia conditions rather than the endophyte status ***per se***.

Total non-structural carbohydrate. Total non-structural carbohydrate (TNC) concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.05$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 8). Concentration of TNC was highest ($P < 0.01$) in stockpiled tall fescue and lowest in 5-wk regrowth after harvest (at bloom). Concentration of TNC was higher ($P < 0.01$) in prebloom tall fescue as compared to bloom, but lower than stockpiled tall fescue. At prebloom and bloom, TNC concentration was higher ($P < 0.05$) in tall fescue grown at Blackstone, compared to Glade Spring. But, in the regrowth, TNC was higher ($P < 0.05$) in tall fescue grown at Glade Spring as compared to Blackstone. Averaged over site and growth

stage, TNC concentration was higher ($P < 0.01$) in the high as compared to the low endophyte-infected tall fescue. Increasing N fertilization decreased ($P < 0.01$) TNC concentration in tall fescue. Two-way interactions of growth stage at harvest with N fertilization ($P < 0.01$) and endophyte level ($P < 0.01$) were present. Total non-structural carbohydrates were decreased ($P < 0.05$) with increasing N fertilization at both sites at the prebloom harvest and at Blackstone at the bloom harvest. High endophyte-infected tall fescue contained higher ($P < 0.05$) concentration of TNC as compared to low endophyte-infected tall fescue harvested at stockpiled and prebloom growth stages at Blackstone.

Differences measured in TNC between sites reflect differences in yield. Plants utilize TNC reserves for growth (Buckner and Bush, 1979). After harvest, TNC typically decreases as the plant uses the reserve carbohydrates for new vegetative production (Gardner et al., 1985). In the present study, TNC concentration in tall fescue decreased with increasing N fertilization. These results agree with those of Hallock and coworkers (1965 and 1973) and Belesky and coworkers (1984). The decrease was related to higher yields with N fertilization, diluting the TNC concentration. Nitrogen fertilization stimulates plant growth, resulting in utilization of TNC and decreased TNC reserves in the plant (Gardner et al., 1985). Carbohydrates accumulate when environmental conditions are adequate for photosynthesis but not optimal for growth (Gardner et al., 1985). Total non-structural carbohydrate was higher in stockpiled tall fescue and lower in 5-wk regrowth. The influence of endophyte on TNC concentration measured in the present study agree with observations from greenhouse study (see previous section). In the greenhouse study, TNC was numerically but not significantly higher in endophyte-infected, compared to non-infected tall fescue. and the accumulation of TNC was higher ($P < 0.05$) in endophyte-infected as compared to non-infected tall fescue. In the present study, TNC was higher in high endophyte-infected, compared to low endophyte-infected tall fescue. Results from the present study agree with those of Buckner and coworkers (1967) who reported higher TNC in the fall and lower TNC in summer.

Neutral detergent fiber. Neutral detergent fiber (NDF) content of tall fescue was influenced by growth stage at harvest ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 9). Neutral detergent fiber was highest ($P < 0.01$) in tall fescue at the bloom harvest (58.5

Table 8. Tall fescue total non-structural carbohydrate concentration as affected by growth stage, nitrogen and endophyte infection.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/100g (dry basis)						
Stockpiled								
	Glade Spring	25.9	23.3	24.4	22.5	22.5	22.5	0.9
	Blackstone[5]	22.0	18.9	23.0	18.6	22.3	18.0	1.0
Prebloom[6]								
	Glade Spring[7,8]	15.6	13.1	11.7	11.5	10.6	9.7	0.6
	Blackstone[7,8,9]	23.4	21.6	19.6	16.8	16.2	14.5	1.4
Bloom[6]								
	Glade Spring	10.5	10.2	9.6	9.4	13.6	17.2	1.6
	Blackstone[7,8]	18.2	17.2	16.9	14.8	13.3	12.8	1.1
Regrowth[6]								
	Glade Spring	7.8	8.1	8.3	8.2	8.3	8.3	0.2
	Blackstone	6.0	4.2	5.4	6.4	4.9	5.3	1.2
Pooled[9]		16.2	14.6	14.9	13.5	13.9	13.5	0.5

[1]Means of stockpiled > prebloom > bloom > regrowth (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect of endophyte level ($P < 0.05$).
[6]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[7]Indicates effect of N fertilization ($P < 0.05$).
[8]Indicates linear effect N fertilization ($P < 0.05$).
[9]Pooled over site and growth stage. Indicates difference due to date ($P < 0.01$), site ($P < 0.05$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$), and two-way interaction of growth stage x site ($P < 0.01$), growth stage x endophyte level ($P < 0.01$) and growth stage x N fertilization ($P < 0.01$) and site x N fertilization ($P < 0.01$).

g/100g) and lowest in stockpiled tall fescue (46.6 g/100g). Neutral detergent fiber was higher ($P < 0.01$) in prebloom tall fescue than 5-wk regrowth but lower than bloom-cut tall fescue. High and low endophyte-infected tall fescue swards contained 53 and 55 g/100g NDF, respectively. Nitrogen fertilization decreased ($P < 0.01$) NDF in tall fescue. Interactions of site with growth stage at harvest ($P < 0.05$) and N fertilization ($P < 0.01$) were present. Stockpiled tall fescue and 5-wk regrowth at Glade Spring was lower ($P < 0.05$) in NDF as compared to Blackstone. At Blackstone, NDF in the 5-wk regrowth was decreased ($P < 0.01$) linearly by N fertilization. High endophyte-infected tall fescue was lower ($P < 0.05$) in NDF as compared to low endophyte-infected tall fescue in the stockpiled harvest at both locations. At prebloom, NDF of tall fescue grown at Glade Spring was higher ($P < 0.05$), compared to tall fescue grown at Blackstone. Low endophyte-infected tall fescue at Glade Spring, was higher ($P < 0.05$) in NDF as compared to high endophyte-infected tall fescue.

Hemken (1983) reported no difference in NDF of 'G1-307' (endophyte-infected) and 'G1-306' (non-infected) tall fescue. Jackson and coworkers (1984) reported no difference in NDF of endophyte-infected and non-infected 'Kenhy' tall fescue. In a 3-yr study with 'Kenhy' (non-infected), 'Kentucky-31' (36%), 'G1-306' (25%) and 'G1-307' (76% infected), Bond and coworkers (1984) reported no consistent difference in NDF of the varieties. However, in one of the years (1977), NDF of endophyte-free 'Kenhy' was higher ($P < 0.01$) than the other endophyte-infected fescues.

Acid detergent fiber. Acid detergent fiber concentration was influenced by site ($P < 0.01$), growth stage at harvest ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 10). Tall fescue grown at Blackstone was higher ($P < 0.01$) in ADF as compared to Glade Spring. Bloom stage tall fescue contained 34.2 g/100g ADF whereas, stockpiled tall fescue contained only 24.2 g/100g. Concentration of ADF in 5-wk regrowth was lower than in bloom-cut but higher than prebloom harvested tall fescue ($P < 0.01$). Low endophyte-infected tall fescue was higher ($P < 0.01$) in ADF content than high endophyte-infected tall fescue. Increasing N fertilization decreased ADF content in tall fescue. Interactions of growth stage at harvest with site ($P < 0.01$), endophyte level ($P < 0.05$) and N fertilization ($P < 0.01$) were present. Acid detergent fiber was higher ($P < 0.05$) in

Table 9. Tall fescue neutral detergent fiber as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - - g/100g (dry basis) - - - - - - - - - -						
Stockpiled[5]								
	Glade Spring[6]	42.9	44.7	41.8	44.7	42.7	43.2	0.8
	Blackstone[7]	50.2	52.0	47.8	51.4	47.5	50.4	1.0
Prebloom[5]								
	Glade Spring[7]	56.1	57.6	56.6	57.9	54.0	59.5	1.9
	Blackstone	51.6	52.1	50.0	51.5	50.7	51.6	0.7
Bloom								
	Glade Spring	57.4	59.6	60.3	59.8	56.1	59.1	1.4
	Blackstone	57.3	60.8	56.2	57.9	58.6	58.7	1.2
Regrowth[5]								
	Glade Spring	55.2	57.6	53.9	55.2	54.1	55.1	1.4
	Blackstone[8,9]	61.8	62.4	57.9	57.6	54.8	55.6	1.4
Pooled[10]		54.0	55.8	53.1	54.5	52.3	53.8	0.4

[1]Means of bloom > prebloom > regrowth > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[6]Indicates effect of endophyte level ($P < 0.01$).
[7]Indicates effect of endophyte level ($P < 0.05$).
[8]Indicates effect of N fertilization ($P < 0.01$).
[9]Indicates a linear response to N fertilization ($P < 0.01$).
[10]Pooled over site and growth stage. Indicates difference due to date differ ($P < 0.01$), effect of N fertilization ($P < 0.01$), linear effect of N fertilization ($P < 0.01$), two-way interaction of site x N fertilization ($P < 0.05$), growth stage x site ($P < 0.01$).

stockpiled tall fescue grown at Blackstone as compared to Glade Spring. At prebloom ADF was higher ($P < 0.01$) in tall fescue grown at Glade Spring, compared to Blackstone. High endophyte-infected tall fescue was lower ($P < 0.05$) in ADF content than low endophyte-infected tall fescue at prebloom. Tall fescue grown at Blackstone contained higher ($P < 0.05$) ADF in the 5-wk regrowth as compared to tall fescue grown at Glade Spring.

Reeves (1987) reported lowest ADF concentration in tall fescue on 13 May (28.8 g/100g) and the highest on 11 August (40.3 g/100 g). In his study, the tall fescue was cut for hay on 3 June. In the present research, ADF concentration of tall fescue prior to hay-cut (bloom) stage was also lower than the ADF in regrowth after hay-cut.

Low endophyte-infected ADF concentration was higher than high endophyte-infected tall fescue at prebloom at both sites. This may indicate earlier maturation of low as compared to high endophyte-infected tall fescue. Hoveland (1988) reported accelerated maturation rate of endophyte-infected tall fescue. Thus it seems more likely that the difference observed in ADF in the present study was related to cultivar difference. Other researchers have reported no influence of endophyte infection on ADF (Bond et al., 1984; Jackson et al., 1984; and Strahan et al., 1987).

Cellulose. Cellulose content of tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.01$), endophyte level ($P < 0.01$), and N fertilization ($P < 0.01$) (Table 11). Tall fescue harvested at bloom (29.7 g/100g) contained the highest ($P < 0.01$) cellulose and stockpiled tall fescue contained the lowest (21.5 g/100g). Prebloom and 5-wk regrowth tall fescue were similar in cellulose concentration and higher than stockpiled tall fescue ($P < 0.01$). Cellulose concentration in tall fescue grown at Blackstone (26.7 g/100g) was higher ($P < 0.01$) than tall fescue grown at Glade Spring (24.9 g/100g). Low endophyte-infected tall fescue contained higher ($P < 0.01$) cellulose as compared to high endophyte-infected tall fescue. Nitrogen fertilization decreased ($P < 0.01$) cellulose concentration of tall fescue, averaged over growth stage at harvest, site and endophyte level. Interactions of growth stage at harvest with site ($P < 0.01$), endophyte level ($P < 0.05$) and N fertilization ($P < 0.01$) were present. Cellulose was higher ($P < 0.01$) in stockpiled tall fescue grown at Blackstone but lower ($P < 0.01$) at prebloom, compared to tall fescue grown at Glade Spring. In the regrowth, cellulose content of tall fescue grown at Blackstone was higher

Table 10. Tall fescue acid detergent fiber as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha						
		0		40		80		
		HE[2]	LE[3]	HE	LE	HE	LE	SE[4]
		g/100g (dry basis)						
Stockpiled[5]								
	Glade Spring	21.5	22.6	20.2	21.9	20.3	21.0	0.4
	Blackstone	27.7	28.6	26.1	27.7	25.7	27.4	0.5
Prebloom[5]								
	Glade Spring[6]	29.1	31.8	30.0	32.0	29.0	30.3	0.5
	Blackstone[6]	28.9	29.0	27.8	28.7	28.2	27.9	0.3
Bloom								
	Glade Spring	33.1	35.0	33.6	34.6	31.8	35.0	0.9
	Blackstone	33.0	36.0	34.2	34.3	33.4	36.0	1.0
Regrowth[7]								
	Glade Spring	32.3	25.3	28.5	26.0	27.6	26.2	1.1
	Blackstone	34.4	35.1	29.9	28.8	29.3	28.0	1.2
Pooled[8]		30.0	31.3	28.8	29.5	28.2	29.3	0.2

[1]Means of bloom > regrowth > prebloom > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[6]Indicates effect endophyte level ($P < 0.05$).
[7]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[8]Pooled over site and date. Indicates difference due to date ($P < 0.01$), site ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilzation ($P < 0.01$) and two-way interaction of date x site ($P < 0.01$), date x endophyte level ($P < 0.05$) and date x N fertilization ($P < 0.01$).

($P < 0.05$) as compared to tall fescue grown at Glade Spring. Nitrogen fertilization linearly decreased ($P < 0.01$) cellulose in tall fescue at both locations in the 5-wk regrowth after harvesting at the bloom stage.

Hemicellulose. Growth stage at harvest influenced tall fescue hemicellulose concentration ($P < 0.01$) (Table 12). Regrowth contained highest ($P < 0.01$) hemicellulose (26.4 g/100g) and stockpiled tall fescue contained the lowest hemicellulose (22.4 g/100g) concentration. There was no significant difference in hemicellulose concentration between prebloom and bloom. A two-way interaction was present between growth stage at harvest and site ($P < 0.01$). At prebloom, Blackstone was lower ($P < 0.05$) in hemicellulose, compared to Glade Spring.

Lignin. Lignin content of tall fescue was influenced by growth stage at harvest ($P < 0.01$) and endophyte level ($P < 0.01$) (Table 13). Tall fescue harvested at bloom (4.0 g/100g) contained the highest ($P < 0.01$) and stockpiled the lowest (1.9 g/100g) concentration of lignin. There was no significant difference between lignin concentration of bloom and 5-wk regrowth. Low endophyte-infected tall fescue was higher ($P < 0.01$) in lignin as compared to high endophyte-infected tall fescue. Interactions of growth stage with site ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) were present. Lignin content of tall fescue grown at Blackstone was higher ($P < 0.05$) in stockpiled and bloom growth stages but lower ($P < 0.05$) at the prebloom harvest, compared to fescue grown at Glade Spring. At bloom, high endophyte-infected tall fescue was lower ($P < 0.05$) in lignin as compared to high endophyte-infected tall fescue. Nitrogen fertilization increased lignin in tall fescue grown at Blackstone at the bloom harvest. Lignin was lower ($P < 0.05$) in 'G1-307' (high endophyte-infected) as compared to 'G1-306' (low endophyte-infected) tall fescue (Bond et al., 1984). The results of the present study agree with those of Bond and coworkers (1984).

Nitrogen. Nitrogen concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.05$) and N fertilization ($P < 0.01$) (Table 14). Interactions of growth stage at harvest with site ($P < 0.01$) and N fertilization ($P < 0.01$) were present. Tall fescue harvested at bloom (19.1 g/kg) contained the lowest ($P < 0.01$) and 5-wk regrowth tall fescue contained the highest (27.8 g/kg) concentration of N. There was no significant difference between stockpiled and

Table 11. Tall fescue cellulose content as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/100g (dry basis)						
Stockpiled[5]								
	Glade Spring	18.9	19.9	18.0	19.3	18.0	18.8	0.3
	Blackstone	24.8	24.9	23.0	24.9	23.1	24.7	0.7
Prebloom[5]								
	Glade Spring	26.1	28.0	26.8	27.9	25.9	26.9	0.3
	Blackstone	25.8	25.9	24.9	25.7	25.4	25.3	0.2
Bloom								
	Glade Spring	26.8	30.8	30.0	30.3	27.9	30.4	1.6
	Blackstone	29.3	32.4	29.6	29.2	29.4	30.5	0.8
Regrowth[5]								
	Glade Spring[6,7]	26.8	26.4	23.1	24.1	22.3	23.9	0.7
	Blackstone[6,7]	29.4	29.3	26.5	26.8	25.3	24.0	1.0
Pooled[8]		26.0	27.2	25.1	25.9	24.7	25.4	0.2

[1]Means of bloom > prebloom = regrowth > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.01$), averaged over endophyte infection and N fertilization.
[6]Indicates effect N fertilization ($P < 0.01$).
[7]Indicates linear effect N fertilization ($P < 0.01$).
[8]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$), site ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilzation ($P < 0.01$) and two-way interaction of growth stage x site ($P < 0.01$), growth stage x endophyte level ($P < 0.05$), growth stage x N fertilization ($P < 0.01$) and site x endophyte ($P < 0.05$).

Table 12. **Tall fescue hemicellulose content as affected by growth stage, N fertilization, endophyte infection and site.**

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - - g/100g (dry basis) - - - - - - - - - -						
Stockpiled								
	Glade Spring	21.4	22.2	21.6	22.9	22.3	22.3	0.5
	Blackstone[5]	22.5	23.4	21.7	23.6	21.8	23.0	0.5
Prebloom[6]								
	Glade Spring	27.0	25.8	26.6	25.8	25.0	26.2	1.5
	Blackstone	22.7	23.1	22.2	22.8	22.4	23.7	0.6
Bloom								
	Glade Spring	24.4	24.5	26.8	25.2	24.3	24.1	1.4
	Blackstone	24.3	24.8	21.9	23.6	25.2	22.6	1.4
Regrowth								
	Glade Spring	22.8	25.3	25.5	26.0	26.5	26.2	0.7
	Blackstone	27.5	27.2	28.0	28.7	25.5	27.7	0.8
Pooled[7]		24.0	24.5	24.3	24.9	24.1	24.5	0.4

[1]Means of regrowth > prebloom = bloom > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect of endophyte level ($P < 0.01$).
[6]Indicates means of sites differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[7]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$) and two-way interaction of growth stage x site ($P < 0.01$).

Table 13. Tall fescue lignin content as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - - g/100g (dry basis) - - - - - - - - - -						
Stockpiled[5]								
	Glade Spring	1.7	1.8	1.6	1.8	1.7	1.5	0.1
	Blackstone	2.0	2.6	2.4	2.4	2.1	2.4	0.2
Prebloom[5]								
	Glade Spring	2.4	2.9	2.8	3.4	2.7	2.9	0.3
	Blackstone[6]	2.4	2.6	2.5	2.8	2.7	2.8	0.1
Bloom[5]								
	Glade Spring[7]	3.1	3.8	3.3	3.7	3.6	3.9	0.2
	Blackstone[6,8,9]	3.6	4.6	4.2	5.1	4.0	5.4	0.3
Regrowth								
	Glade Spring	4.1	4.2	4.3	3.7	4.4	3.8	0.2
	Blackstone	3.7	4.7	3.3	3.2	3.5	3.4	0.4
Pooled[10]		2.9	3.4	3.0	3.2	3.1	3.3	0.1

[1]Means of prebloom = bloom > regrowth > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[6]Indicates effect of endophyte level ($P < 0.05$).
[7]Indicates effect of endophyte level ($P < 0.01$).
[8]Indicates effect of N fertilization ($P < 0.05$).
[9]Indicates linear effect N fertilization ($P < 0.05$).
[10]Pooled over site and growth stage. Indicates difference due to date differ ($P < 0.01$), endophyte level ($P < 0.01$) and two-way interaction of growth stage x site ($P < 0.01$), growth stage x endophyte level ($P < 0.01$) and growth stage x N fertilization ($P < 0.01$).

prebloom harvested tall fescue N concentration. Nitrogen concentration was higher ($P < 0.05$) in tall fescue grown in Glade Spring (24.2 g/kg) as compared to Blackstone (21.1 g/kg) at all four harvest dates. This affect may reflect a difference in soil available N. Fertilization with N linearly increased ($P < 0.01$) the concentration of N in tall fescue tissue at all four harvest dates and both locations.

The influence of N fertilization on N concentration in tall fescue has been documented by others (Duncan et al., 1969; Hannaway et al., 1982). Endophyte infection did not influence N concentration in tall fescue. In the greenhouse study (see previous section), N concentration was higher ($P < 0.05$) in non-infected, compared to endophyte-infected tall fescue. In other research, crude protein concentration of high and low endophyte-infected tall fescue harvested from the Glade Spring location and ensiled on 21 Oct. 1987 was not significantly different (Zylka, 1989). Bush and Burrus (1988) also reported no effect of endophyte infection on crude protein. Belesky and coworkers (1984) reported no difference in total N concentration in seeds of 'Kenhy' and 'Kentucky-31' that varied in endophyte infection from 11 to 60%.

Phosphorus. Phosphorus concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 15). Tall fescue harvested at 5-wk regrowth was highest ($P < 0.01$) and stockpiled tall fescue was lowest in P concentration. Nitrogen fertilization decreased ($P < 0.01$) P concentration in tall fescue. Interaction of growth stage at harvest with site ($P < 0.01$) and N fertilization ($P < 0.01$) were present. At prebloom and bloom, P concentration was higher ($P < 0.05$) in tall fescue grown at Glade Spring as compared to Blackstone. In 5-wk regrowth however, P concentration was higher ($P < 0.01$) in tall fescue grown at Blackstone, compared to Glade Spring. A two-way interaction of site and N fertilization ($P < 0.01$) was also present. Phosphorus concentration in the 5-wk regrowth at both sites was decreased ($P < 0.01$) linearly ($P < 0.01$) by N fertilization. Low endophyte-infected, stockpiled tall fescue grown at Blackstone contained higher ($P < 0.01$) P as compared to high endophyte-infected tall fescue. High endophyte-infected tall fescue grown at Glade Spring was higher ($P < 0.05$) in P concentration, compared to low endophyte-infected tall fescue at prebloom.

Table 14. Tall fescue nitrogen concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/kg (dry basis)						
Stockpiled[5]								
	Glade Spring[6,7]	19.8	21.3	23.1	23.4	26.2	25.6	0.9
	Blackstone[6,7]	17.0	18.8	20.7	20.1	22.1	23.1	1.4
Prebloom[5]								
	Glade Spring[6,7]	20.1	20.5	22.7	23.0	28.2	28.2	1.2
	Blackstone[6,7]	16.4	18.0	18.6	19.8	22.7	26.6	1.2
Bloom[8]								
	Glade Spring[6,7]	18.2	17.8	20.7	20.7	26.5	23.2	1.2
	Blackstone[6,7]	14.7	13.3	16.6	17.7	20.6	19.2	1.1
Regrowth[5]								
	Glade Spring[6,7]	23.9	23.6	30.2	29.8	32.5	31.8	0.7
	Blackstone[6,7]	20.3	21.6	26.8	26.3	33.0	33.5	1.1
Pooled[9]		18.8	19.4	22.4	22.6	26.5	26.4	0.4

[1]Means of regrowth > stockpiled = prebloom > bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Indicates effect of N fertilization ($P < 0.01$).
[7]Indicates linear effect N fertilization ($P < 0.01$).
[8]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[9]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$), site ($P < 0.05$), and N fertilization ($P < 0.01$), and two-way interactions of growth stage x site ($P < 0.01$) and growth stage x N fertilization ($P < 0.01$).

Table 15. Tall fescue phosphorus concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/kg (dry basis)						
Stockpiled								
	Glade Spring	1.8	1.8	1.9	1.8	1.9	1.9	0.1
	Blackstone[5]	1.9	2.0	1.8	2.0	1.7	2.1	0.1
Prebloom[6]								
	Glade Spring[7]	2.9	2.5	2.8	2.5	2.9	2.6	0.1
	Blackstone	2.4	2.4	2.4	2.3	1.9	2.3	0.2
Bloom[8]								
	Glade Spring	2.4	2.5	2.5	2.6	2.7	2.4	0.1
	Blackstone	2.4	2.2	1.8	2.0	1.8	2.2	0.2
Regrowth[5]								
	Glade Spring[6,7]	3.4	3.1	2.3	2.3	2.3	2.0	0.2
	Blackstone[9,10]	4.5	4.5	2.5	2.8	2.3	2.4	0.2
Pooled[11]		2.7	2.6	2.2	2.3	2.2	2.2	0.0

[1]Means of regrowth > prebloom > bloom > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect of endophyte level ($P < 0.01$).
[6]Indicates means of sites differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[7]Indicates effect of endophyte level ($P < 0.05$).
[8]Indicates means of sites differ ($P < 0.01$), pooled over endophyte level and N fertilization.
[9]Indicates effect of N fertilization ($P < 0.01$).
[10]Indicates linear effect N fertilization ($P < 0.01$).
[11]Pooled over site and growth stage. Indicates difference due to date ($P < 0.01$) and N fertilization ($P < 0.01$), and two-way interaction of growth stage x site ($P < 0.01$), growth stage x N fertilization ($P < 0.01$) and site x N fertilization ($P < 0.01$).

Results from the present study support data reported by Belesky and coworkers (1984) that N fertilization decreased P in tall fescue. No consistent effect of endophyte infection on tall fescue P concentration was measured. In the greenhouse study (see previous section), endophyte infection did not influence P concentration in tall fescue. Zylka (1989) also did not report any influence of endophyte on P concentration of tall fescue silage. Hoveland and coworkers (1984b) reported no influence of endophyte infection on P concentration in tall fescue.

Potassium. Potassium concentration in tall fescue was not significantly influenced by growth stage at harvest, site, N fertilization or endophyte level (Table 16). Results reported from the present study do not support the findings of Reid and Jung (1965) that N fertilization increased K concentration in tall fescue. The results of the present study also do not agree with Wilkinson (1987) that endophyte infection increased K concentration in tall fescue. In the greenhouse study (see previous section), K concentration was higher ($P < 0.05$) in endophyte-infected, compared to non-infected, composited tall fescue. Results of the present study agree with Odom and coworkers (1981) and Zylka (1989) that endophyte infection did not influence K concentration in tall fescue.

Calcium. Calcium concentration was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 17). The 5-wk regrowth of tall fescue had the highest ($P < 0.01$) concentration of Ca (6.0 g/100g) and the lowest occurred at bloom (2.9 g/100g). Concentration of Ca was not significantly different between prebloom and bloom harvested tall fescue. Nitrogen fertilization increased ($P < 0.01$) Ca concentration in tall fescue. A two-way interaction of growth stage at harvest and N fertilization ($P < 0.01$) was present. Calcium concentration was increased ($P < 0.01$) by N fertilization at Glade Spring at prebloom, bloom and 5-wk regrowth but, only increased ($P < 0.05$) Ca concentration in the 5-wk regrowth at Blackstone.

The influence of N fertilization on increasing Ca concentration in the present study agree with those of Hannaway et al. (1982) and Belesky et al. (1984). Endophyte infection did not significantly influence Ca concentration in tall fescue. Odom et al. (1981) and Hoveland et al. (1984) also reported no signficant affect of endophyte infection on Ca concentration in tall fescue. In the greenhouse study (see previous section), Ca concentration was lower ($P < 0.05$) in endophyte-infected, compared to non-infected tall fescue.

Table 16. Tall fescue potassium concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/kg (dry basis)						
Stockpiled								
	Glade Spring	20.8	18.6	19.2	19.2	22.2	18.8	1.2
	Blackstone	24.9	28.3	20.3	27.1	24.2	21.8	0.1
Prebloom								
	Glade Spring	23.6	23.4	25.2	23.3	24.6	23.6	2.2
	Blackstone	19.5	22.9	19.4	22.5	22.3	27.8	1.0
Bloom								
	Glade Spring	23.9	21.9	24.2	25.4	25.2	23.4	2.0
	Blackstone	19.2	21.2	21.0	22.7	19.9	21.8	1.4
Regrowth								
	Glade Spring	20.8	19.3	18.8	21.1	19.6	16.9	1.9
	Blackstone	20.7	23.4	25.4	24.8	22.5	24.1	2.1
Pooled[5]		21.6	22.4	21.7	23.3	22.6	22.3	0.6

[1]Means of stockpiled, prebloom, bloom and regrowth not different by Tukey pairwise comparison.
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Data pooled over site and growth stage.

Table 17. Tall fescue calcium concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha						
		0		40		80		
		HE[2]	LE[3]	HE	LE	HE	LE	SE[4]
		g/kg (dry basis)						
Stockpiled								
	Glade Spring	4.2	4.2	4.6	4.3	4.4	4.4	0.1
	Blackstone	4.3	3.4	5.0	3.7	4.3	3.7	0.2
Prebloom								
	Glade Spring[5,6]	3.1	2.8	3.0	3.0	3.7	3.5	0.1
	Blackstone	3.0	2.8	3.2	2.9	3.4	3.1	0.2
Bloom								
	Glade Spring[5,6]	2.5	2.5	3.1	3.0	3.7	3.2	0.3
	Blackstone	2.8	2.2	2.8	2.8	3.2	2.9	0.2
Regrowth								
	Glade Spring[6,7]	5.6	5.5	6.4	6.1	7.2	6.4	0.3
	Blackstone[6,7]	5.6	5.2	6.1	5.5	6.6	5.8	0.4
Pooled[8]		3.9	3.6	4.3	3.9	4.6	4.1	0.1

[1]Means of regrowth > stockpiled > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect N fertilization ($P < 0.01$).
[6]Indicates linear effect N fertilization ($P < 0.01$).
[7]Indicates effect of N fertilization ($P < 0.05$).
[8]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$) and N fertilization ($P < 0.01$) and endophyte level ($P < 0.06$) and two-way interaction of growth stage x N fertilization ($P < 0.01$).

Magnesium. Magnesium concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.05$) and N fertilization ($P < 0.01$) (Table 18). The highest ($P < 0.01$) concentration of Mg occurred in the 5-wk regrowth (5.2 g/kg) and the lowest (2.5 g/kg) concentration occurred in the tall fescue harvested at the bloom stage. Magnesium concentration was not significantly different between prebloom and bloom harvested tall fescue. Concentration of Mg in tall fescue harvested from Glade Spring (3.7 g/kg) was higher ($P < 0.05$) as compared to Blackstone (3.4 g/kg). Nitrogen fertilization increased ($P < 0.01$) Mg concentration in tall fescue. A two-way interaction of growth stage at harvest and N fertilization ($P < 0.05$) was present. Concentration of Mg in tall fescue harvested from Glade Spring was higher ($P < 0.05$), compared to Blackstone. Stockpiled and bloom harvested tall fescue contained higher ($P < 0.05$) concentration of Mg at Glade Spring as compared to Blackstone, averaged over growth stage. Nitrogen fertilization increased ($P < 0.01$) Mg concentration in tall fescue at both sites in prebloom, bloom and 5-wk regrowth harvests.

The difference in Mg concentration in tall fescue due to site effects were probably related to soil Mg. Availability of soil Mg was higher at Glade Spring, compared to Blackstone. Nitrogen fertilization increased Mg concentration in tall fescue. Belesky et al. (1984) also reported increased Mg concentration in tall fescue with N fertilization. In the greenhouse study (see previous section), endophyte-infected tall fescue plants contained lower ($P < 0.01$) concentration of Mg, compared to non-infected tall fescue plants. In the present study, endophyte infection had no effect on Mg concentration in tall fescue plants.

Sulfur. Sulfur concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 19). The highest ($P < 0.01$) concentration of S in tall fescue occurred at 5-wk regrowth (3.1 g/kg) and the lowest occurred at bloom (1.6 g/kg). Sulfur concentration was not significantly different at stockpiled and prebloom harvests. A two-way interaction of growth stage at harvest with site ($P < 0.01$) and N fertilization ($P < 0.01$) was present. At bloom, S concentration in tall fescue grown at Glade Spring was higher ($P < 0.05$), compared to Blackstone. At 5-wk regrowth after harvest, S concentration in tall fescue grown at Blackstone was higher ($P < 0.05$) as compared to Glade Spring. Nitrogen fertilization increased ($P < 0.05$) S concentration

Table 18. **Tall fescue magnesium concentration as affected by growth stage, N fertilization, endophyte infection and site.**

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - g/kg (dry basis) - - - - - - - - -						
Stockpiled[5]								
	Glade Spring	3.6	3.6	4.4	3.8	4.1	4.4	0.3
	Blackstone	3.2	3.0	3.8	3.3	3.7	3.5	0.3
Prebloom								
	Glade Spring[6,7]	2.6	2.2	2.7	2.6	3.1	3.3	0.2
	Blackstone[7,8]	2.1	2.3	2.4	2.6	2.9	3.2	0.1
Bloom[9]								
	Glade Spring[6,7]	2.1	2.3	2.8	2.8	3.4	3.1	0.2
	Blackstone[7,8]	2.0	2.0	2.1	2.5	2.7	2.9	0.1
Regrowth								
	Glade Spring[6,7]	4.8	4.5	5.9	5.5	5.9	5.5	0.3
	Blackstone[6,7]	4.6	4.8	5.1	5.3	5.9	4.9	0.3
Pooled[10]		3.1	3.1	3.6	3.5	4.0	3.8	0.1

[1]Means of regrowth > stockpiled > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Indicates effect N fertilization ($P < 0.01$).
[7]Indicates linear effect N fertilization ($P < 0.01$).
[8]Indicates effect of N fertilization ($P < 0.05$).
[9]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[10]Pooled over site and growth stage. Indicates difference due to date ($P < 0.01$), site ($P < 0.05$), and N fertilization ($P < 0.01$), and two-way interaction of growth stage x N fertilization ($P < 0.05$).

at both locations at prebloom, bloom and 5-wk regrowth. Results of the greenhouse study (see previous section) agree with the present study that endophyte infection had no effect on S concentration in tall fescue.

Aluminium. Aluminium concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.01$), and N fertilization ($P < 0.05$) (Table 20). Averaged over site, N fertilization, and endophyte level, Al concentration was 79.2 and 70.3 mg/kg in 5-wk regrowth and stockpiled tall fescue, respectively and 37.1 and 27.4 mg/kg in prebloom and bloom tall fescue, respectively. Aluminium concentration was not significantly different between stockpiled and 5-wk regrowth or between prebloom and bloom harvested tall fescue. Averaged over growth stage, Al concentration was 43% higher ($P < 0.01$) at Glade Spring (75.0 mg/kg) as compared to Blackstone (32.0 mg/kg). A two-way interaction of growth stage at harvest and site ($P < 0.01$) was present. Aluminium concentration in tall fescue grown at Glade Spring was higher ($P < 0.05$) as compared to tall fescue grown at Blackstone at all four harvest dates. Nitrogen fertilization linearly ($P < 0.01$) increased Al concentration in tall fescue grown at Glade Spring at the bloom harvest. Aluminium may interfere with phosphate metabolism by the formation of stable Al-phosphate complexes (Mengel and Kirkby, 1987). In the present study, Al concentration was numerically but not significantly higher in low, compared to high endophyte-infected tall fescue. Results of the present study agree with the greenhouse study (see previous section), that non-infected tall fescue contained higher concentration of Al, compared to endophyte-infected tall fescue.

Boron. Boron concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 21). The highest ($P < 0.01$) B concentration occurred in tall fescue harvested at 5-wk regrowth (8.5 mg/kg) and the lowest at bloom (5.1 mg/kg). Boron concentration was not significantly different between stockpiled and prebloom harvested tall fescue. An interaction of growth stage at harvest and site ($P < 0.01$) was present. Boron concentration was higher ($P < 0.05$) in tall fescue harvested at Blackstone, compared to Glade Spring at the prebloom and 5-wk regrowth harvests. At Glade Spring, low endophyte-infected tall fescue was higher ($P < 0.05$) in B concentration as compared to high endophyte-infected tall fescue at the bloom stage. This effect was not significant at other harvest dates, but B concentration in low

Table 19. Tall fescue sulfur concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		g/kg (dry basis)						
Stockpiled								
	Glade Spring	1.8	2.0	1.9	2.1	2.1	2.1	0.1
	Blackstone	2.0	2.1	1.9	1.9	2.1	2.1	0.1
Prebloom								
	Glade Spring[5,6]	1.8	1.8	1.9	1.9	2.1	2.2	0.1
	Blackstone[7,8]	1.8	1.8	1.7	1.9	2.0	2.2	0.1
Bloom[9]								
	Glade Spring[7,8]	1.5	1.6	1.8	1.8	1.9	1.8	0.1
	Blackstone[5,6]	1.5	1.3	1.5	1.6	1.7	1.7	0.1
Regrowth[9]								
	Glade Spring[7,8]	3.3	3.5	2.6	2.7	2.5	2.3	0.1
	Blackstone[7,8]	4.2	4.1	3.1	3.2	2.8	2.8	0.1
Pooled[9]		2.3	2.3	2.1	2.1	2.1	2.2	0.1

[1]Means of regrowth > bloom > stockpiled = prebloom (Tukey pairwise co mparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect N fertilization ($P < 0.05$).
[7]Indicates effect N fertilization ($P < 0.01$).
[6]Indicates linear effect N fertilization ($P < 0.05$).
[8]Indicates linear effect N fertilization ($P < 0.01$).
[9]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[10]Pooled over site and growth stage. Indicates difference due to date ($P < 0.01$) and N fertilzation ($P < 0.01$) and two-way interactions of growth stage x site ($P < 0.01$) and growth stage x N fertilization ($P < 0.01$).

Table 20. Tall fescue aluminium concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - mg/kg (dry basis) - - - - - - - - - -						
Stockpiled[5]								
	Glade Spring	81.3	96.3	80.9	98.6	91.7	105.2	17.9
	Blackstone	56.7	72.1	39.8	42.1	41.3	37.9	6.8
Prebloom[6]								
	Glade Spring	52.7	42.4	42.8	41.2	48.5	57.8	6.4
	Blackstone	39.6	28.8	26.5	24.6	21.7	24.3	7.2
Bloom[5]								
	Glade Spring[7,8]	27.7	37.1	34.1	40.1	45.8	40.9	5.3
	Blackstone	27.7	14.5	13.3	16.3	12.6	18.6	7.3
Regrowth[6]								
	Glade Spring	111.9	209.3	103.0	86.9	95.5	134.3	40.0
	Blackstone	33.9	37.7	34.7	39.9	25.0	38.4	7.1
Pooled[9]		54.0	67.3	46.9	48.7	47.7	57.2	6.1

[1] Means of stockpiled = regrowth > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2] HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3] LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4] Standard error of mean.
[5] Indicates means averaged over site differ ($P < 0.01$).
[6] Indicates means averaged over site differ ($P < 0.05$).
[7] Indicates effect N fertilization ($P < 0.05$).
[8] Indicates linear effect N fertilization ($P < 0.01$).
[9] Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$), site ($P < 0.01$) and N fertilization ($P < 0.05$) and two-way interaction of growth stage x site ($P < 0.01$).

endophyte-infected tall fescue was numerically higher, compared to high endophtye-infected tall fescue in regrowth at both sites and prebloom harvest at Blackstone. The lower B concentration observed in high endophyte-infected agrees with Wilkinson (1987) who reported lower B concentration in endophyte-infected plants as compared to non-infected plants.

Copper. Copper concentration in tall fescue was influenced by growth stage at harvest ($P < 0.05$) and N fertilization ($P < 0.01$) (Table 22). The highest ($P < 0.05$) concentration of Cu in tall fescue occurred in the 5-wk regrowth (5.5 mg/kg) and the lowest at the bloom harvest (3.9 mg/kg). There were no differences between prebloom and bloom harvested tall fescue Cu concentration. Nitrogen fertilization linearly increased ($P < 0.01$) Cu in tall fescue. An interaction of growth stage at harvest and site ($P < 0.01$) was present. Copper concentration was higher ($P < 0.05$) in stockpiled and prebloom harvested tall fescue grown at Glade Spring, compared to tall fescue grown at Blackstone. Nitrogen linearly increased ($P < 0.05$) Cu concentration in tall fescue at both locations in stockpiled, prebloom and 5-wk regrowth harvests and at Glade Spring at bloom harvest. Copper concentration in stockpiled tall fescue was higher ($P < 0.05$) in low endophyte-infected than high endophyte-infected tall fescue grown at Glade Spring, but not at Blackstone. Results of the present study agree with the greenhouse study (see previous section) that non-infected tall fescue contained higher ($P < 0.01$) concentration of Cu, compared to endophyte-infected tall fescue.

Iron. Iron concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.01$) and endophyte level ($P < 0.05$) (Table 23). The 5-wk regrowth (100.9 mg/kg) harvested tall fescue contained the highest ($P < 0.01$) and tall fescue harvested at bloom (52.9 mg/kg) contained the lowest concentration of Fe. Iron concentration was not significantly different between prebloom and bloom harvests. Iron concentration in tall fescue grown at Glade Spring (93.2 mg/kg) was higher ($P < 0.01$) than tall fescue grown at Blackstone (56.1 mg/kg). Low endophyte-infected tall fescue contained higher ($P < 0.05$) concentration of Fe, compared to high endophyte-infected tall fescue. An interaction between growth stage at harvest and site ($P < 0.01$) was present. Tall fescue grown at Glade Spring (93.2 mg/kg) was higher ($P < 0.05$) in Fe, compared to tall fescue grown at Blackstone (56.1 mg/kg) at all four harvests. The Fe concentration in tall fescue grown at Glade Spring was nearly two times higher than at Blackstone in stockpiled (101 vs. 61 mg/kg)

Table 21. Tall fescue boron concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		mg/kg (dry basis)						
Stockpiled								
	Glade Spring	6.6	7.0	7.2	7.1	6.8	7.1	0.3
	Blackstone	7.0	6.8	6.7	7.1	7.4	7.0	0.3
Prebloom[5]								
	Glade Spring	5.9	5.9	6.4	5.6	5.8	5.9	0.5
	Blackstone	6.7	7.0	7.2	7.8	6.7	8.2	0.6
Bloom								
	Glade Spring[6]	4.5	4.9	5.3	6.8	4.9	5.5	0.4
	Blackstone	4.6	4.1	5.4	5.1	4.5	4.9	0.3
Regrowth[5]								
	Glade Spring	7.5	7.7	7.6	8.2	7.7	7.8	0.2
	Blackstone	8.6	9.5	9.1	10.4	8.9	9.2	0.4
Pooled[7]		6.4	6.6	6.9	7.3	6.6	6.9	0.1

[1]Means of regrowth > stockpiled = prebloom > bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blacks tone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Indicates effect of endophyte level ($P < 0.05$).
[7]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$) and N fertilization ($P < 0.01$) and two-way interaction of growth stage x site ($P < 0.01$).

Table 22. Tall fescue copper concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha: 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		mg/kg (dry basis)						
Stockpiled[5]								
	Glade Spring[6,7,8]	4.4	4.9	5.0	5.4	5.3	6.1	0.1
	Blackstone[9,10]	4.4	4.2	4.3	4.4	4.7	4.8	0.2
Prebloom[5]								
	Glade Spring[9,10]	4.0	4.2	4.3	4.7	4.9	5.6	0.3
	Blackstone[7,9]	3.1	3.3	3.8	3.5	4.4	4.2	0.2
Bloom								
	Glade Spring[6,7]	3.5	3.9	3.8	4.7	4.4	5.3	0.3
	Blackstone	2.7	2.8	3.2	4.2	4.1	3.9	0.5
Regrowth								
	Glade Spring[9,10]	5.1	5.4	5.7	6.0	5.5	6.0	0.2
	Blackstone[6,7]	4.5	5.3	4.7	5.8	6.0	5.4	0.5
Pooled[11]		3.9	4.3	4.5	4.8	4.9	5.2	0.1

[1]Means of regrowth > stockpiled > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of site differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[6]Indicates effect of N fertilization ($P < 0.01$).
[7]Indicates linear effect of N fertilization ($P < 0.05$).
[8]Indicates effect endophyte level ($P < 0.05$).
[9]Indicates effect of N fertilization ($P < 0.05$).
[10]Indicates linear effect of N fertilization ($P < 0.05$).
[11]Data pooled over site and growth stage. Indicates difference of means averaged over growth stage ($P < 0.05$) and N fertilization ($P < 0.01$) and a two-way interaction of growth stage x site ($P < 0.01$).

and regrowth (135 vs. 66 mg/kg) but at prebloom (72 vs. 54 mg/kg) and bloom (63 vs. 42 mg/kg), the Fe concentration in tall fescue grown at Glade Spring and Blackstone were similar. Nitrogen linearly ($P < 0.01$) increased Fe in tall fescue grown at Glade Spring at prebloom and bloom harvests.

Iron is a constituent of electron transport enzymes active in photosynthesis (Garner et al., 1985). Iron also funtions as a catalyst for polymerization of phenols to lignin. Deficiency of Fe could inhibite cell wall formation and lignification and allow accumulation of phenolic compounds (Mengel and Kirkby, 1987). In the greenhouse study (see previous section), Fe concentration was numerically but not significantly higher in non-infected, compared to endophte-infected tall fescue.

Manganese. Manganese concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$) (Table 24). The 5-wk regrowth (116.8 mg/kg) was highest ($P < 0.01$) in Mn concentration and lowest in the bloom harvest (59.4). Manganese concentration was not significantly different between prebloom and bloom harvests of tall fescue. An interaction between growth stage at harvest and site ($P < 0.01$) was present. The 5-wk regrowth of tall fescue grown at Glade Spring was higher ($P < 0.05$) in Mn concentration as compared to tall fescue grown at Blackstone. In the greenhouse study (see previous section), Mn concentration was higher ($P < 0.01$) in non-infected, compared to endophyte-infected tall fescue. In the present study, endophtye infection did not influence Mn concentration in tall fescue.

Sodium. Sodium concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$), site ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 25). Stockpiled (456.8 mg/kg) tall fescue contained the highest ($P < 0.01$) and prebloom (226.4 mg/kg) harvested the lowest but was not significantly different from bloom and 5-wk regrowth tall fescue Na concentration. Tall fescue grown at Glade Spring (403.1 mg/kg) was two-fold higher ($P < 0.01$) in Na concentration, compared to tall fescue grown at Blackstone (203.7 mg/kg). High endophyte-infected (170.2 mg/kg) tall fescue was lower ($P < 0.01$) in Na concentration as compared to low endophyte-infected tall fescue (436.6 mg/kg). Nitrogen fertilization increased ($P < 0.01$) Na concentration in tall fescue. Two-way interactions of endophyte level with growth stage at harvest ($P < 0.05$) and site ($P < 0.01$) were present. Sodium concentration was higher ($P < 0.05$) in low

Table 23. Tall fescue iron concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		mg/kg (dry basis)						
Stockpiled[5]								
	Glade Spring	88.5	100.0	95.2	106.9	102.8	114.6	12.3
	Blackstone	58.6	75.1	55.5	60.7	58.6	60.8	5.0
Prebloom[6]								
	Glade Spring[7,8]	71.3	67.6	66.9	67.8	81.2	85.6	4.5
	Blackstone	53.9	49.9	48.5	54.0	53.5	64.9	5.9
Bloom[5]								
	Glade Spring[8,9]	51.5	60.3	59.3	66.6	72.9	69.1	4.9
	Blackstone	40.3	38.0	39.1	45.0	43.5	49.6	3.4
Regrowth[5]								
	Glade Spring	128.9	203.6	116.6	110.8	114.4	140.1	22.1
	Blackstone	59.2	60.9	66.0	68.0	65.9	77.1	4.0
Pooled[10]		68.9	81.9	68.4	72.5	74.1	82.7	4.5

[1]Means of regrowth > stockpiled > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.01$), pooled over endophyte level and N fertilization.
[6]Indicates means of sites differ ($P < 0.05$), pooled over endophyte level and N fertilization.
[7]Indicates effect N fertilization ($P < 0.05$).
[8]Indicates linear effect N fertilization ($P < 0.01$).
[9]Indicates effect N fertilization ($P < 0.01$).
[10]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$), site ($P < 0.01$), and endophyte level ($P < 0.05$) and two-way interaction of growth stage x site ($P < 0.01$).

Table 24. Tall fescue manganese as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - mg/kg (dry basis) - - - - - - - - - -						
Stockpiled								
	Glade Spring	134.2	124.4	137.1	117.5	119.5	136.6	13.4
	Blackstone	68.4	59.2	74.9	49.2	67.1	61.2	9.4
Prebloom								
	Glade Spring	62.4	68.4	66.9	67.1	66.2	77.8	7.4
	Blackstone	52.3	48.3	62.9	36.1	54.4	77.7	17.7
Bloom								
	Glade Spring	63.4	64.4	65.2	74.3	62.3	75.4	4.9
	Blackstone	65.8	30.8	49.0	29.5	66.5	66.0	12.2
Regrowth[5]								
	Glade Spring	164.2	153.7	144.0	157.8	124.2	168.1	19.1
	Blackstone	77.5	83.2	88.6	63.5	82.7	93.5	13.5
Pooled[6]		86.8	79.1	86.1	74.4	80.4	94.5	6.4

[1]Means of regrowth > stockpiled > prebloom = bloom (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Pooled over site and growth stage. Indicates differences due to growth stage ($P < 0.01$) and two-way interaction growth stage x site ($P < 0.01$).

endophyte-infected tall fescue as compared to high endophyte-infected tall fescue grown at Glade Spring in all four harvests. Results of the present study agree with the greenhouse study (see previous section) that non-infected tall fescue contained higher concentration of Na, compared to endophyte-infected tall fescue. Nitrogen fertilization linearly ($P < 0.05$) increased Na in tall fescue grown at Glade Spring in the prebloom and 5-wk regrowth harvests.

Zinc. Zinc concentration in tall fescue was influenced by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 26). Tall fescue harvested at prebloom (17.9 mg/kg) was highest ($P < 0.01$) and stockpiled (14.4 mg/kg) was the lowest but was not significantly different from prebloom or bloom harvested tall fescue in Zn concentration. Nitrogen fertilization increased ($P < 0.01$) Zn concentration in tall fescue. Nitrogen fertilization linearly ($P < 0.01$) increased Zn concentration in tall fescue grown at Glade Spring (stockpiled) and Blackstone (prebloom and 5-wk regrowth). Interactions of site with endophyte level ($P < 0.05$) and growth stage at harvest ($P < 0.01$) were present. Low endophyte-infected tall fescue was higher ($P < 0.05$) in Zn concentration as compared to high endophyte-infected tall fescue grown at Blackstone and harvested at prebloom and 5-wk regrowth after harvest (at bloom). In the greenhouse study (see previous section), Zn concentration was higher ($P < 0.01$) in non-infected, compared to endophyte-infected tall fescue.

Nutritional value of stockpiled and bloom-cut tall fescue for steers. Composition of high and low endophyte-infected, stockpiled and bloom-cut tall fescue were similar for most minerals (Table 27). Comparing the dietary requirements (NRC, 1984) of a 227 kg steer gaining 0.45 kg/day to mineral composition of high and low endophyte-infected, stockpiled tall fescue, indicates potential mineral imbalances in grazing steers. Nitrogen concentration in stockpiled tall fescue was 25% higher than the requirement. Bloom-cut tall fescue N concentration was lower than stockpiled but was also above the dietary requirement for steers. Phosphorus concentration in stockpiled tall fescue was similar to dietary requirements for steers. In bloom-cut tall fescue, P was slightly higher than required by steers. Potassium concentration in stockpiled and bloom-cut tall fescue was approximately 4 times higher than steer requirements. High dietary K interfers with Mg absorption and can lead to hypomagnesemic tetany (Fontenot et al., 1973). Calcium concentration in stock-

Table 25. Tall fescue sodium concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		- - - - - - mg/kg (dry basis) - - - - - - - - - -						
Stockpiled[5]								
	Glade Spring[6]	152.4	748.0	286.1	789.3	270.9	1073.8	105.8
	Blackstone	229.4	180.4	319.1	456.8	392.8	532.9	147.8
Prebloom[5]								
	Glade Spring[7,8,9]	118.4	302.0	153.2	461.6	188.0	611.8	127.7
	Blackstone	76.5	111.3	105.2	186.4	159.6	246.2	37.4
Bloom[10]								
	Glade Spring[7]	106.2	506.4	167.3	638.5	198.5	721.0	155.7
	Blackstone	162.7	124.4	89.7	206.1	126.4	252.8	65.2
Regrowth[10]								
	Glade Spring[6,8,9]	73.5	296.5	216.4	602.1	174.7	770.0	131.9
	Blackstone	58.3	80.7	81.3	285.2	181.5	243.2	67.6
Pooled[11]		122.3	300.0	177.3	453.3	211.6	556.5	32.7

[1]Means of stockpiled > prebloom = bloom = regrowth (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates means of sites differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Indicates effect of endophyte level ($P < 0.01$).
[7]Indicates effect of endophyte level ($P < 0.05$).
[8]Indicates effect of N fertilization ($P < 0.05$).
[9]Indicates linear effect of N fertilization ($P < 0.05$).
[10]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[11]Data pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$), site ($P < 0.01$), endophyte level ($P < 0.01$) and N fertilization ($P < 0.01$) and two-way interactions growth stage x endophyte level ($P < 0.05$) and site x endophyte level ($P < 0.01$).

Table 26. Tall fescue zinc concentration as affected by growth stage, N fertilization, endophyte infection and site.

Growth stage[1]	Site	Nitrogen fertilization, kg/ha 0 HE[2]	0 LE[3]	40 HE	40 LE	80 HE	80 LE	SE[4]
		mg/kg (dry basis)						
Stockpiled								
	Glade Spring[5,6]	13.9	13.4	15.3	14.4	15.0	16.2	0.8
	Blackstone	13.2	14.2	12.7	14.4	14.8	15.8	1.0
Prebloom[7]								
	Glade Spring	19.7	19.7	19.3	18.9	20.9	21.4	0.9
	Blackstone[5,8]	12.5	14.5	14.3	16.9	16.6	20.2	0.5
Bloom								
	Glade Spring	14.4	17.0	14.9	18.0	28.1	19.1	4.8
	Blackstone	14.4	14.1	14.3	16.8	17.7	19.8	1.2
Regrowth								
	Glade Spring	17.1	15.8	16.7	16.9	17.0	17.3	0.7
	Blackstone[5,9]	15.1	15.8	16.7	16.9	17.0	17.3	0.7
Pooled[10]		15.1	15.9	15.6	17.0	18.7	18.7	1.0

[1]Means of prebloom = bloom = regrowth > stockpiled (Tukey pairwise comparison, $P < 0.01$).
[2]HE = high endophyte-infection; 73% at Glade Spring and 81% at Blackstone.
[3]LE = low endophyte-infection; 2% at Glade Spring and 0% at Blackstone.
[4]Standard error of mean.
[5]Indicates effect N fertilization ($P < 0.01$).
[6]Indicates linear effect N fertilization ($P < 0.01$).
[7]Indicates means of sites differ ($P < 0.01$), averaged over endophyte level and N fertilization.
[8]Indicates effect of endophyte level ($P < 0.01$).
[9]Indicates effect of endophyte level ($P < 0.05$).
[10]Pooled over site and growth stage. Indicates difference due to growth stage ($P < 0.01$) and N fertilization ($P < 0.01$) differ and two-way interaction of growth stage x site ($P < 0.01$).

piled tall fescue was higher than steer dietary requirement, but in bloom-cut tall fescue, Ca was below the dietary requirement of steers. The ratio of Ca/P is important in relation to utilization of these two minerals. The ratio of Ca/P in the diet should not be less than 1/1 nor greater than 7/1 for normal animal performance (NRC, 1984). The Ca/P ratio of stockpiled and bloom-cut tall fescue was within the recommended range. Magnesium concentration in stockpiled tall fescue was higher than required for steers. In bloom-cut tall fescue, the Mg was close to dietary requirements. High dietary levels of Al, K, P or Ca can increase cattle Mg requirements (NRC, 1984). Potassium concentrations in stockpiled and bloom-cut tall fescue were very high, compared to animal requirements but were within 'normal' sufficiency ranges for plant growth (Mengel and Kirkby, 1987). Greene and coworkers (1983) reported that increasing dietary K from 0.6 to 2.4% decreased apparent absorption of Mg by 33% in lambs. Sulfur concentration in stockpiled and bloom-cut tall fescue would meet dietary requirement of steers. The N to S ratios of the stockpiled and bloom-cut tall fescue were close to the recommended ratio of 10 to 1 (Moir et al., 1968).

Dietary Cu of less than 3 to 5 mg/kg is considered deficient and could result in low Cu in serum and liver (NRC, 1984). Steers grazed on the fescue from the present study could need Cu supplementation to prevent Cu deficiency. Absorption and utilization of Cu can be depressed by high dietary S and Mo (Goodrich and Tillman, 1966). Copper deficiencies in animals could be exasperated by the application of S containing fertilizers to prevent S deficiencies in plants. In the present study, Mo was not measured and S was not high enough to interfere with Cu absorption. Copper absorption can be reduced by high dietary Zn (Hill and Matron, 1970), but would probably was not a factor in this study. The low endophyte-infected tall fescue contained higher ($P < 0.05$) concentration of Fe, compared to high endophyte-infected stockpiled and bloom-cut tall fescue. Iron concentration in stockpiled tall fesuce would meet dietary requirement of steers, but bloom-cut tall fescue would be borderline deficient. High and low endophyte-infected, stockpiled tall fescue contained twice the Mn required by steers. Manganese concentration in bloom-cut tall fescue was not as high as in stockpiled tall fescue. Ruminants can tolerate very high (1000 mg/kg) levels of Mn without toxicity (NRC, 1984). Sodium concentration in stockpiled high endophyte-infected tall fescue would not be adequate for grazing steers. In low endophyte-infected tall fescue, the Na

concentration would meet the lower dietary level. In bloom-cut tall fescue, Na concentration was also higher ($P < 0.01$) in low endophyte-infected, compared to high endophyte-infected tall fescue Concentration in both low and high endophyte-infected, bloom-cut tall fescue were below dietary requirements for steers, however, concentration of Na in forages is typically lower than cattle requirements (NRC, 1984) and sodium chloride is normally provided to grazing cattle. The stockpiled and bloom-cut tall fescue from this study would not provide adequate Zn for grazing steers. Aluminium and B concentration (potentially toxic minerals to grazing livestock) in stockpiled and bloom-cut tall fescue were well below the maximum tolerable levels for steers.

Comparison of dietary requirements for steers grazed on stockpiled tall fescue and mineral composition of high and low endophyte-infected tall fescue suggests the need for supplementation with Cu, Na and Zn. Steers fed bloom-cut tall fescue may need supplementation with Ca, Cu, Na and Zn. No biologically significant difference in the nutritional value between high and low endophyte-infected tall fescue (except for Na in stockpiled tall fescue) for growing steers was measured. Copper and Zn may be slightly improved in low endophyte-infected tall fescue but the increase is insufficient to overcome deficiencies among animals.

SUMMARY

Yield and chemical composition of endophyte-infected and non-infected tall fescue fertilized with N was studied at two locations. Virginia is divided into five physiographic regions based on geology, topography and soil characteristics. One site, Glade Spring is located in the Ridge and Valley region and the other site, Blackstone, is located in the Southern Piedmont region of Virginia. Selection of two sites enabled evaluation of tall fescue growth and chemical composition under different environmental conditions. Tall fescue grown at Glade Spring was higher in N, Mg, Al,

Table 27. Composition of stockpiled and bloom-cut tall fescue compared to dietary requirements of cattle.

Item	Dietary requirement[2]	Stockpiled[1] HE[3]	LE[4]	SE[5]	Bloom-cut HE	LE	SE
			g/kg				
Nitrogen	15.2	21.5	22.0	0.5	19.5	18.7	0.4
Phosphorus	1.9	1.8	1.9	0.1	2.3	2.3	0.0
Potassium	5-7	21.9	22.3	0.9	22.2	22.7	0.7
Calcium	3.3	4.5	3.9	0.1	3.0	2.8	0.1
Magnesium	0.5-2.5	3.8	3.9	0.1	2.5	2.6	0.1
Sulfur	0.8-1.5	1.9	2.1	0.1	1.6	1.6	0.0
			mg/kg				
Copper	4-10	4.7	5.0	0.1	3.6	4.1	0.2
Iron[6]	50-100	76.5	86.4	9.0	51.1	54.8	1.3
Mangenese	20-50	100.2	91.3	4.1	62.0	56.7	3.6
Sodium[6]	600-1000	275.1	638.6	45.0	141.8	408.2	46.8
Zinc	20-40	14.2	14.7	0.4	17.3	17.5	1.1
Aluminum[7]	< 1000	65.7	75.4	9.6	26.8	27.9	1.8
Boron[7]	< 150	6.9	7.0	0.1	4.9	5.2	0.2

[1]Mean of mineral concentration in tall fescue, averaged over two locations in VA and three levels of N fertilization.
[2]Dietary requirements of 227 kg steer, gaining .45kg/day (NRC, 1984).
[3]HE = high (77%, average) endophyte-infected tall fescue.
[4]LE = low (1%, average) endophyte-infected tall fescue.
[5]Standard error of mean.
[6]Indicates difference between means ($P < 0.05$) of endophyte levels.
[7]Maximum tolerable levels in cattle.

Cu, Fe and Mn, compared to Blackstone. Differences in mineral composition between the sites probably reflect a higher initial soil fertility at Glade Spring, compared to Blackstone.

Nitrogen fertilization decreased NDF, ADF and cellulose in tall fescue. Concentration of N, Mg, Ca, B, Cu, Na and Zn were increased and P and S were decreased in tall fescue in response to N fertilization. The influence of N fertilization on mineral composition of plants depends on many factors including soil availablility of the mineral, possible interactions with other minerals present in the soil and effects of N on soil pH. This makes interepretation of N influence on tall fescue tissue composition difficult.

Neutral detergent fiber, ADF, cellulose, and lignin were higher in low endophyte-infected as compared to high endophyte-infected tall fescue. This indicates a higher quality of the high endophyte-infected tall fescue as compared to the low endophyte-infected tall fescue but may also be related to cultivar and/or growth stage (maturity). Averaged over growth stage at harvest and site, low endophyte-infected tall fescue was higher in concentration of Fe and Na as compared to high endophyte-infected tall fescue. Sodium was higher in the low as compared to the high endophyte-infected tall fescue at the Glade Spring site at all four harvests. Zinc was higher in the low as compared to the high endophyte-infected tall fescue at Blackstone in prebloom and 5-wk regrowth harvests. However, the influence of endophyte on tall fescue chemical composition could not be separated from cultivar effects. The high endophyte-infected tall fescue was 'Alta' and the low endophyte-infected was 'Kentucky-31'.

In a greenhouse (see previous section), endophyte-infected tall fescue plants (composited by endophyte level and individually harvested) contained lower concentrations of N, Ca, Mg, Al, B, Mn and Zn, compared to non-infected tall fescue. The only significant effect of endophyte infection on mineral composition in both the greenhouse and field studies was on Zn concentration of tall fescue. The influence of endophyte-infection on concentrations of TNC, N, Fe, Al, Cu, B and Na were similar in both the greenhouse and field studies. Concentrations of P, K, S, Mg, Ca and Mn did not response the same to endophyte-infection of tall fescue in the greenhouse and field studies.

In a separate study, high and low endophyte-infected tall fescue was harvested from the Glade Spring location on 21 Oct. 1987 and ensiled (Zylka, 1989). Tall fescue silages were fed to wethers in a metabolism trial. Endophyte infection did not influence digestibility of DM, CP, NDF, ADF, cellulose or hemicellulose. Apparent absorption of N, P and K were lower for sheep fed the high endophtye-infected tall fescue silage, compared to the low endophyte-infected tall fescue silage. Forage quality is defined in terms of the presence of essential nutrients and the palatability to the animal (Heath, 1985). This defination implies that the best measure of forage quality is animal performance. Further animal studies with endophyte-infected tall fescue need to be conducted to measure the influence of the endophyte on the utilization of the forage nutrients.

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SERUM HORMONE AND MINERAL COMPOSITION OF STEERS FED ENDOPHYTE-INFECTED TALL FESCUE SILAGE AND HAY

INTRODUCTION

Infection of tall fescue with the endophyte fungus, ***Acremonium coenophilum***, Morgan-Jones and Gams has been associated with tall fescue toxicosis (Bacon et al., 1975). Endophyte-infected tall fescue may depress forage intake (Goetsch et al., 1987a) and performance (Hoveland, 1983) of cattle. The substance(s) in tall fescue that cause reduced performance and toxic symptoms have not been identified, but alkaloids have been implicated in the etiology of tall fescue toxicosis. Perloline has been reported to inhibit ruminal cellulose digestibility and volatile fatty acid (VFA) production, ***in vitro*** (Bush et al., 1972). ***In vivo***, perloline (fed at 0.5% of diet) decreased ($P < 0.05$)

crude protein and cellulose digestibility in lambs (Boling et al., 1975). The decrease in digestibility may reflect changes in ruminal microfauna population or activity. Perloline inhibited growth of the ruminal microorganisms, ***Bacteroides succinogenes, Bityrivibrio fibrisolvens, Ruminoccus albus and Ruminocuccus flavefaciens*** (Bush et al., 1972). Another class of alkaloids in tall fescue, the pyrrolizidine alkaloids, have been associated with insect resistance in plants (Johnson et al., 1985). In addition, ergopeptine alkaloids have been isolated from endophyte grown in culture (Bacon et al., 1979). These alkaloids are similar to those produced from ***Claviceps purpurea*** and symptoms of tall fescue toxicosis are similar to ergotism (Bacon et al., 1975).

Changes in serum prolactin and cholesterol may be associated with fescue toxicosis. Serum prolactin has been reported to be supressed in cattle fed endophyte-infected tall fescue. Cows grazing tall fescue had lower prolactin levels than those grazing orchardgrass (Daniels et al., 1983). Steers fed non-infected fescue had serum prolactin levels of 15.0 ng/ml whereas those fed infected fescue did not have detectable levels of prolactin (Goetsch et al., 1987b). Heifers grazed on endophyte-infected 'Kentucky-31' and 'G1-307' tall fescue had lower ($P < 0.01$) serum prolactin as compared to those grazed on non-infected 'Kentucky-31', 'Kenhy', or 'Johnstone' tall fescue (Bolt and Bond, 1985). Holstein calves fed 'G1-307' tall fescue had lower basal prolactin (1.8 ng/ml) as compared to those fed 'G1-306' tall fescue (6.0 ng/ml) (Hurley et al., 1981). The 'G1-307' tall fescue was higher in endophyte infection as compared to 'G1-306' tall fescue.

Serum prolactin concentration in cattle can be altered by environmental and biochemical processes such as, photoperiod (Fitzgerald et al., 1982; Thompson et al., 1987), temperature (Smith et al., 1977; Hurley et al., 1981), milking and parturition (Smith et al., 1974). Both naturally occuring and synthetic compounds can act as prolactin-inhibiting or prolactin-releasing factors. Naturally occurring compounds have been reported to influence serum prolactin concentration (Karg and Schams, 1974). Seretonin stimulates prolactin secretion indirectly by activating the prolactin-releasing hormone from the hypothalamus which stimulates prolactin secretion (Clemens and Shaar, 1980). Dopamine, a brain catecholamine, inhibits prolactin secretion from the pituitary gland (Macleod and Lehmeyer, 1974).

Tall fescue, ***Festuca arundinacea*** Shreb., infected with the endophytic fungus, ***Acremonium coenophialum***, Morgan-Jones and Gams has been shown to supress serum prolactin in cattle (Bolt et al., 1983; Daniels et al., 1983; Goetsch et al., 1987ab and Thompson et al., 1987). The factor(s) responsible for the depressed serum prolactin in animals fed endophyte-infected tall fescue are not known but, the ergopeptine alkaloids have been suggested as causitive agents (Bacon et al., 1986). Ergopeptine alkaloids are produced by the endophyte fungus grown in culture (Porter et al., 1981) and have been isolated from endophyte-infected tall fescue plant tissue (Yates et al., 1985). In addition, ergot alkaloids are known to stimulate dopamine receptors (Porter et al., 1985).

Serum prolactin depression may serve as an indicator of tall fescue toxicosis. Serum prolactin decreased over time (a seasonal affect) in heifers grazed on tall fescue (Bolt and Bond, 1985). But, prolactin was depressed to a greater extent ($P < 0.01$) in heifers grazed on endophyte-infected tall fescue as compared to those grazed on non-infected tall fescue. Holstein calves fed 'G1-307' tall fescue had lower basal prolactin concentration (1.8 ± 0.1 ng/ml) compared to those fed 'G1-306' tall fescue (6.0 ± 1.2 ng/ml) (Hurley et al., 1981). The variety 'G1-307 had a higher level of endophyte as compared to 'G1-306'. Increasing ambient temperature from 10 C to 34 C, increased basal prolactin levels in calves fed 'G1-306' (2.3 ± 0.3 to 12.1 ± 1.3 ng/ml , respectively) but not in calves fed 'G1-307' (1.8 ± 0.1 to 1.6 ± 0.1 , respectively).

Other compounds have been administered to animals that change serum prolactin concentration. Yohimbine hydrochloride (YOH), an indole alkaloid, is an alpha$_2$ adrenergic blocking agent that stimulates seretonin receptors and blocks D_2-dopamine recepters. Fed orally to rats, YOH decreased serum prolactin by 35% but, injected intraperitineally, increased prolactin 7.8 fold (Jernigan et al., 1986). Possible effects on ruminants are not known.

The ergot-like compound, 2-Br-alpha-ergokryptin (CB154) lowered serum prolactin (17ng/ml) in rats dosed via intragastrin cannula (Porter et al., 1985). Holstein cows treated with CB154 reduced basal serum prolactin concentrations by 80% and blocked normal surges in prolactin at parturition and during milking (Akers et al., 1981). Average milk production was also decreased in cows treated with CB154 as compared to controls. Serum prolactin in Holstein cows adminstered ergocryptine in 50% ethanol were lower ($P < 0.01$) as compared to controls (ethanol

only) (Smith et al., 1974). In the control cows, serum prolactin increased from 15 ng/ml prior to milking to 28 ng/ml within 10 minutes after milking. Cows administered ergocryptine showed no increase in serum prolactin in response to milking. In addition, depression of prolactin by ergocryptine was effective for 5 days after treatment.

Thyrotropin-releasing hormone (TRH) promotes prolactin release (Chen and Meites, 1975). Serum prolactin, stimulated by TRH was increased in cattle fed 'G1-306' tall fescue but only slightly increased in those fed endophyte-infected, 'G1-307' tall fescue (Hurley et al., 1981). L-dopa, a metabolic precursor of brain catecholamines, acts on pituitary cells and blocks TRH stimulation of prolactin (Macleod and Lehmeyer, 1974). Ergopeptine alkaloids in endophyte-infected tall fescue may function in a similar manner to dopamine, suppressing serum prolactin.

Depression of serum cholesterol may also be an indication of tall fescue toxicosis in livestock. Serum cholesterol levels were lower in steers grazed on the tall fescue line, 'G1-307' as compared to 'G1-306' or varieties 'Kentucky-31' or 'Kenhy' (Stuedemann et al., 1985). Steers grazing the tall fescue line 'G1-307' exibited symptoms of summer toxicosis. The 'GI-307' had the highest endophyte level compared to the other line and varieties grazed. Plants from this pasture also contained a low concentration of perololine and a high concentration of N-formyl and N-acetyl loline.

Interpretation of some studies in the literature is difficult since many did not include a non-tall fescue control. Also, the influence of endophyte-infected tall fescue on serum mineral concentration in cattle has not been reported in the literature. Any influence of endophyte infection on the chemical composition of tall fescue plants would also change the nutritional value of tall fescue to the animal. These changes may be reflected in the hormone and mineral levels in cattle.

Preservation of endophyte-infected tall fescue by ensiling or drying (hay) may alter the substance(s) responsible for tall fescue toxicosis in cattle. The process of ensiling, may change the toxicity of endophyte-infected tall fescue fed to cattle. In a study with growing beef calves fed high and low endophyte-infected 'Kentucky-31' tall fescue silage, there was no difference in calf performance but rectal temperatures differed indicating toxicity differences (Gay et al., 1986). Ensiling endophyte-infected (90%) tall fescue in November, decreased the level of loline alkaloids (Gay et

al., 1985). Endophyte-infected hay contained 980 and 1415 ng/g and endophyte-infected silage contained 686 and 980 ng/g of N acetyl loline and N-formyl loline, respectively. Dry matter intake was similar in steer calves fed the endophyte-infected hay and silage but was lower ($P < 0.01$) than calves fed non-infected tall fescue hay (Gay et al., 1985). Rectal temperature, measured at ambient temperature of 32.2 C, was higher in calves fed endophyte-infected hay and silage as compared to those fed non-infected tall fescue hay. The ensiling of the low endophyte-infected tall fescue was poor (Gay et al., 1986). In addition, the toxicity of tall fescue ensiled in spring may differ from fescue ensiled in the fall after stockpiling.

Lack of a non-tall fescue control has limited conclusions concerning the influence of endophyte-infection on basal and TRH-stimulated serum prolactin levels. Normal fluctuations in serum prolactin in cattle with season or photoperiod have also confused interperetion of some data. In addition, the influence of ensiling endophyte-infected tall fescue in spring or after stockpiling in the fall on TRH-stimulated serum prolactin has not been reported.

The objectives of this research were 1) to determine the influence of preservation of endophyte-infected tall fescue as hay or silage on steer serum hormone and mineral composition; 2) to investigate seasonal effects of toxicity of ensiled tall fescue by comparing ensiled spring-cut and stockpiled fall-cut tall fescue; 3) to determine the influence of feeding endophyte-infected tall fescue on basal and TRH-stimulated serum prolactin in steers; and 4) to investigate the seasonal effect of time of ensiling on toxicity of basal and TRH-stimulated serum prolactin by comparing spring-cut and stockpiled fall-cut tall fescue silage fed to steers.

MATERIALS AND METHODS

Phase 1. Feeding studies were conducted at the Middleburg Research Station, Virginia (Fauquier county) in 1986, 1987 and 1988 to measure basal serum hormone and mineral concen-

trations in steers fed endophyte-infected tall fescue silage and hay. 'Kenhy' tall fescue hay and silage were harvested from a 'toxic' tall fescue pasture on the station. Cattle grazing in this pasture developed symptoms of summer syndrome (Byrant and Hammes, 1981). Angus steers (four per treatment per year) were blocked by weight (average 222 kg) and randomly allotted to treatments within blocks. Experimental diets were fed 24 March to 25 April in 1986, 10 April to 5 May in 1987 and 25 March to 19 April in 1988. Experimental diets were, 1) (control) orchardgrass/alfalfa hay , 2) endophyte-infected 'Kenhy' tall fescue ensiled in spring, and 3) endophyte-infected tall fescue ensiled in fall after stockpiling. In 1987 and 1988, an additional treatment, 4) spring-cut endophyte-infected tall fescue hay was included. Treatment 4 was not included in year 1 because of rain damage during field drying. Animals were tethered in separate stalls in an enclosed barn. Diets were fed ad libitum and cattle had free access to water. Ambient temperature was recorded daily with a solar-thermal unit computer[6] .

The pasture was fertilized with 89.7 kg N/ha in mid-March or mid-April of 1984, 1985, 1986 and 1987. On 20 Sept. 1985, 33.6 kg P_2O_5/ha and 67.3kg K_2O/ha was applied. In May 1986, the field was fertilized with 140 kg K_2O/ha. Phosphorus (56.1 kg P_2O_5/ha) and K (69.5 kg K_2O/ha) were applied in April 1987. The soil was a Chester-Brandywine loam, 2-7% slope.

Tall fescue was harvested on 4 June 1985 (spring-cut) and on 5 Dec. 1985 after stockpiling, for the 1986 trial. For the 1987 trial, tall fescue (spring-cut) was harvested for hay and silage on 17 June 1986 and in December for stockpiled silage. For the 1988 trial, tall fescue was havested on 29 May 1987 for spring-cut hay and silage and on 3 December 1987 for stockpiled silage. Spring-cut silage was wilted to reduce moisture content and ensiled in upright concrete silos. Stockpiled tall fescue was not wilted prior to ensiling as suggested by previous research (Torres, 1983). Hay was baled with a New Holland Baler and ensiled forages were cut with a Fox Forage Harvester. Orchardgrass (***Dactylis glomerata*** L.)/alfalfa (***Medicago sativa*** L.) hay (purchased locally) was fed during a seven day adjustment period to all steers. Blood was sampled initially (end of the preliminary period, day 0), on day 11 (1987 and 1988), day 25 (1986, 1987 and 1988) and

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[6] Model AG-12, AG-Tech instrument Company, Savannah, Georgia

day 32 (1986) by jugular puncture. On day 25 of the 1988 trial, blood was withdrawn from jugular catheters that were inserted on day 24 of the trial (see phase 2).

Feeds were sampled daily and frozen prior to analysis for chemical composition. At the completion of the trial (1988), the daily feed samples were subsampled (200 g per daily sample) and composited as described below. The alfalfa/orchardgrass hay from the adjustment period was composited. Feeds were composited by diet from day 0 to day 11 and from day 12 to day 25. Silage composite samples were subdivided and kept frozen for pH, water-soluble carbohydrate (Dubois et al., 1956; Johnson et al., 1966) and lactic acid (Barker and Summerson, 1941; Pennington and Sutherland, 1960). Feed composite samples from the 1988 trial were dried (60 C) and ground (1-mm) in a Wiley mill prior to chemical analysis. Neutral detergent fiber (Van Soest and Wine, 1967 and Goering and Van Soest, 1970), acid detergent fiber (Van Soest, 1963; Goering and Van Soest, 1970), lignin and cellulose (Van Soest and Wine, 1968; Goering and Van Soest , 1970) were determined on composited samples. Total N was determined colorimetrically as ammonia-salicylate with a Technicon Autoanalyzer[7] (Technicon Industrial Systems, 1976) on plant material (200 mg) digested in a salt/catalyst mixture (94% K_2SO_4, 4% HgO, 1% $CuSO_4$ and 1% pumice) with 2.5 ml H_2SO_4 at 400 C for approximately 40 minutes (McKenzie and Wallace, 1954). Minerals (P, K, Ca, Mg, S, Na, Al, B, Cu, Fe, Mn, Se, and Zn) were determined by an inductively-coupled plasma optical emission spectrophotometer (ICP-OES) on plant material (0.5 g) digested with nitric and perchloric acid (3:1 ratio) (Muchovej et al., 1986). Total ergopeptine alkaloids were determined colorimetrically (nitrate procedure) as ergovaline maleate (Michelon and Kellehoer, 1963) on extracted material (Porter et al., 1979) with the following modifications. Dried and ground plant material (25 g) was made basic with 4 N NaOH (50 ml) and extracted with chloroform (100 ml). A sidearm flask fitted with a Buchner funnel lined with Whatman 42 filter paper, attached to a vacuum pump was used to separate the chloroform filtrate from the plant material. The chloroform filtrate was extracted with 2% (w/v) tartartic acid (50 ml) in a separatory funnel. The tartartic acid extract was adjusted to a pH of 10 with 4 N NaOH. The basic tartartic

[7] Technicon Industrial Systems, Tarrytown, NY 10591

acid extract was extracted with chloroform in a seperatory funnel. The chloroform fraction was harvested and evaportated to dryness over low heat. The resulting residue was diluted with 10-ml of a weak acid and analyzed colorimetrically as ergovaline maleate.

Hemoglobin was determinined by the cyanmethemoglobin method (Fisher Diagnostics) on whole blood. Hematocrit was determined on whole blood by centrifuging blood in Micro-Hematocrit Capillary Tubes[8] for 5 minutes and measuring the percentage of packed cell volume with a hematocrit reader. Hemoglobin and hematocrit were not determined on samples from the 1987 trial. Serum prolactin was determined by the double-antibody radioimmunoassay (Koprowski and Tucker, 1971) except the stock solution of radiolabelled prolactin hormone was iodinated as described by Akers and Keys (1984). Serum cholesterol was measured enzymatically in a modified method of Allain and coworkers (1974) (Sigma Diagnostic). Serum minerals (Ca, Mg, P, K, S, Na, Cu, Fe and Zn) were determined (ICP-OES) on serum (1 ml) diluted with 0.1 M HCl (9 ml) for years 1987 and 1988. Calcium and Mg concentrations in serum from the 1986 trial were determined by atomic absorption spectrophotometry on serum diluted with 0.1% lanthanium chloride to prevent P interference. Serum inorganic P was determined by the colorimetric procedure of Fiske and Subbarow (1925) for the 1986 trial.

Data were analyzed by the general linear method (SAS, 1985) with regard to trial (year). The experimental design was a randomized complete block design. The residual error was used to test the significance of the main effects of treatment (diet) and animal block. Orthogonal contasts for the 1986 trial were 1) orchardgrass-alfalfa hay vs. spring-cut and fall-cut silages and 2) spring-cut silage vs. fall-cut silage. In 1987 (initial and day 11 but not day 25) and 1988 trial, an additional treatment (experimental diet) was added and an additional orthogonal contrast was included, 3) fescue hay vs. spring and fall-cut silages.

Phase 2. A feeding study to measure basal and thyrotropin-releasing hormone (TRH) stimulated prolactin in steers fed endophyte-infected tall fescue hay and silage was conducted at the Middleburg Research Station in the spring of 1988. Sixteen Angus steers (four per treatment) were

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[8] Lancer, Division of Sherwood Medical. St. Louis, MO

blocked by weight and randomly assigned to treatments within blocks. Animals were tethered in separate stalls in an enclosed barn and had free access to water.

Experimental diets were, 1) (control) orchardgrass/alfalfa hay , 2) endophyte-infected 'Kenhy' tall fescue ensiled in spring, 3) endophyte-infected tall fescue ensiled in fall after stockpiling and 4) spring-cut endophyte-infected tall fescue hay. Animals were fed experimental diets for 25 days before administeration of TRH. On the day prior to administering the TRH, animals were fitted with jugular cannulaes (Thompson et al., 1987). A puncture in the jugular vein was made with a sterilized 11 gauge needle and plastic tubing (1.90 mm) was inserted through the needle into the jugular vein. The tubing was sutured to the neck to allow easy removal of the blood samples. Catheters were flushed with 5% sodium citrate in saline solution to prevent blood clotting in the tubing. Blood samples were taken every half hour for 4 hours to establish baseline prolactin. Immediately after the 4-hr sampling, TRH (33 ug/kg body weight), dissolved in saline (Johke, 1978), was injected into the catheters and the catheters were flushed with saline. Blood was sampled every 10 minutes for the first hour and every 20 minutes for the second hour after administration of TRH. Serum prolactin was determined by the double-antibody radioimmunoassay (Koprowski and Tucker, 1971) except the stock solution of radiolabelled prolactin hormone was iodinated as described by Akers and Keys (1984).

Data were analyzed by the general linear method (SAS, 1985) with regard to sampling time. The experimental design was a randomized complete block design. The residual error was used to test the significance of the main effects of treatment (diet) and animal block. Orthogonal contasts were 1) orchardgrass-alfalfa hay vs. spring-cut and fall-cut silages and 2) spring-cut silage vs. fall-cut silage and 3) fescue hay vs. spring and fall-cut silages.

RESULTS

Phase 1.

Ambient temperature. Ambient temperature, average of the high and low temperature recorded daily, was 13.9 C on day 0 (initial), 14.4 C on day 25 and 12.8 C on day 32 in the 1986 trial; 11.7 C on day 0 (initial), 17.8 C on day 11 and 9.4 C on day 25 in 1987; and 18.3C on day 0 (initial), 16.7 C on day 11 and 7.2 C on day 25 in 1988.

Chemical composition of diets. Chemical composition was different among the two hay and two silage diets fed to steers in the 1988 trial (Table 28). The pH was slightly lower in spring-cut as compared to the fall-cut tall fescue silage. Lactic acid was 2.5 fold higher in fall-cut as compared to spring-cut fescue silage. Total non-structural carbohydrate (TNC) was 30% higher in fall-cut as compared to the spring-cut silage. Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin were similar for spring-cut tall fescue hay and silage. The orchardgrass/alfalfa hay had higher concentrations of N, P and Ca compared to the fescue diets. Magnesium and S were highest in the fall-cut silage and lowest in the fescue hay. Sodium was higher in the orchardgrass/alfalfa hay and fall-cut fescue silage compared to the spring-cut fescue hay and silage. The fall-cut fescue silage contained the highest levels of Al, Fe, Mn and Zn, possibly indicating soil contamination. Boron and Cu were higher in the orchardgrass/alfalfa hay compared to the fescue diets. Total ergopeptine alkaloids were 10-fold higher in the silages as compared to the hays. The orchardgrass/alfalfa hay had the lowest level and the spring-cut fescue silage had the highest level of ergopeptine alkaloids.

Prolactin. In the 1986 trial, initial serum prolactin concentration among steers were not significantly different (Fig. 3). On day 25 of the trial, serum prolactin in steers fed tall fescue silage decreased whereas those fed orchardgrass/alfalfa hay did not change. At the end of the trial (day 32), serum prolactin was higher ($P < 0.01$) in steers fed orchardgrass/alfalfa hay as compared to those fed spring-cut fescue silage. There was not enough fall-cut fescue silage available to feed steers

Table 28. Chemical composition of diets for animal trial, 1988.

Item	Diet			
	Hay		Silage	
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue
pH	--	--	4.06±0.14	4.46±0.18
	% as fed basis			
Lactic acid	--	--	0.7±0.1	1.9±0.3
Total non-structural carbohydrate	--	--	7.0±2.8	23.3±2.5
	g/100g, dry basis			
Netural detergent fiber	55.4±1.2	70.8±0.5	68.0±0.9	53.1±1.3
Acid detergent fiber	40.3±1.1	43.9±0.1	43.8±0.8	33.6±0.4
Cellulose	36.2±1.5	37.2±0.1	36.8±0.5	26.1±0.5
Hemicellulose	15.1±0.5	26.9±0.4	24.2±0.1	19.4±0.6
Lignin	4.1±0.4	5.8±0.0	6.2±0.3	3.5±0.1
	g/kg, dry basis			
Nitrogen	23.2±.1	16.4±.1	18.2±.1	21.9±.1
Phosphorous	2.7±.1	2.2±.1	2.6±.1	2.4±.1
Potassium	23.2±.2	22.1±.1	23.3±1.4	20.6±.3
Calcium	8.1±.2	2.6±.1	3.2±.0	6.0±.1
Magnesium	2.08±.11	1.74±.02	2.04±.01	3.18±.08
Sulfur	1.8±.1	1.6±.0	1.9±.0	2.2±.0
Sodium	0.6±.1	0.3±.0	0.3±.0	0.6±.0
	ug/g, dry basis			
Aluminium	95.1±42.3	72.6±6.0	38.4±5.4	589.9±45.5
Boron	22.7±1.2	6.4±.4	6.9±.2	7.5±.2
Copper	6.3±.4	3.7±.2	4.4±.2	5.0±.0
Iron	123.1±35.8	127.6±21.1	94.0±14.6	650.4±47.0
Mangenese	47.2±4.0	81.2±1.2	125.7±11.9	197.5±1.5
Zinc	19.5±.2	14.2±.1	20.0±1.7	22.1±.1
Ergopeptine alkaloids	0.7±.4	2.7±1.2	27.2±.2	18.2±3.6
	ratio			
Ca/P	2.9	1.1	1.2	2.5
N/S	12.6	10.3	9.4	10.0

for 32 days and this treatment was terminated after the steers were bled on day 25. In the 1987 trial, serum prolactin tended ($P < 0.08$) to be higher on day 11 in steers fed orchardgrass/alfalfa hay as compared to those fed fescue hay or silage (Fig. 4). On day 25 of the 1987 trial, prolactin in steers fed orchardgrass/alfalfa hay was numerically but not significantly higher as compared to steers fed fescue diets. On day 11 of the 1988 trial, serum prolactin was higher ($P < 0.05$) in steers fed orchardgrass/alfalfa hay as compared to those fed fescue hay or silage (Fig. 5). On the last day of the trial (day 25), serum prolactin tended to be higher ($P < 0.08$) in steers fed orchardgrass/alfalfa hay as compared to those fed fescue hay or silage.

Cholesterol. Cholesterol concentration in the initial samples did not differ significantly among steers, in all three trials (Table 29). On day 11 of the 1987 trial, serum cholesterol in steers fed orchardgrass/alfalfa hay was higher ($P < 0.05$) as compared to steers fed the tall fescue hay or the tall fescue silage diets. Serum cholesterol was lower ($P < 0.01$) in steers fed spring-cut tall fescue hay, compared to steers fed spring-cut or fall-cut tall fescue silage on day 11 of the 1987 trial. In the 1988 trial, on day 11, serum cholesterol was numerically but not significantly lower in steers fed fescue hay as compared to those fed fescue silages. By day 25, serum cholesterol was higher ($P < 0.05$) in steers fed orchardgrass/alfalfa hay, compared to steers fed fescue diets in the 1986 and 1987 trials. In 1988, on day 25, serum cholesterol in steers fed orchardgrass/alfalfa hay was numerically but not significantly higher compared to steers fed fescue diets. On day 32 of the 1986 trial, serum cholesterol was numerically but not significantly higher in steers fed orchardgrass/alfalfa hay, compared to steers fed spring-cut fescue silage.

Blood hematocrit. Hematocrit was not influenced by diet in the 1986 or 1988 trials (Table 30). Hematocrit was not measured in the 1987 trial.

Blood hemoglobin. Hemoglobin in initial blood samples were not different among steers in 1986 and 1988 trials (Table 31). On day 25 of 1986 trial, hemoglobin in steers fed orchardgrass/alfalfa hay was lower ($P < 0.01$), compared to steers fed fescue diets. Hemoglobin was lower ($P < 0.01$) in steers fed fall-cut as compared to spring-cut fescue silage on day 25 of the 1986 trial. On day 25 of the 1988 trial, hemoglobin in steers fed spring-cut fescue silage was numerically

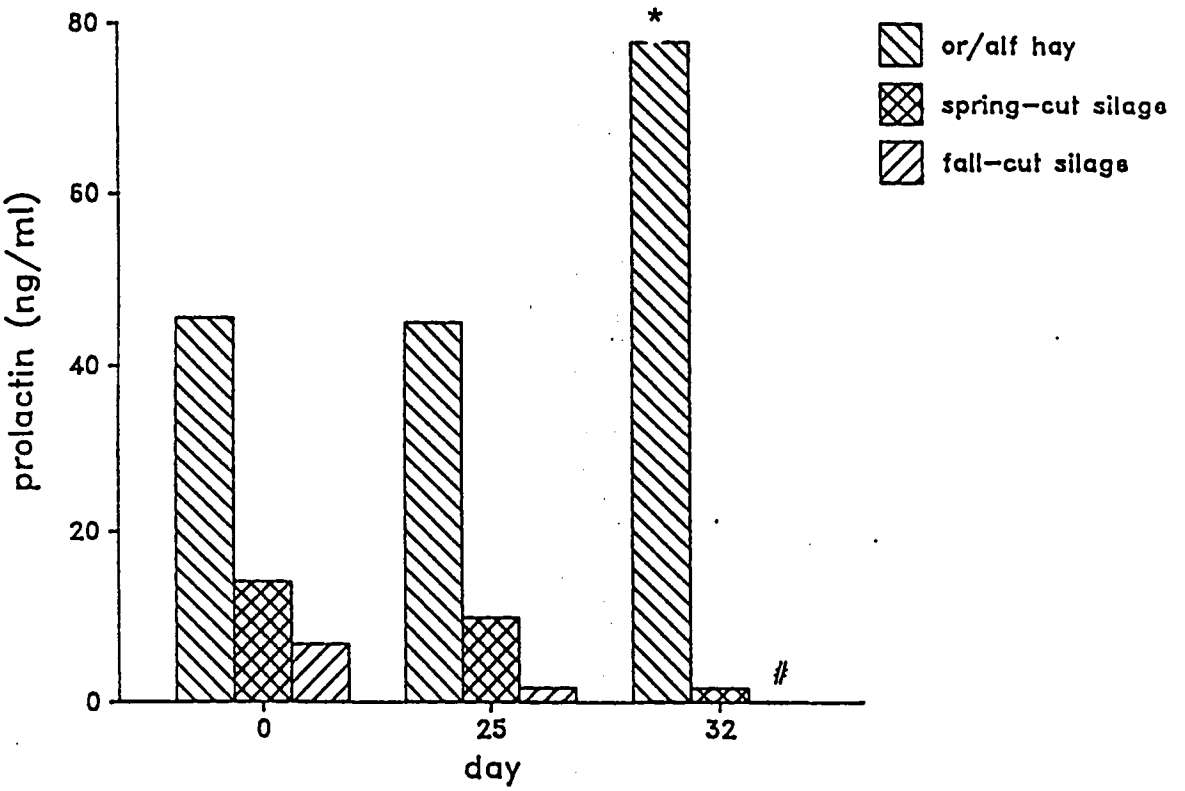

Figure 3. **Serum prolactin in steers, 1986 trial:** The influence of serum prolactin in steers fed endophyte-infected tall fescue ensiled in spring or after stockpiling in the fall as compared to orchardgrass/alfalfa (or/alf) hay in the 1986 trial. Standard errors of the treatment means were 13.6, 23.8 and 18.2 for the initial, day 25 and day 32 samples, respectively. * Means for orchardgrass/alfalfa hay differed from spring-cut tall fescue silage ($P < 0.05$). # Indicates no sample taken on this date.

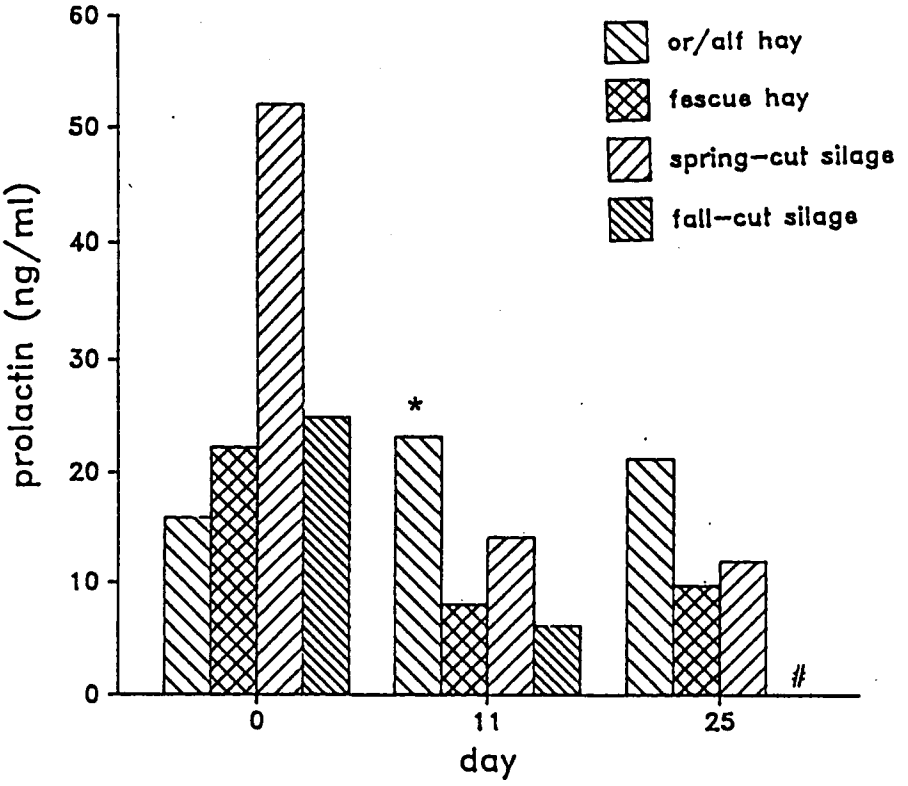

Figure 4. Serum prolactin in steers, 1987 trial: Serum prolactin in steers fed orchardgrass/alfalfa (or/alf) hay as compared to endophyte-infected tall fescue hay, spring-cut silage, and fall-cut silage, in the 1987 trial. Standard errors of the treatment means were 13.1, 6.4 and 3.7 for the initial, day 11 and day 25 samples, respectively. * Means for the orchardgrass/alfalfa hay were different from the fescue hay and silages ($P < 0.08$). # Indicates no sample from this date.

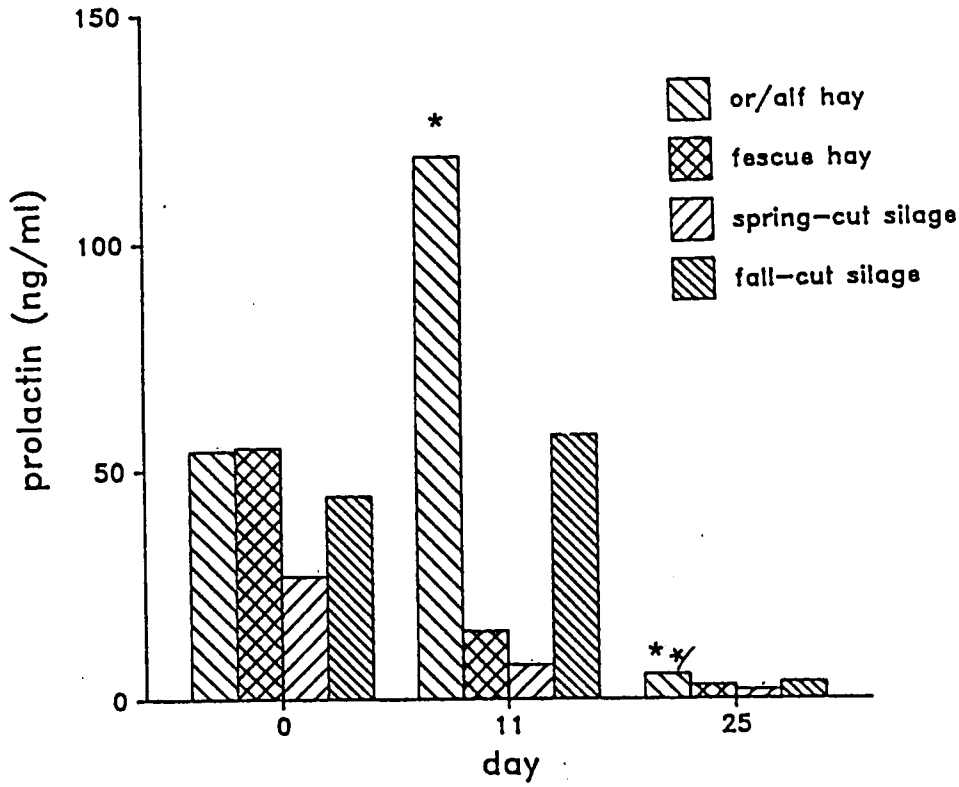

Figure 5. Serum prolactin in steers, 1988 trial: Serum prolactin in steers fed orchardgrass/alfalfa (or/alf) hay as compared to endophyte-infected tall fescue hay, spring-cut silage, and fall-cut silage, in the 1988 trial. Standard errors of the treatment means were 13.6, 33.8 and 1.2 for the initial, day 11 and day 25 samples, respectively. * Means for the orchardgrass/alfalfa hay were different from the fescue hay and silages ($P < 0.05$). ** Means for the orchardgrass/alfalfa hay were different from the fescue hay and silages ($P < 0.08$).

Table 29. Serum cholesterol in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	SE[1]
	mg/dl				
Initial:					
1986	163.5	--[4]	166.1	182.6	18.2
1987	122.2	94.4	83.8	101.2	6.9
1988	86.1	86.4	89.4	87.6	12.0
Day 11:					
1987[2,3]	106.5	72.4	82.9	101.0	6.9
1988	87.6	84.5	103.7	88.6	10.1
Day 25:					
1986[2]	218.9	--[4]	140.3	152.4	20.4
1987[2]	125.5	94.9	98.1	--[4]	4.4
1988	106.5	102.9	104.9	92.2	13.2
Day 32:					
1986	242.9	--[5]	186.1	--[4]	22.7

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silages ($P < 0.05$).
[3]Difference between fescue hay and fescue silages ($P < 0.05$).
[4]No sample taken at this date.

but not significantly higher, compared to steers fed fall-cut silage. Hemoglobin was not measured in the 1987 trial.

Calcium. The normal range for serum Ca is 9 to 12 mg/dl (Church, 1979). In the 1986 and 1987 trials, serum Ca was within the normal range and there were no differences due to experimental diet (Table 32). Serum Ca for the 1987 and 1988 trials tended to be lower as compared to the 1986 trial. On day 11 of the 1988 trial, serum Ca in steers fed fall-cut silage was higher ($P < 0.05$) than those fed the spring-cut silage. Serum Ca was numerically but not significantly higher on day 25 in steers fed the fall-cut as compared to those fed the spring-cut silage.

Magnesium. Normal range for serum Mg in cattle is 1.8 to 3.0 mg/dl (Church, 1979). Serum Mg was higher ($P < 0.01$) in steers fed the fall-cut as compared to those fed the spring-cut silage on day 25 of the 1986 trial (Table 33). On day 11 of the 1987 trial, serum Mg was lower ($P < 0.01$) in steers fed fescue hay as compared to steers fed the fescue silages (spring-cut and fall-cut). Serum Mg was also lower ($P < 0.05$) in steers fed the fescue hay as compared to fescue silages on day 11 of the 1988 trial. On day 25 of the 1988 trial, serum Mg of steers fed the spring-cut silage was lower ($P < 0.05$) than those fed the fall-cut silage.

Phosphorus. Normal serum P ranges from 4 to 9 mg/dl (Church, 1979). On day 25 of the 1986 trial, serum P was lower ($P < 0.05$) in steers fed fall-cut silage as compared to those fed the spring-cut silage (Table 34). No differences in serum P due to diet were measured in the 1987 or 1988 trials. The levels of P were considerably higher in the 1987 and 1988 trials as compared to the 1986 trial. This difference can be attributed to the different methods used to determine P in 1986, compared to 1987 and 1988. The method used in 1986 measured inorganic P and in 1987 and 1988 total P was determined.

Potassium. Normal potassium ranges from 14 to 18 mg/dl (Church, 1979). In the 1987 trial, serum K in steers fed orchardgrass/alfalfa hay were significantly lower ($P < 0.05$) on day 11 and numerically lower on day 25 as compared to steers fed fescue hay or silage (Table 35). This effect was not measured in the 1988 trial.

Table 30. Blood hematocrit in steers fed endophtye-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	------- % --------				
Initial:					
1986	34.7	--[2]	36.5	34.2	2.3
1988	31.6	29.6	30.9	31.4	1.2
Day 11:					
1988	33.0	32.0	34.1	33.4	1.3
Day 25:					
1986	36.0	--[2]	38.5	35.8	1.3
1988	28.2	26.8	28.8	25.5	1.4
Day 32:					
1986	34.4	--[2]	37.4	--[2]	1.3

[1]Standard error of mean.
[2]No sample taken on this date.

Table 31. Blood hemoglobin in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	g/dl				
Initial:					
1986	11.0	--[4]	12.1	12.0	0.6
1988	11.6	10.9	11.6	11.6	0.4
Day 11:					
1988	12.2	11.8	12.7	12.4	0.5
Day 25:					
1986[2,3]	12.7	--[4]	15.5	13.4	0.5
1988	10.3	9.6	10.5	9.8	0.3
Day 32:					
1986	12.0	--[4]	13.3	--[4]	0.4

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.01$).
[3]Difference between spring-cut and fall-cut fescue silages ($P < 0.01$).
[4]No sample taken at this date.

Table 32. Serum calcium in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	mg/dl				
Initial:					
1986	11.50	--[3]	11.17	11.02	0.23
1987	9.25	9.27	9.25	9.04	0.29
1988	9.35	9.13	9.30	9.47	0.15
Day 11:					
1987	9.05	9.20	9.16	9.31	0.21
1988[2]	9.36	9.17	9.09	9.61	0.16
Day 25:					
1986	12.05	--[3]	11.68	11.82	0.19
1987	9.74	9.28	9.28	--[3]	0.15
1988	9.13	9.11	8.83	9.29	0.27
Day 32:					
1986	11.35	--[3]	11.58	--[3]	0.19

[1]Standard error of mean.
[2]Difference between spring-cut and fall-cut silages ($P < 0.01$).
[3]No sample taken at this date.

Table 33. Serum magnesium in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	SE[1]
	mg/dl				
Initial:					
1986	2.25	--[5]	2.25	2.32	0.08
1987	1.75	1.63	1.81	1.63	0.06
1988	1.91	1.85	1.87	1.88	0.07
Day 11:					
1987[3]	1.88	1.69	1.88	1.86	0.04
1988[2]	1.91	1.60	1.84	1.92	0.09
Day 25:					
1986[4]	2.27	--[5]	2.18	2.53	0.08
1987	1.87	1.95	1.93	--[7]	0.06
1988[4]	1.97	1.65	1.62	1.91	0.10
Day 32:					
1986	2.25	--[5]	2.18	--[7]	0.08

[1]Standard error of mean.
[2]Difference between fescue hay and fescue silages ($P < 0.05$).
[3]Difference between fescue hay and fescue silages ($P < 0.01$).
[4]Difference between spring-cut and fall-cut silages ($P < 0.05$).
[5]No sample taken at this date.

Table 34. Serum phorphorus in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	mg/dl				
Initial:					
1986	3.45	--[4]	3.55	3.63	0.15
1987[2]	11.76	10.05	10.31	11.31	0.33
1988	11.03	11.26	11.65	10.99	0.69
Day 11:					
1987	11.40	11.46	11.90	13.31	0.50
1988	12.44	12.01	12.49	12.39	0.77
Day 25:					
1986[3]	3.40	--[4]	3.65	3.28	0.10
1987	13.24	11.50	11.69	--[4]	0.54
1988	12.24	12.35	12.78	12.06	0.66
Day 32:					
1986	3.85	--[4]	3.61	--[4]	0.11

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.05$).
[3]Difference between spring-cut and fall-cut silages ($P < 0.05$).
[4]No sample taken at this date.

Table 35. Serum potassium in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	SE[1]
	---------- mg/dl ----------				
Initial:					
1987	15.41	17.08	15.53	16.30	0.67
1988	15.34	15.56	16.18	16.36	0.51
Day 11:					
1987[2]	18.46	21.51	24.20	23.08	1.49
1988	17.40	17.28	18.92	17.79	0.65
Day 25:					
1987	25.47	28.29	27.30	--[3]	1.70
1988	17.28	17.98	18.13	17.52	0.48

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.05$).
[3]No sample taken at this date.

Sulfur. On day 25 of the 1987 trial, serum S was lower ($P < 0.01$) in steers fed the fescue diets as compared to those fed the orchardgrass/ alfalfa hay (Table 36). No influence of diet on serum S was measured in the 1988 trial.

Sodium. Concentration of Na in serum is generally about 300 mg/dl (Church, 1979). Sodium was within normal range in steers in the 1987 and 1988 trials (Table 37). In the 1988 trial, on day 25, Na was lower ($P < 0.05$) in steers fed fescue hay and silage as compared to those fed orchardgrass/alfalfa hay.

Copper. Serum Cu is normally about 0.1 mg/dl (Church, 1979). Values for serum Cu in this study were lower than the expected level, indicating a possible Cu deficiency in steers in both 1987 and 1988 (Table 38).

Iron. Normal serum Fe level is generally about 0.15 mg/dl (Church, 1979). This indicates that Fe was above normal in steers in the 1987 trial (Table 39). In the 1987 trial, serum Fe was significantly lower ($P < 0.01$) and numerically lower on days 11 and 25, respectively in steers fed the orchardgrass/alfalfa hay as compared to those fed fescue hay or silage. This effect was also measured in the 1988 trial. On day 11, serum Fe was lower ($P < 0.05$) in steers fed the orchardgrass/alfalfa hay as compared to those fed the fescue hay or silages and was lower ($P < 0.05$) in steers fed the silages as compared to those fed fescue hay.

Zinc. Normal Zn in serum ranges from 0.08 to 0.12 mg/dl (Church, 1979). Zinc was within normal ranges in steers on all diets in the 1987 and 1988 trials (Table 40). Serum Zn was lower ($P < 0.01$) in steers fed orchardgrass/alfalfa hay, compared to steers fed fescue diets on day 11 of the 1987 trial. On day 11 of the 1988 trial, Zn concentration in serum of steers fed orchardgrass/alfalfa hay was lower ($P < 0.05$), compared to steers fed fescue diets. Zinc concentration in serum of steers fed spring-cut fescue hay was higher ($P < 0.05$), compared to steers fed fescue silages.

Phase 2. **Ambient temperature.** Ambient temperature was 7.2 C on the day of the TRH challenge study.

Serum prolactin. Serum prolactin in steers fed orchardgrass/alfalfa hay was significantly higher ($P < 0.05$) as compared to those fed fescue silage or hay at time = 0, 30, 60, 90, 120, 150, 180

Table 36. Serum sulfur in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	mg/dl				
Initial:					
1987	80.00	79.15	80.02	74.54	1.14
1988	75.46	74.94	74.78	77.85	2.44
Day 11:					
1987	79.37	80.19	80.05	78.22	1.87
1988	79.71	81.44	78.84	81.01	2.64
Day 25:					
1987[2]	84.83	78.32	80.57	--[3]	0.92
1988	74.57	79.11	74.30	72.86	0.23

[1]Standard error of mean.
[2]Indicated difference between treatment means ($P < 0.05$).
[3]No sample taken at this date.

Table 37. Serum sodium in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	mg/dl				
Initial:					
1987	329.00	332.00	324.92	330.50	3.94
1988	331.65	328.70	333.25	329.65	4.05
Day 11:					
1987	302.78	310.30	313.92	305.87	4.22
1988	339.52	337.75	339.10	342.95	2.21
Day 25:					
1987[2]	313.40	307.30	298.68	--[3]	3.81
1988	334.17	341.37	345.42	341.67	2.23

[1]Standard error of mean.
[2]Indicated difference between treatment means ($P < 0.05$).
[3]No sample taken at this date.

Table 38. Serum copper in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	SE[1]
	mg/dl				
Initial:					
1987	0.07	0.06	0.06	0.06	0.01
1988	0.06	0.07	0.06	0.07	0.01
Day 11:					
1987	0.06	0.04	0.04	0.05	0.01
1988	0.05	0.05	0.04	0.05	0.01
Day 25:					
1987	0.04	0.03	0.04	--[2]	0.00
1988	0.05	0.05	0.04	0.05	0.01

[1]Standard error of mean.
[2]No sample taken at this date.

Table 39. Serum iron in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	SE[1]
	mg/dl				
Initial:					
1987	0.21	0.25	0.25	0.21	0.02
1988	0.12	0.17	0.13	0.20	0.02
Day 11:					
1987[2]	0.22	0.34	0.36	0.33	0.03
1988[3,4]	0.12	0.27	0.18	0.20	0.03
Day 25:					
1987	0.59	0.72	0.70	--[5]	0.11
1988	0.12	0.13	0.14	0.11	0.01

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.01$).
[3]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.05$).
[4]Difference between fescue hay and fescue silages ($P < 0.05$).
[5]No sample taken at this date.

Table 40. Serum zinc in steers fed endophyte-infected tall fescue hay and silage.

Item	Diet				SE[1]
	Hay		Silage		
	Orchardgrass-alfalfa	Spring-cut fescue	Spring-cut fescue	Fall-cut fescue	
	mg/dl				
Initial:					
1987	0.10	0.09	0.08	0.10	0.01
1988	0.06	0.06	0.06	0.06	0.01
Day 11:					
1987	0.12	0.13	0.12	0.13	0.01
1988[2,3]	0.05	0.06	0.07	0.07	0.00
Day 25:					
1987	0.14	0.13	0.14	--[5]	0.01
1988[4]	0.07	0.07	0.09	0.08	0.00

[1]Standard error of mean.
[2]Difference between orchardgrass/alfalfa hay vs. fescue hay and silage ($P < 0.05$).
[3]Difference between fescue hay and fescue silages ($P < 0.05$).
[4]Difference between spring-cut and fall-cut silages ($P < 0.05$).
[5]No sample taken at this date.

and 210 minutes (Fig. 6). Thyrotropin-releasing hormone was administered at time = 240 minutes. At time = 320 minutes, serum prolactin increased in steers fed fescue hay and spring-cut silage.

Basal serum prolactin (mean of time = 0 to time = 240 minutes) was significantly higher ($P < 0.05$) in steers fed orchardgrass/alfalfa hay as compared to those fed fescue hay or silage (Fig. 7). Average of serum prolactin, 1-hr post-TRH administration, was similar to basal levels in steers fed orchardgrass/alfalfa hay and fall-cut fescue silage. Serum prolactin in steers fed spring-cut fescue silage and hay increased by 24 and 20% units (expressed as a percent of basal orchardgrass/alfalfa hay), respectively. In the second hour post-TRH administration, serum prolactin was increased 30 and 127% units in steers fed fall-cut fescue silage and spring-cut fescue hay, respectively. But in steers fed spring-cut silage, serum prolactin had decreased to basal levels by 2-hr post-TRH. Steers fed orchardgrass/alfalfa hay did not appear to respond to TRH.

DISCUSSION

Environmental Temperature. Ambient temperature was cool and animals did not experience heat stress. High temperatures have been associated with increased basal prolactin in non-infected but not in endophyte-infected tall fescue (Hurley el al., 1981). Substance(s) in endophyte-infected tall fescue may block temperature related increases in basal prolactin (Hurley el al., 1981).

Diet. The orchardgrass/alfalfa hay appeared to be higher in quality than the fescue diets. The spring-cut fescue hay and silage were similar in fiber composition, indication that ensiling did not change the quality of the fescue. However, the fall-cut fescue silage appeared to be higher in quality as compared to the spring-cut fescue silage. The fall-cut silage had lower NDF, ADF, cellulose, hemicellulose and lignin and higher TNC compared to the spring-cut silage. In cooler temperatures, plant respiration is slowed more than photosynthesis (Heath et al., 1985). Tall fescue stockpiled in the fall accumulates carbohydrates, which may lead to increased quality. Ensiling did

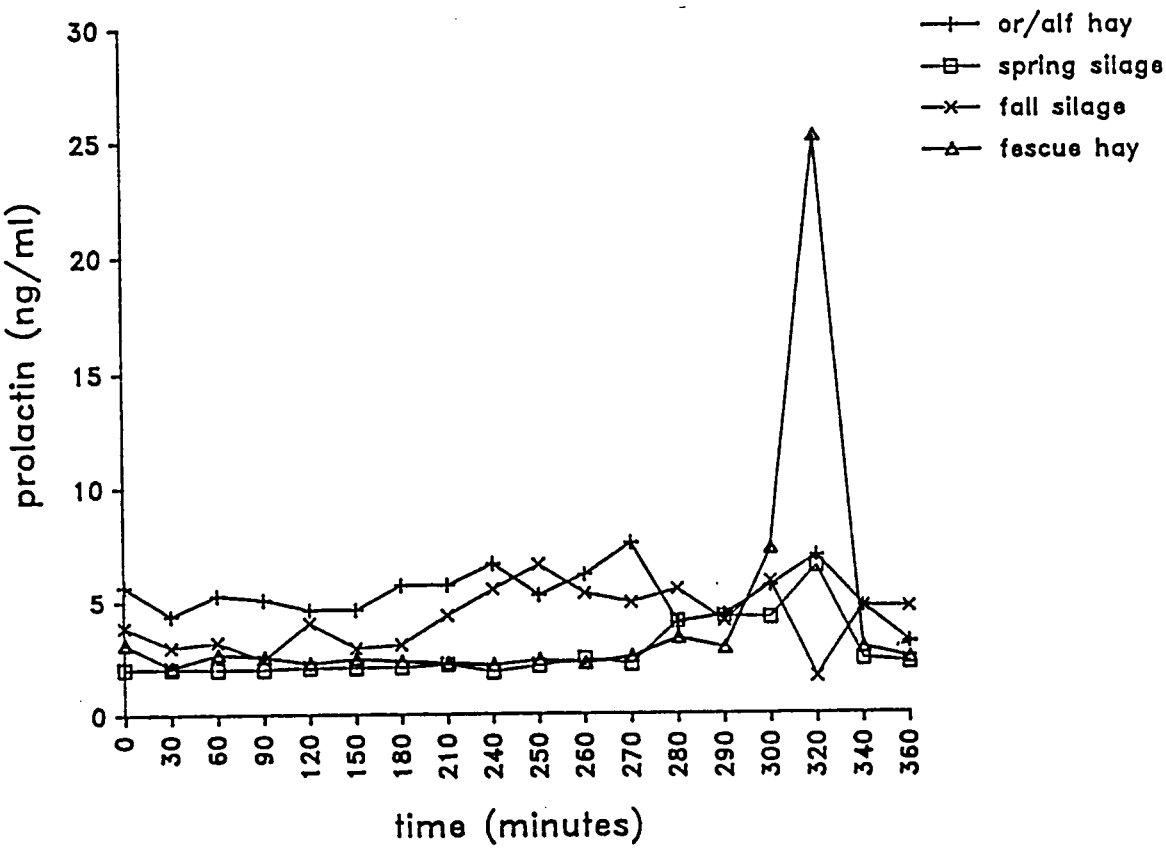

Figure 6. **Serum prolactin in steers administered thyrotropin-releasing hormone:** Thyrotropin-releasing hormone administered at time = 240 minutes to steers fed orchardgrass/alfalfa (or/alf) hay as compared to endophyte-infected tall fescue ensiled in the spring or fall and endophyte-infected tall fescue hay cut in the spring.

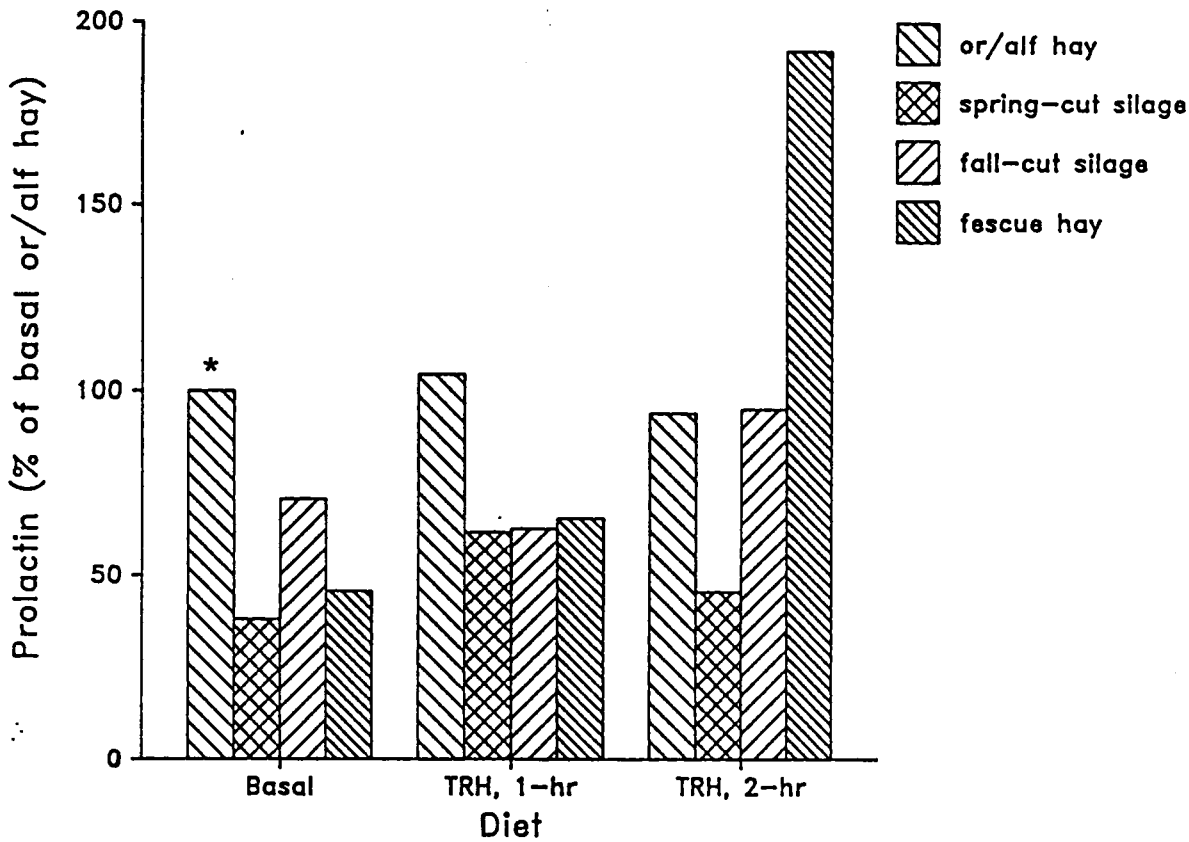

Figure 7. **Basal and thyrotropin-releasing hormone (TRH)-stimulated serum prolactin in steers:** Basal serum prolactin, 1-hr post-TRH and 2-hr post-TRH administration to steers fed orchardgrass/alfalfa (or/alf) hay as compared to endophyte-infected tall fescue ensiled in the spring or fall or spring-cut hay. Standard errors of treatment means were 7.0, 2.8 and 3.8 for basal, 1-hr post TRH and 2-hr post TRH, respectively. * Indicates difference of means of orchardgrass/alfalfa hay vs. fescue hay and silages ($P < 0.05$).

not appear to influence the levels of fiber components. Fiber components were lower in fall-cut fescue silage as compared to spring-cut silage, indicating a higher quality. Copper concentration in all diets was low and may indicate a Cu deficiency (NRC, 1985). The fescue hay and silages contained ergopeptine alkaloids. The spring-cut silage had the highest concentration and the orchardgrass/alfalfa hay had the lowest concentration of ergopeptine alkaloids.

The presence of ergopeptine alkaloids in the orchardgrass/alfalfa hay was unexpected. The value reported could be an artifact of the procedure. It is possible however, that the alkaloids are actually present. Orchardgrass is a small genus in the *Festuceae* tribe (Heath et al., 1985). The endophyte in tall fescue belongs to the *Balansia* family, these species have been isolated from most of the *Festuceae* tribe (Bacon et al., 1986). Weeds in the orchardgrass/alfalfa hay are endophyte-infected and contain the ergopeptine alkaloids. Many grass-like weeds found in pastures can be infected by endophytic fungi (Bacon et al., 1986). The presence of endophyte-infected grass-like weeds in the hay could also account for the alkaloids detected in the orchardgrass/alfalfa hay.

Serum prolactin and cholesterol. Time of ensiling or method of preservation (silage vs. hay) did not appear to influence serum prolactin in steers. Prolactin tended to be lower in steers fed fescue hay and silages as compared to orchardgrass/alfalfa hay. Results reported from this study support observations in the literature. Hurley and coworkers (1981) reported suppressed serum prolactin in Holstein calves fed toxic 'G1-307' tall fescue (1.8 mg/ml) as compared to serum prolactin in calves fed 'G1-306' (6.0 mg/ml). Thompson and coworkers (1987) reported suppressed serum prolactin in steers grazed on endophyte-infected tall fescue.

Cholesterol also tended to be higher in steers fed orchardgrass/alfalfa hay as compared to those fed fescue hay or silage. Results from this study support the work of Stuedemann and coworkers (1986). They reported lower cholesterol in steers fed tall fescue that was 76% infected with the endophyte as compared to levels in steers fed non-infected tall fescue. These researchers attributed the decline in serum cholesterol to the accumulation of cholesterol esters in necrotic fat, leading to lipomatosis. Fat necrosis was not observed in the steers from the present study.

Prolactin may trigger biochemical events that change serum lipid concentration. Prolactin has been reported to influence the amount of cholesterol substrates available for steriod synthesis

(Armstrong et al., 1970). These changes appear to occur as a result of the action of serum prolactin on the number of prolactin-recepters in cell membranes. Prolactin acts to regulate its own uptake by modulating the prolactin binding sites. Changes in prolactin recepter levels is related to changes in the fluidity of membranes. Administration of ovine prolactin to adult male rats increased serum lipid fluidity in a dose-dependent response (Dave and Witorsch, 1985). Prolactin also increased cholesterol levels in corpora lutea of hypophysectomized rats (Armstrong et al, 1970). Akers and Keys (1984) reported lowered rates of fatty acid biosynthesis in tissue from cows with low prolactin. They also measured lower activities of acetyl coenzyme A carboxylase and fatty acid synthetase. Acetyl coenzyme A carboxylase acts in the initial conversion of acetyl coenzyme A to cholesterol precursers (McGilvery, 1983). The lower prolactin and cholesterol in steers fed endophyte-infected tall fescue may be related.

Ergot alkaloids have been reported to inhibit prolactin secretion in cattle (Karg and Schams, 1974; Smith et al., 1974). Tissue from cows treated with CB154 (2-Br-alpha-ergokryptin) had lower rates of fatty acid synthesis as compared to the control (Akers et al., 1981). Activities of acetyl CoA carboxylase and fatty acid synthetase were lower in tissue from cows treated with CB154. The ergopeptine alkaloids are similar in structure to the ergot alkaloids. In the present study, the orchardgrass/alfalfa hay contained the lowest amount of ergopeptine alkaloids compared to the fescue hay and silages. Steers fed the orchardgrass/alfalfa hay had higher prolactin and cholesterol as compared to those fed the endophyte-infected fescue hay or silage. These results may be explained by the following mechanism. Prolactin acts on membranes to increase the number of prolactin-receptor sites in membranes, this action increases the lipid fluidity of the membrane, activating enzymes involved in cholesterol biosynthesis. Ergopeptine alkaloids may suppress prolactin to levels which inhibit the above sequence of events, resulting in decreased cholesterol synthesis and lowered serum cholesterol levels.

Blood hematocrit and hemoglobin. Hemoglobin levels in steers in the present study were within the expected range, of 11 to 12 g/dl (Church and Pond, 1978). Early work by Jacobson and coworkers (1963) indicated that ethanol extract of toxic tall fescue, 'G1-43' caused elevated hematocrit and hemoglobin. But, they also reported a depression of hemoglobin in steers admin-

istered ethanol extract of 'Kentucky-31'. Jacobson and coworkers (1963) concluded that the changes in blood components were not related to the toxicity of the forage or extract.

Serum minerals. Serum Ca tended to be higher in steers fed fall-cut silage as compared to spring-cut silage, reflecting the higher dietary Ca in the fall-cut silage. Excess dietary PO_4 and Mg can depress Ca absorption (Church, 1979) but do not appear to be involved in the present study. Over the 3-yrs of trials, two trends in serum Mg were observed. Serum Mg in steers fed fall-cut silage was higher than those fed spring-cut silage and serum Mg was higher in steers fed the silages as compared to the fescue hay. Dietary Mg (1988) was higher in fall-cut as compared to spring-cut silage and also higher in silages as compared to fescue hay. High K has been shown to decrease apparent absorption of Mg (Fontenot et al., 1960). But, in the present study, all diets were similar in K concentration. High P and low Ca may depress serum Mg (Church, 1979). These factors do not appear to be causitive agents in the present study, since the orchardgrass/alfalfa hay had the highest P and Ca but also had high levels of serum Mg. Magnesium absorption is enhanced by increasing Mg and Ca intake (Church, 1979). Phosphorus absorption is directly related to dietary P (Church and Pond, 1978). Levels of P in the serum were above the normal range expected in the 1987 and 1988 trials, because total rather than inorganic P was measured by the procedure used. The influence of experimental diet on serum K is not clear. In the 1987 trial, serum K values were above normal. Ruminants can consume large quantities of K without detrimental effects (Church, 1979). However, high dietary K can depress Mg absorption (Church, 1979). Serum Cu levels indicate a deficiency of Cu in the diet. Copper interacts with other minerals, decreasing the availability of Cu. Sulfur may decrease available Cu by reacting with Cu to form cupric sulfide, a relatively insoluble compound. Dietary Ca and Zn also influence Cu absorption and utilization. Iron was higher in fescue hay and silage as compared to orchardgrass/alfalfa hay and higher in fescue hay as compared to the silages. The diets in 1988 were within the requirements of cattle.

TRH-stimulated Prolactin. Basal serum prolactin concentration in Holstein calves was 2.3 in non-infected and 1.8 ng/ml in endophyte-infected tall fescue (Hurley et al., 1981). Thyrotropin-releasing hormone stimulated serum prolactin was 5.6 and 45.7 ng/ml in calves fed endophyte-infected and non-infected tall fescue, respectively. Ambient temperature was 10 C,

which was similar to that of the present study, 7 C. The present study did not include a non-endophyte infected tall fescue control, but basal prolactin was higher in steers fed orchardgrass/alfalfa hay compared to steers fed endophyte-infected tall fescue hay or silages. Wallner and coworkers (1983) reported no response of serum prolactin to TRH in Holstein cows administered endophyte culture medium that contained 0.11 mg/ml of ergopeptine alkaloids (ergonovinine, ergonovine, elymoclavine and chanoclavine). In the present study, there was no response in serum prolactin concentration in steers fed orchardgrass/alfalfa hay. The orchardgrass/alfalfa hay contained the lowest ergopeptine alkaloid concentration, compared to the other diets fed to steers in the study. Ergopeptine alkaloid concentration was highest in the spring-cut fescue silage. Serum prolactin in steers fed spring-cut fescue silage had returned to basal levels with 2-hr after TRH administration.

Results support the hypothesis that compound(s) in endophyte-infected tall fescue suppress basal serum prolactin. It is spectulated that ergopeptine alkaloids in endophyte-infected tall fescue hay and silage may act to depress serum prolactin. Results indicate there may be a difference in response to TRH-stimulated serum prolactin in steers fed spring-cut vs. fall-cut tall fescue silage.

Thompson and coworkers (1987) reported maximal TRH-stimulated prolactin within 10 minutes after administration of the hormone. In the present study there appeared to be a delayed response of serum prolactin to TRH in steers fed fescue hay. No response was measured on serum prolactin to TRH in steers fed orchardgrass/alfalfa hay. It appears that the orchardgrass/alfalfa hay may contain other compounds that also act to supress prolactin.

Results from this study indicate endophyte-infected hay and silage may change the levels of serum prolactin and cholesterol in steers. Ensiled endophyte-infected tall fescue contained a higher concentration of ergopeptine alkaloids, compared to the fescue hay. Time of ensiling (spring vs. fall) did not appear to change the toxicity of the forage to steers. Differences in the mineral compostion of hays and silages were reflected in serum mineral levels in steers.

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EVALUATION OF A DISC METER FOR USE IN ESTIMATING YIELD OF TALL FESCUE SWARDS

INTRODUCTION

Destructive and non-destructive methods are available for the determination of forage sward yield. The selection of the appropriate method will depend on many factors including the objectives of the particular research, the sample unit, the accuracy required and the available facilities and labor. Direct measurement of yield can be accomplished by cutting forage either by hand or with a power-driven tool such as a tractor or lawn mower, from a known area. Problems with this method include variation in cutting height with hand-cut forage, restriction of cutting height with mechanical devices, contamination of sample with litter, soil or fecal material and intensive labor.

In pastures, forage availability is generally not uniform over the entire field as a result of grazing patterns, selective grazing and soil-plant factors that may limit or promote plant growth.

Non-uniform areas of forage availability in pastures may bias the estimation of yield, unless many areas in the pasture are sampled. High labor and equipment investments with direct measurement of yield may limit the number of samples that can be taken in a pasture and, compromise the precision of this method due to inadequate sampling.

Non-destructive methods indirectly estimate yield and as such include unavoidable errors. Visual estimation is the simplest and with training and practice can be accomplished with precision (Haydock and Shaw, 1975). Other methods use capacitance (Burzlaff et al., 1973), beta-attenuation and spectral analysis ('tMannetje, 1978).

Plant height and density have also been related to forage yield (Baker et al., 1981). Most procedures available that are based on the relationship between plant height and yield require direct yield measurement to calibrate and convert the readings to yield estimates. Michalk and Herbert (1977) reported a positive correlation between dry matter yield and forage height times percentage ground cover. These researchers used a plywood board with a hole cut in the center to measure the height of the board above the forage. Others have used aluminium discs (Castle, 1976) and hardboard paneling (Baker et al., 1981).

The estimation of yield with a disc meter has several advantages over destructive methods of yield determination. With the disc meter, many estimates can quickly and easily be made over a large area. In addition, areas of pastures not easily acessible to harvesting equipment could be estimated with the disc meter. The disc meter is light-weight and easy to transport. Estimation of yield with a disc meter would simplify and potentially improve the estimation of the yield over a large pasture.

The disc meter method needs to be evaluated under Virginia conditions. The influence of N fertilization of tall fescue on the accuracy of this method should be measured. In addition, the influence of infection of tall fescue with the endophyte, ***Acremonium coenophilum*** on disc meter estimate of yield should be measured. Researchers have reported changes in morphology of tall fescue infected with the endophyte. Arechareleta and coworkers (1987) reported endophyte-infected plants had thicker and more narrow leaves than non-infected plants. Endophyte-infected plants have also been reported to have more upright growth and deeper placement of crowns in the soil as compared

to non-infected plants (Hill et al., 1987). These changes could influence the density of the sward and bias yield estimates by the disc meter method.

The objectives of this project were: 1) to examine the correlation between a disc meter and yield of tall fescue harvested from a known area and 2) elucidate the effects of growth stage, location, endophyte infection and N fertilization on the accuracy of disc meter estimates of yield.

MATERIALS AND METHODS

The experimental design was a split-plot with endophyte infection as the main plot and N fertilization as subplots. The experiment was replicated at two sites, Glade Spring (Ridge and Valley physiographic region) and Blackstone (Southern Piedmont region), VA. Tall fescue was established as described previously in the section titled, 'Tall fescue growth and chemical composition as affected by growth stage, site, endophyte infection and nitrogen fertilization'. The high and low endophyte infection levels were 73 and 2% Glade Spring and 81 and 0% at Blackstone, respectively. Endophyte treatments, were randomized within four blocks, for a total of eight plots at each site. Each plot was 3 by 6 m was and divided into three equal subplots (2 x 3 m, each). Plots were mowed (Gravelly tractor) to remove excess and overmature forage prior to each N application. Nitrogen treatments (0, 40 and 80 kg N/ha as ammonium nitrate) were randomly assigned to the subplots in each main plot. Nitrogen treatments were applied on 13 August 1987, 22 March 1988 and 16 May 1988 to Glade Spring and on 20 August 1987, 14 March 1988 and 13 May 1988 to Blackstone.

Plant density was measured with a disc meter contructed as described by Castle (1976) and Baker et al. (1981) with the following modifications. The disc meter used in this study was made of a shaft of PVC pipe over which was fitted a free sliding PVC pipe collar. One end of the pipe was fitted with a cap to prevent the collar from sliding off. The sliding shaft was marked in 0.5 cm

intervals for ease in determining the height of the sward under the disc. The disc was 51-cm by 51-cm square of clear plastic plexiglass which weighed 2 kg.

Plant density was determined by taking five disc meter readings in each plot at each sampling date. To determine plant density, the disc was allowed to settle to a constant position above the plant canopy, the height of the disc at this position was recorded. Immediately following disc meter measurement, yield was determined by clipping the forage in a 0.5 m^2 area with hand-operated sheep shears. The clipping height was 3-cm. Measurements with the disc meter and clipped yield were sampled on: prebloom (9 May 1988, Glade Spring and 4 May 1988, Blackstone), full bloom, hay-cut stage (16 May 1988, Glade Spring and 13 May 1988, Blackstone), spring growth after hay-cut (24 June 1988, Glade Spring and 22 June 1988, Blackstone) and summer growth after hay-cut (3 August 1988, Glade Spring and 6 August 1988, Blackstone).

Two regression equations to predict yield from disc meter measurements were chosen from the literature. The regression line that best fit the data reported by Castle (1976) was linear. The regression equation was:

$$\text{yield} = a + bx$$

where x= disc meter reading, a=intercept and b=slope. Coeffieients of regression equation were used from the pooled data from perennial ryegrass hay experiments over a range of N fertilization treatments. The intercept used was -1.69 and the slope used was 1.38.

Baker and coworkers (1981) developed a regression equation to estimate yield of mixed swards. This method uses 14 categories based on the growth stage and relative percentages of grass and lequmes in the pasture. The dominant grass was orchardgrass (***Dactylis golmerata*** L.) but, timothy (***Phleum pratense*** L.), bluegrass (***Poa*** species) and meadow fescue (***Festuca elatier*** L.). legumes in the pastures included red clover (***Trifolium pratense***), alfalfa (***Medicago sativa***) and white clover (***Trifolium repens*** L.). Slopes of the regression lines of each category were similar but the intercepts differed. The regression equation used to describe the relationship between yield and disc meter readings was:

$$Yield = a + bx + cx^2$$

where x = disc meter reading, a = intercept for the category, b = 183 and c = -1.73. The relationship between dry matter yield and disc meter reading was curvilinear but linear for forage yield less than 4000 kg/ha. Both regression equations were used to predict yield from the disc meter readings.

Statistical analysis. Possible treatment effects of endophyte level and N fertilization on yield and disc meter reading were examined using an analysis of variance (SAS, 1985) for a split-plot design. Data were analyzed separately with regard to sampling date. The main effect of site was tested with the error term, block (nested within site). The main effect of endophyte level and the two-way interaction of site and endophyte level were tested with the error term, endophyte by block (nested within site). The main effect of N fertilization rate and the two-way interaction of site by N fertilization rate were tested with the error term, N fertilization by block (nested within site). The two-way interaction of endophyte level by N fertilization and the three-way interaction of endophyte level by N fertilization by site were tested using the residual error term. Regression analyses were performed to examine the relationship of disc meter reading and yield using the general linear model (SAS, 1985). Criteria for the 'best fit' line through the data utilized the concept of least squares. With this method, the vertical deviation of each point from the regression line is considered. The 'best fit' regression has the smallest value for the sum of squares of these deviations which are called the residual sum of squares. The homogenity of the variences, called homoscedasticity, indicates that each of the sample variences is an estimate the sample population varience. Homoscedasticity can be checked by plotting the residual sum of squares as a function of corresponding disc meter reading. If homoscedasticity exists, the residuals should be evenly distributed above and below zero (ie at zero, actual yield equals predicted yield). If the distribution is patterned in someway, this indicated a linear regression is an improper model to describe the data. The regression coefficient is the slope of the 'best fit' regression line. The coefficient of determination (r^2) was used as a measure of the strength of the straight line relationship. Both linear and quadratic prediction equations were considered.

RESULTS AND DISCUSSION

Measured yield. Measured yield of tall fescue was affected by growth stage at harvest ($P < 0.01$), site ($P < 0.01$), endophyte infection ($P < 0.05$) and N fertilization ($P < 0.01$) (Table 41). Tall fescue measured yield was highest ($P < 0.01$) at bloom and lowest at 5-wk regrowth. Averaged over growth stage, endophyte level and N fertilization, measured yield was higher ($P < 0.01$) at Glade Spring (2744 kg/ha), compared to Blackstone (2227 kg/ha). Yield of low endophyte-infected tall fescue (2663 kg/ha) was higher ($P < 0.05$), compared to high endophyte-infected (2308 kg/ha). Nitrogen fertilization linearly ($P < 0.01$) increased measure yield of tall fescue as both locations. Two-way interactions of date with site ($P < 0.01$) and N fertilization ($P < 0.01$) were present. Tall fescue yield was significantly higher ($P < 0.05$) at the Glade Spring as compared Blackstone at pre-bloom and 10-wk regrowth after cutting at the bloom stage. Yield of tall fescue was numerically higher at the bloom stage but not at the 5-wk growth stage at Glade Spring, compared to Blackstone. Nitrogen fertilization increased ($P < 0.05$) yield of tall fescue at each of the four harvests. Yield of tall fescue was influenced by endophyte infection at 5 and 10-wk regrowth after cutting. Yield of low endophyte-infected (LE) tall fescue was 66% higher ($P < 0.05$) than that of high endophte-infected (HE) tall fescue at 5-wk regrowth and 87% higher at 10-wk regrowth stage.

Disc meter reading. Disc meter measurement of tall fescue was affected by growth stage at harvest ($P < 0.01$) and N fertilization ($P < 0.01$) (Table 41). As with measured yield, the disc meter readings were highest ($P < 0.01$) at bloom and lowest at 5-wk regrowth at harvest. Nitrogen fertilization increased ($P < 0.01$) disc meter readings. This affect was also determined with measured yield. Two-way interactions of growth stage with site, endophyte level and N fertilization were present. An interaction of site with N fertilization was also present. Disc meter reading was significantly higher ($P < 0.05$) at the prebloom growth stage for Glade Spring as compared to Blackstone. The readings were numerically but not significantly higher at bloom and 10-wk regrowth stages, but not 5-wk regrowth stage at Glade Spring, compared to Blackstone. Plant density

Table 41. Yield and disc meter readings of tall fescue as affected by growth stage, site, endophyte infection and N fertilization

	Nitrogen fertilization, kg/ha						
	0		40		80		
Growth stage[1] Site	HE[2]	LE[3]	HE	LE	HE	LE	SE[4]
	- - - - - - Yield (kg/ha, dry basis) - - - - - - - -						
Prebloom[5]							
Glade Spring[6,7]	2102	3138	3747	3752	4694	3835	464
Blackstone[6,7]	813	2298	2250	3331	3169	3501	383
Bloom							
Glade Spring[6,7]	3172	3735	4696	4784	5343	5560	452
Blackstone[6,7]	1651	3322	4580	4354	4676	5539	433
Regrowth, 5-wk							
Glade Spring[6,7]	342	569	491	1045	740	1173	165
Blackstone[6,7]	220	375	790	1073	1033	1198	91
Regrowth, 10-wk[9]							
Glade Spring[6,7]	1435	1736	2264	2645	22451	2418	250
Blackstone[6,7]	552	777	1411	1704	1769	2053	159
	- - - - - Disc meter (cm) - - - - - - - -						
Prebloom[9]							
Glade Spring[6,7,8]	23.2	27.7	28.1	31.0	28.8	29.6	1.3
Blackstone[6,7,8]	16.1	23.8	24.9	28.5	31.6	30.7	1.4
Bloom							
Glade Spring[6,7]	26.7	24.4	32.4	30.7	34.3	30.8	2.4
Blackstone[6,7]	19.3	22.2	29.7	26.8	35.6	31.1	1.6
Regrowth, 5-wk							
Glade Spring	6.9	7.1	7.3	8.0	6.9	8.2	0.7
Blackstone[6,7]	6.4	6.0	8.7	8.5	9.4	9.1	0.4
Regrowth, 10-wk							
Glade Spring	9.8	9.2	10.5	10.3	11.5	11.4	0.9
Blackstone[6,7]	9.2	8.0	10.2	10.3	11.1	12.6	

[1]Means of bloom > stockpiled, prebloom > 10-wk regrowth > 5-wk regrowth (Tukey pairwise comparison, $P < 0.01$).

[2]HE = high (77%) endophyte-infection.
[3]LE = low (1%) endophyte-infection.
[4]Standard error of mean.
[5]Indicates means of site differ ($P < 0.05$), averaged over endophyte level and N fertilization.
[6]Indicates effect of N fertilization ($P < 0.01$).
[7]Indicates linear effect of N fertilization ($P < 0.01$).
[8]Indicates effect of endophyte level.
[5]Indicates means of site differ ($P < 0.01$), averaged over endophyte level and N fertilization.

as measured by the disc meter was also increased ($P < 0.05$) by N fertilization at prebloom, bloom, 5-wk regrowth and 10-wk regrowth stages. The disc meter readings were only influenced by endophyte-infection at the prebloom stage. The disc meter reading at prebloom stage for the LE tall fescue was 28.4 cm, averaged over site and N fertilization, was higher ($P < 0.05$) as compared to the HE which was 25.4 cm. In general, effects of site, endophyte level and N fertilization on tall fescue yield at four growth stages were also reflected in the disc meter reading.

In the present study, each vertical cm of sward measured by the disc meter corresponded to approximately 133 kg dry matter/ha. Calculations from the data of Baker and coworkers (1981) show that 1-cm measured 150 kg/ha and from Castle (1976), 1-cm corresponded to 160 kg dry matter/ha. The amount of forage estimated by 1-cm of the disc meter in the present study was similar to reports in the literature. Differences in the literature may be related to differences in the disc meters or in the morphology of the different forages measured.

Regression equations. Plotting disc meter readings vs. measured yield revealed a positive relationship between these values (Fig. 8). Linear and quadratic regression equations were fit to the data. The formula for the linear equation was:

$$y = 140.6x - 106.3$$

The linear regression line and actual data points are presented in Fig. 9. The coefficient of determination (r^2) was 0.72. Plotting the residual sum of squares vs. the disc meter readings showed a distribution of data above and below the zero line (Fig. 10). This indicates a good fit of the data. A quadratic regression was also fit to the data, but there was no improvement in the r^2. The formula of the quadratic line was:

$$y = 0.4x^2 + 124.6x + 10.$$

A positive, linear relationship between yield and disc meter readings was measured. This agrees with the work of Castle (1976) and Earle and McGowan (1979). Others have reported a

curvilinear response at high yields (Bransby et al., 1977; Baker et al., 1981). A quadratic regression equation may predict yield more accurately when the response is curvilinear.

In the present study, the coefficient of determination was 0.72, indicating that the regression model did not explain 28% of the variation between yield and forage density as measured by the disc meter. Castle (1976) reported a r^2 of 0.80 in hay fields but only 0.61 in grazing experiments. Standard error was also high in the grazing experiments, ranging from 403 to 694 kg dry matter/ha. Baker et al. (1981) reported the regression explained 82.4% of the variation in the model. These workers had 708 paired observations of yield and disc meter readings. The higher number of observations probably provided a better estimate of the population and a better fit of the regression to the data.

The literature differs concerning the use of a universal regression equations for all pastures. Bransby and coworkers (1977) and Earle and McGowan (1979) suggest that the meter should be recalibrated for conditions at each location. More recently, Baker and coworkers (1981) suggest that recalibration is not necessary. These researchers did not measure any change in the slope of the regression line in forage in the reproductive as compared to the vegetative growth stages. Considering the precision of the method, recalibration does not appear to be necessary.

Predicted yield. Four regression equations were fit to the data, the linear and quadratic equations suggested by the data, and two equations from the literature; the linear formula of Castle (1976) and the quadratic equation of Baker and coworkers (1981) (Fig. 11). All four equations for predicted yield were effected by growth stage ($P < 0.01$). Interactions of growth stage with site ($P < 0.01$) and endophyte level ($P < 0.01$) and site with N fertilization ($P < 0.01$) were present. At prebloom, predicted yields were higher ($P < 0.01$) at Glade Spring, compared to Blackstone. This agrees with measured yield data. At bloom and 5-wk regrowth, site and endophyte infection did not significantly influence predicted yields. Nitrogen fertilization increased measured and predicted yields ($P < 0.01$) at bloom, 5-wk and 10-wk regrowth. Predicted yield of 10-wk regrowth was numerically but not significantly higher at Blackstone, compared to Glade Spring.

Effects of growth stage, site, endophyte level and N fertilization on disc meter readings were reflected in predicted yields. All four equations for predicted yield were effected by N fertilization

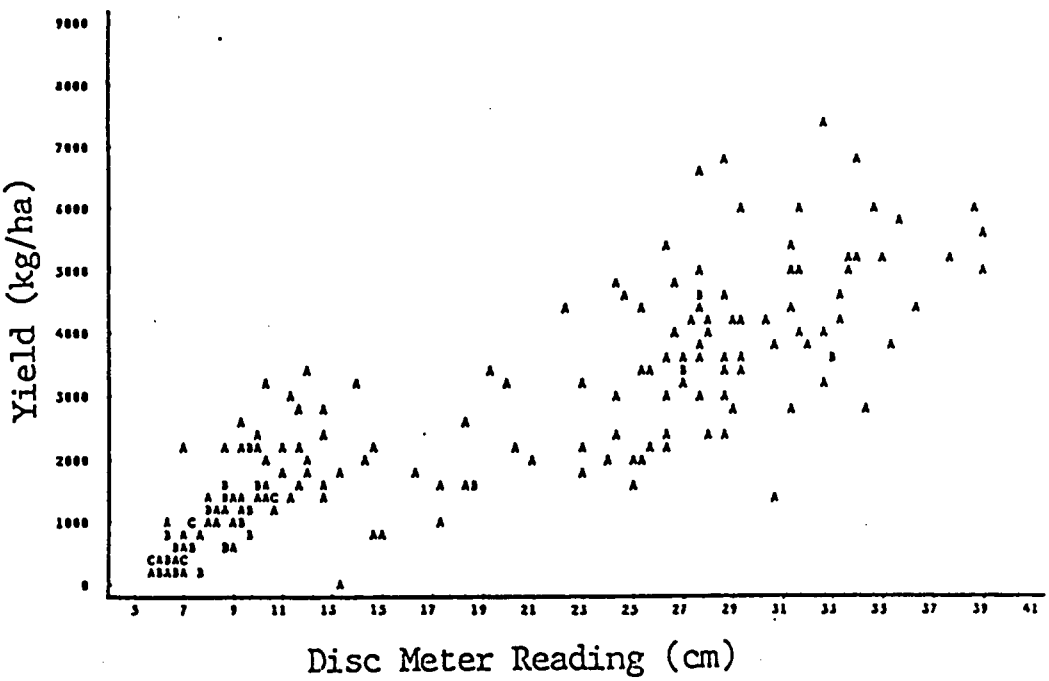

Figure 8. Relationship between disc meter reading and measured yield.: Letters represent data correlation of disc meter reading and measured yield The letter 'A' represents 1 observation, 'B' represents 2 observations, 'C' represents 3 observations at the indicated data point. Samples were taken at two locations, from plots fertilized at three rates of N (0, 40 and 80 kg/ha), with two levels of endophyte (1% and 77%, average) at four sampling dates over a four month period in 1988.

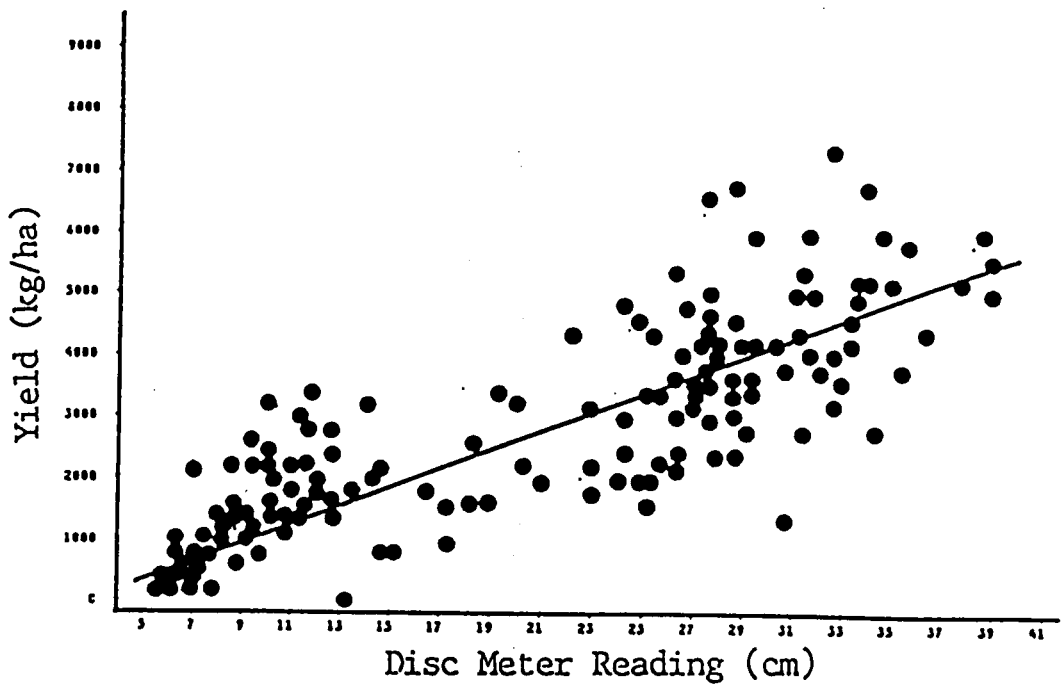

Figure 9. Linear regression line fit to disc meter readings and yield.: Bullets represent actual data points. Line is the linear regression of the data. Equation of the line: $Y = 140.6x - 106.3$ where y = yield and x = disc meter reading. The coefficient of determination ($r^2 = 0.72$).

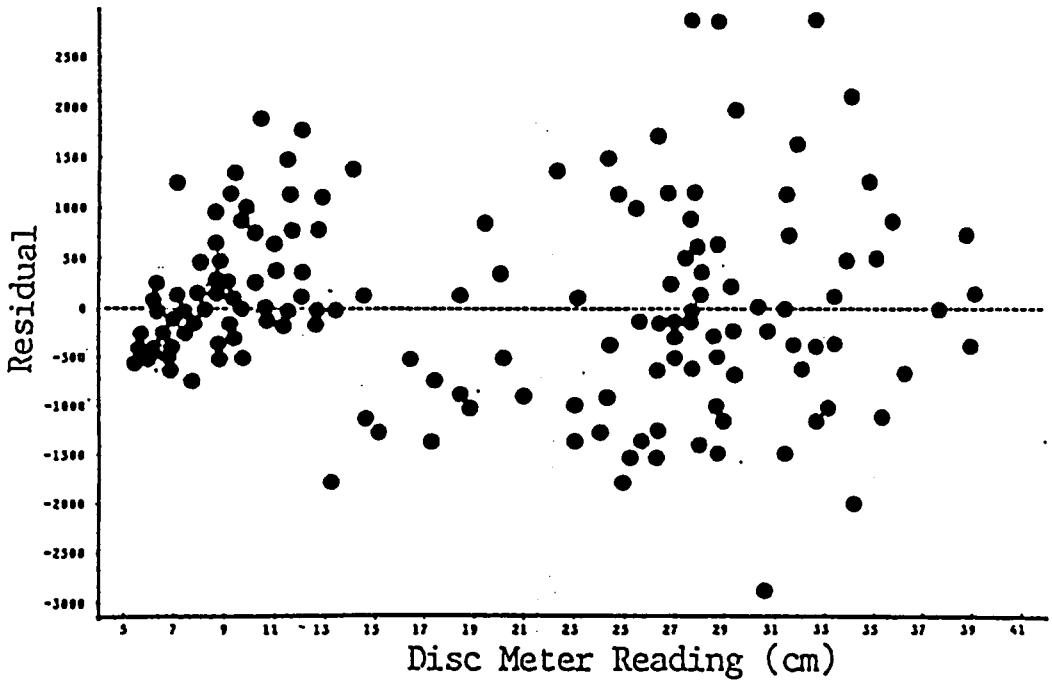

Figure 10. Disc meter reading and residual sum of squares for linear regression

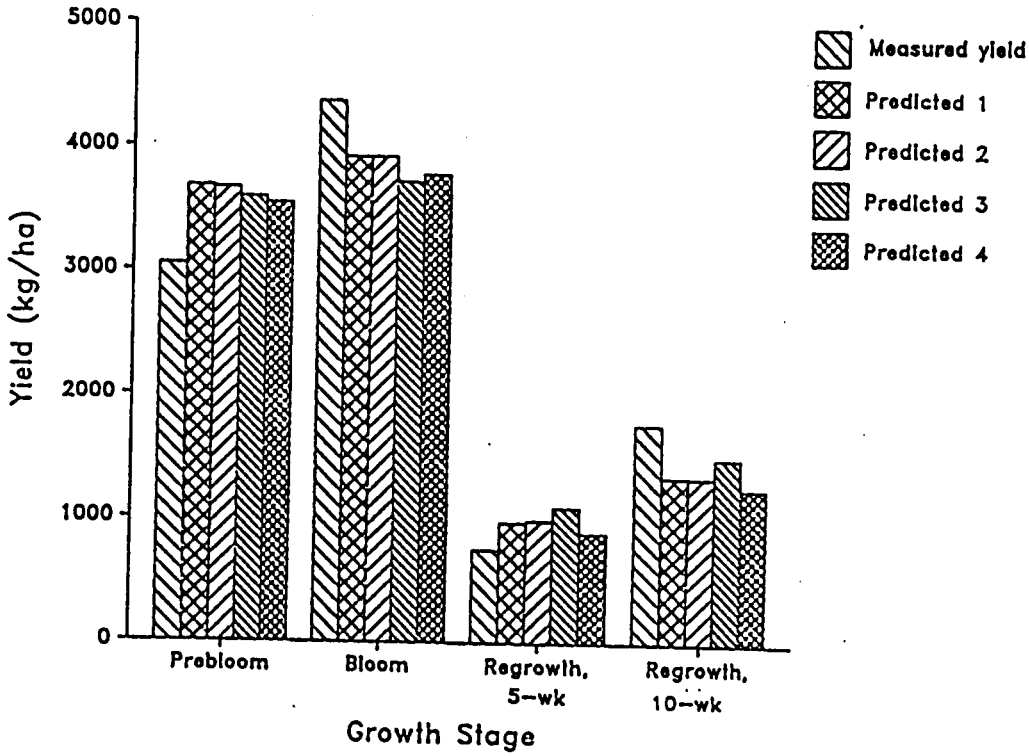

Figure 11. Comparison of measured yield and predicted yield from disc meter readings at four growth stages: Measured yield determined by harvesting, drying and weighing forage cut from a known area. Predication equation 1: linear regression of data, $y = 140.6x - 106.3$; predication equation 2: quadratic regression line, $y = 0.4x^2 + 124.6x + 10$; predication equation 3: $y = -1.73x^2 + 183x - 39$ for prebloom and bloom growth stages and $y = -1.73x^2 + 183x - 202$ for regrowth stages (Baker et al., 1981); predication equation 4: $y = 1.38x - 1.69$ (Castle, 1976) where y = yield and x = disc meter reading.

($P < 0.01$) (Fig. 11). Predication equations over estimated yield at prebloom and 5-wk regrowth and under estimated yield at bloom and 10-wk regrowth. Predication equations under estimated yield at Glade Spring and over estimated yield at Blackstone (Fig. 12). Predication equations over estimated yield of high endophyte-infected tall fescue and under estimated yield of low endophyte-infected tall fescue (Fig. 13). The predicted yield of low endophyte-infected tall fescue was higher ($P < 0.05$), compared to high endophyte-infected tall fescue. This trend was also observed with measured yield, although the difference between high and low endophyte-infected tall fescue was not significantly different. Effect of N fertilization on measured yield were also reflected in predicted yield (Fig. 14). Prediction equations over estimated yield of tall fescue not fertilized with N and under estimated yield of tall fescue fertilized with 40 and 80 kg/ha.

Effect of growth stage, site, endophyte and N fertilization on disc meter readings were reflected in predicted yields. Effect of N fertilization on measured yield were also reflected in predicted yields. A high level of accuracy for the estimation of yield by the disc meter can not be claimed. The disc meter would be preferable to visual estimation of yield because it is unbiased and requires little training prior to use. The disc meter is inexpensive and simple to use. This method is particularly useful when non-destructive measures of yield are necessary or when many estimates are required. The disc meter may be useful as a tool to determine forage availability and help to make descisions of when to begin and end grazing in intensive rotation grazing systems. The meter could also be helpful to evaluate pasture and hay field productivity.

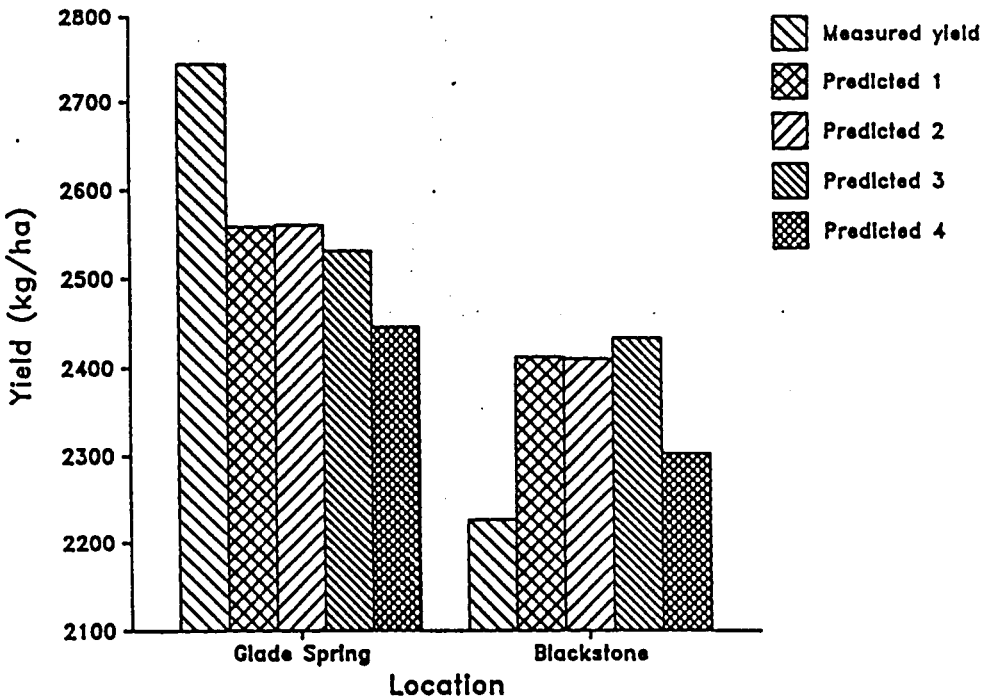

Figure 12. **Comparison of measured yield and predicted yield from disc meter readings at two locations:** Measured yield determined by harvesting, drying and weighing forage cut from a known area. Predication equation 1: linear regression of data, $y = 140.6x - 106.3$; predication equation 2: quadratic regression line, $y = 0.4x^2 + 124.6x + 10$; predication equation 3: $y = -1.73x^2 + 183x - 39$ for prebloom and bloom growth stages and $y = -1.73x^2 + 183x - 202$ for regrowth stages (Baker et al., 1981); predication equation 4: $y = 1.38x - 1.69$ (Castle, 1976) where y = yield and x = disc meter reading.

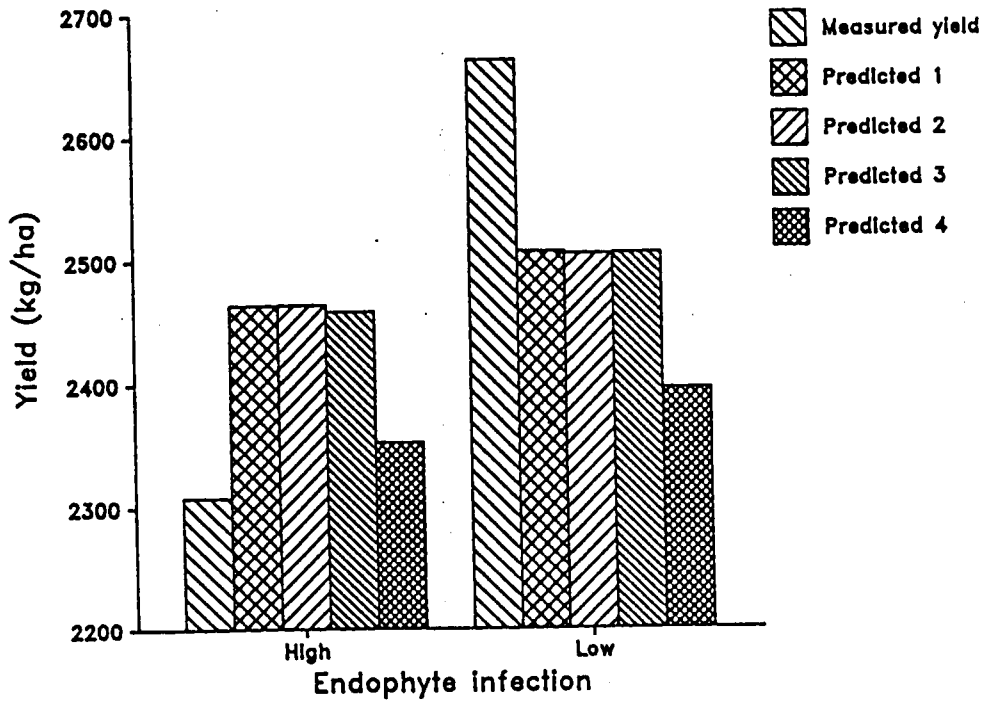

Figure 13. **Comparison of measured yield and predicted yield from disc meter readings at two endophyte levels:** Measured yield determined by harvesting, drying and weighing forage cut from a known area. Predication equation 1: linear regression of data, $y = 140.6x - 106.3$; predication equation 2: quadratic regression line, $y = 0.4x^2 + 124.6x + 10$; predication equation 3: $y = -1.73x^2 + 183x - 39$ for prebloom and bloom growth stages and $y = -1.73x^2 + 183x - 202$ for regrowth stages (Baker et al., 1981); predication equation 4: $y = 1.38x - 1.69$ (Castle, 1976) where y = yield and x = disc meter reading.

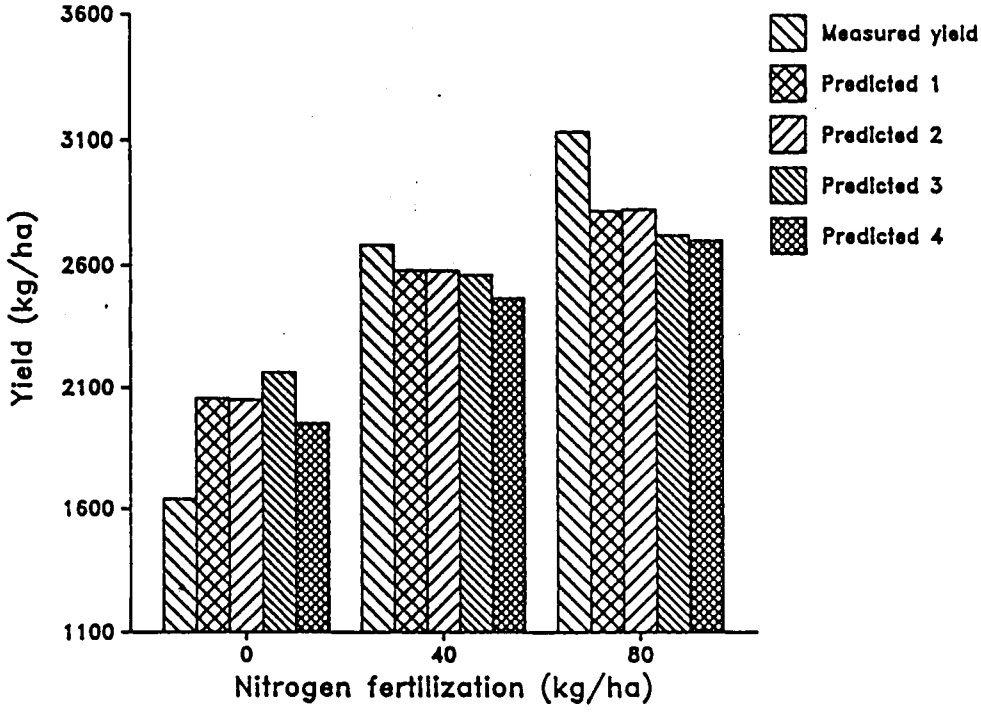

Figure 14. **Comparison of measured yield and predicted yield from disc meter readings of tall fescue fertilizated with three N rates:** Measured yield determined by harvesting, drying and weighing forage cut from a known area. Predication equation 1: linear regression of data, $y = 140.6x - 106.3$; predication equation 2: quadratic regression line, $y = 0.4x^2 + 124.6x + 10$; predication equation 3: $y = -1.73x^2 + 183x - 39$ for prebloom and bloom growth stages and $y = -1.73x^2 + 183x - 202$ for regrowth stages (Baker et al., 1981); predication equation 4: $y = 1.38x - 1.69$ (Castle, 1976) where y = yield and x = disc meter reading.

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GENERAL DISCUSSION

The overall conclusion from a review of the literature is that the cause(s) of tall fescue toxicosis are unknown. What is known is that an endophyte fungus is involved in the etiology of tall fescue toxicosis. This endophytic fungus has been named *Acremonium coenophialum* Morgan-Jones and Gams. A number of biotypes of the endophyte may occur. The biotypes may react differently in the host plant, resulting in conflicting observations in the literature. In addition, the effect of the microenvironment of the plant, including soil fertility and climate may exert confounding and unmeasured effects. The nature of the interaction between the endophyte, plant and environment and animal syndromes requires further investigation. May of the early experiments were not designed to test the influence of the endophyte on plant and animal parameters. The interpretation of many research studies is also complicated because greenhouse studies often do not reflect conditions in the field. Results obtained with a single clone grown in the greenhouse may be insignificant and not observed in the field. Tall fescue is genetically variable and genetic factors may confound observations. The difficulty in obtaining infected and non-infected tall fescue seed from the same source has limited large scale research to evaluate the influence of the endophyte on plant and animal management and performance.

The present study measured effects of endophte infection on tall fescue growth, chemical compostion and insect resistance in greenhouse and field conditions. In addition, the influence of

endophyte-infected tall fescue (hay and silage) on serum prolactin (basal and TRH-stimulated), cholesterol and minerals were measured. In the greenhouse, endophyte-infected tall fescue contained higher K concentration, compared to non-infected tall fescue. Composited by endophyte level, non-infected plants were higher in concentration of Ca, Mg, B, Mn and Zn, compared to infected tall fescue. Individually harvested, non-infected tall fescue plants were higher in concentration of N, Mg, Al, B, Cu, Mn and Zn, compared to endophyte-infected tall fescue. In the field study, low endophyte-infected tall fescue was higher in concentration of Na, Fe and Zn, compared to high endophyte-infected tall fescue. Zinc was lower in endophyte-infected as compared to non-infected in all three studies. Zinc is a component of metalloenzmyes and deficiency of dietary Zn impairs normal reproduction in cattle (NRC, 1984).

Animal performance is improved when fed endophyte-free as compared to endophyte-infected tall fescue (Bacon and Siegel, 1988). Should all endophyte-infected pastures be renovated with non-infected fescue? The answer appears to be no. Simple removal of the endophyte may cause more problems than it solves. Also, removal of the endophyte appears to reduce insect and disease resistance in tall fescue. Replacing endophyte-infected pastures with non-infected tall fescue is costly and has been estimated at $371/ha (Gerken et al., 1986). If renovating endophyte-infected with non-infected tall fescue, the best strategy may be to replace sections of a farm each year. Prevention of re-infecting the non-infected pastures must be taken. Care taken not to re-infect the pastures: by equipment carrying infected seed, by cattle (seeds in feces) or by allowing fescue to produce seedheads. In addition, non-infected tall fescue may be difficult to establish and maintain the stand. Endophyte-free varieties adapted to Virginia conditions must be used in pasture renovation. Johnstone, Kenhy and endophyte-free Kentucky-31 are recommended, whereas, AU-Trimph, Fawn, Alta and Endo-Phyte are not recommended. Low-infected pastures may increase in percentage of infection over time due to survival of the infected plants and death of non-infected plants.

Tall fescue can survive without the endophyte. But can endophyte-free tall fescue thrive and continue to be managed and relied upon as a basis of our forage systems in Virginia? In New

Zealand, endophyte (*Acremonium loline*)-free perennial ryegrass was not able to survive attack by the Argentine stem weevil (*Listronotus bonarensis*) (Barker, 1984).

The possibility exists to alter the form of the toxin(s) (alkaloids?) in tall fescue such that the positive aspects (insect and disease resistance) are retained but are not toxic to the animal This type of manipulation would involve modifying the genetics (DNA) of the endophyte and also possibly the plant. The techniques of biotechnology to do this are available, but, the knowledge of the etiology of the toxicosis to direct bioengineering is lacking.

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Appendix A. Ergopeptine alkaloids

I. Procedure for extraction of ergopeptine alkaloids from forage.

The procedure described below is a modification of the procedure of Porter et al. (1979). The procedures of Porter et al. (1979) and Bacon et al. (1981) were developed for analysis of fungus culture medium. These procedures will not work for plant material because pigments are not removed and interfer with the colorimetric quantification of the alkaloids. The extraction of pigments was accomplished by modifying the procedure of Farnsworth and Euler (1962) who suggest extracting the chloroform extract (alkaline) with HCl.

1. In a beaker, mix 25-g forage, 50-ml of 4 N sodium hydroxide and 100-ml of chloroform. Cover to prevent evaporation of chloroform.

2. Extract for 30 minutes.

3. Filter by vacuum in Buchner funnel lined with Whatman no. 41 filter paper.

4. Discard forage.

5. In a sepatatory funnel, add extractant and 50-ml of tartartic acid.

6. Drain off and discard bottom phase (chloroform).

7. To tartartic acid add 4 N sodium hydroxide to neutralize pH to 10. (Usually requires 3-4 ml of sodium hydroxide).

8. Add 50 ml of chloroform to separtary funnel.

9. Keep chloroform extactant.

10. Evaporate to dryness.

11. Redilute with tartartic acid (10-ml).

II. Nitrite procedure for the determination of ergopeptine alkaloids.

The colorimetric procedure of Michelon and Kelleher (1963) was used to quantify the total ergopeptine alkaloids n the tall fescue extracts.

1. Add 2.0 ml of suitably diluted alkaloid solution to test tube (containing 5-100 ug total alkaloids).

2. Add 2.0 ml of 0.1% PDAB solution (0.1g para-dimethyl amino benzaldehyde to 1/1 (v/v) sulfuric acid/water solution).

3. Vortex to mix. Allow to react for 10 minutes.

4. Add 0.1 ml of 0.1% sodium nitrite solution (this solution MUST be freshly prepared, use within 1-hr after preparation).

5. Vortex to mix. Allow color (blue) development, 15 minutes.

6. Read on spectrophotometer at 590 nm. Color is stable for 1-hr.

Preparation of stock solution and standards.

A stock solution of 100 ug/ml is prepared by adding 200 mg ergonovine maleate to 2-l volumetric flask, dilute to volume with deionized water. Working solutions are prepared from the stock solution: 2, 5, 10 and 50 ug/ml. For the 2 ug/ml working solution, 2-ml of stock solution is diluted to 100 ml with deionized water.

Prepare standard curve with ergonovine maleate:

Standared curve

Standard (ug)	Absorbance (%)
0	0
19.5	.180
39.0	.370
58.5	.577
78.0	.780
97.5	.958
117.0	1.15
136.5	1.37
156.0	1.60

III. Problems with the procedure.

Extraction. The first problem occured when I used toluene to extract the alkaoids. Toluene is slow to evaporate and requires high temperatures. This chemical was not effective in removing the chlorophyll from the extractant. It was also difficult to adjust the pH to 10. Because of the problems with toluene, chloroform was used as an extractant. Chloroform, however, is not misible with the PDAB solution. This made it impossible to read absorbance with the spectrophotometer. The chloroform had to be completely evaporated and the alkaloids diluted with a weak acid so that the colorimetric procedure to quantify the alkaloids could be used.

The chlorophyll also presented problems, interfering with the colorimetric procedure. This meant that the chlorophyll pigment had to be completely removed. The chloroform was very effective in removing all the green pigment in the extract. The rediluted extracts was slightly yellow, possibly indicating the presence of some pigments.

Samples were spiked with the stock solution of ergonovine maleate to determine the recovery of the alkaloids. Only 60% of the spike was recovered. To improve the recovery of the alkaloids, the procedure was run in subdued light (at night), care was taken to completely remove the chloroform extractant from the forage with vacuum pressure through a Buchner funnel and a very low temperature was used to evaporate the chloroform from the alkaloid residue. These procedures improved recovery.

The pH MUST be adjusted to 10 before the second extraction with chloroform. A few samples precipated out after the final extraction and I believe this was a pH related problem.

IV. Results of samples analyzed.

Composited hay and silages from 1988 feeding study.

Sample	Ergopeptine alkaloids (ug/g)
Orchardgrass/alfalfa hay:	
3/19 to 3/25	1.0
3/26 to 4/5	0.74
4/6 to 4/19	0.33
Tall fescue hay:	
3/26 to 4/5	1.48
3/26 to 4/5	1.48
4/6 to 4/19	3.61
4/6 to 4/19	4.18
Fall-cut tall fescue silage:	
3/26 to 4/5	15.67
3/26 to 4/5	13.73
4/6 to 4/19	21.94
4/6 to 4/19	21.61
Spring-cut tall fescue silage:	
3/26 to 4/5	27.50
3/26 to 4/5	26.51
4/6 to 4/19	26.89
4/6 to 4/19	28.04

High and low endophyte-infected tall fescue from Glade Spring and Blackstone harvested on 19 November 1987.

Sample	Ergopeptine alkaloids (ug/g)
Glade Spring:	
High endophyte	
0 kg/ha	3.36
0 kg/ha	2.70
40 kg/ha	4.10
80 kg/ha	6.21
80 kg/ha	5.16
Low endophyte	
0 kg/ha	15.07
0 kg/ha	0.72 ##
80 kg/ha	1.83
80 kg/ha	1.45
Blackstone:	
High endophyte	
0 kg/ha	6.60
80 kg/ha	0.54 ##
80 kg/ha	3.36 ##
Low endophyte	
0 kg/ha	0.72 ##
0 kg/ha	3.36
80 kg/ha	0.72 ##

Indicates problem suspected with analysis, precipate formation.

Appendix B. Genotype data from greenhouse data

Infected (EI) and non-infected (NI) tall fescue individually harvested and composited by date.

Genotype	Endophyte Treatment	Yield	N	P	K	Ca	Mg	S
		g/pot	- - - - - - g/kg - - - - - -					
1	EI	3.6	32.8	4.0	40.3	4.8	4.3	7.3
	NI	3.9	36.1	3.8	40.5	6.6	5.4	7.6
4	EI	6.0	39.6	3.1	41.7	10.7	3.8	3.9
	NI	4.6	38.2	3.4	41.3	5.2	4.4	4.9
9	EI	3.7	39.6	4.4	38.3	4.3	3.7	4.6
	NI	3.4	37.2	3.7	38.2	4.8	4.1	5.1
10	EI	4.2	43.8	3.9	38.8	2.9	2.6	4.1
	NI	2.9	46.4	3.9	40.3	5.1	4.1	3.4
11	EI	3.3	41.4	3.6	40.1	3.5	3.9	5.2
	NI	3.4	39.9	4.4	38.8	3.6	4.0	5.4
12	EI	5.2	39.0	4.3	45.9	3.8	3.2	7.1
	NI	3.8	40.3	3.8	44.0	4.8	3.9	5.4
14	EI	5.1	37.1	3.8	42.2	5.4	4.7	5.9
	NI	5.0	40.0	3.6	40.0	5.4	5.3	4.2
15	EI	6.8	34.6	3.3	41.6	3.6	3.4	5.9
	NI	2.8	38.7	2.8	35.2	4.7	4.2	3.3
16	EI	4.1	37.5	3.8	38.8	3.9	3.3	5.2
	NI	2.7	39.7	3.6	37.8	5.4	3.8	3.5
21	EI	4.0	37.8	4.1	43.9	3.3	2.8	6.4
	NI	4.6	38.0	4.8	43.9	4.0	3.6	6.0
22	EI	4.7	34.9	3.4	33.6	3.6	2.6	4.3
	NI	2.7	41.9	3.4	40.1	5.2	5.1	5.8
24	EI	4.2	44.7	4.4	43.3	4.3	3.5	5.6
	NI	5.6	42.1	4.0	41.4	4.1	4.4	5.7
25	EI	3.9	38.6	3.8	38.6	4.9	3.4	4.8
	NI	6.0	36.8	3.6	36.4	6.8	3.8	5.4
27	EI	4.3	35.3	3.5	38.4	3.5	3.6	4.8
	NI	4.7	36.5	3.9	40.9	5.4	5.0	5.7
28	EI	4.6	33.9	3.7	38.3	3.4	2.7	5.2
	NI	4.0	38.0	3.2	38.2	5.5	4.0	3.7
32	EI	4.6	42.8	3.4	40.5	5.0	5.4	4.2
	NI	3.8	42.8	3.3	38.9	6.7	5.4	3.7
33	EI	3.3	39.6	3.6	37.2	4.5	3.5	4.4
	NI	4.3	39.3	4.1	35.2	4.9	3.4	4.3

Infected (EI) and non-infected (NI) tall fescue individually harvested and composited by date.

Genotype	Trt	Al	B	Cu	Fe	Na	Mn	Zn
		- - - - - -			ug/g	- - - - - -		
1	EI	13.2	31.2	3.3	71.4	815	37.8	26.3
	NI	16.4	40.1	3.5	72.9	541	58.0	32.1
4	EI	12.3	42.3	3.0	66.2	410	26.6	38.4
	NI	17.5	45.7	3.4	83.9	674	52.0	34.8
9	EI	13.9	26.7	2.6	68.4	417	48.7	35.4
	NI	13.9	34.7	3.4	72.4	570	46.4	35.9
10	EI	10.6	32.3	3.4	87.7	223	41.1	35.5
	NI	15.5	33.4	3.9	74.4	524	34.0	51.3
11	EI	11.5	24.2	2.4	58.1	595	29.9	40.8
	NI	13.0	34.0	2.9	71.9	556	30.7	37.7
12	EI	13.9	35.2	3.0	81.8	360	36.2	28.1
	NI	14.8	30.4	3.7	75.4	355	42.0	47.9
14	EI	16.0	29.3	3.3	74.8	703	37.3	32.9
	NI	18.4	39.4	3.5	67.4	628	44.9	37.8
15	EI	14.2	34.6	2.8	67.4	448	30.6	31.0
	NI	17.1	39.6	2.6	80.9	665	27.6	41.7
16	EI	9.8	27.9	2.4	65.0	211	33.6	37.3
	NI	13.5	24.6	4.0	55.7	182	37.3	48.1
21	EI	12.6	28.6	2.5	88.2	246	32.3	44.7
	NI	14.8	31.9	3.5	80.7	238	59.9	42.3
22	EI	12.2	28.8	2.4	75.8	308	27.6	29.0
	NI	18.5	46.7	2.8	88.1	1443	37.3	41.0
24	EI	12.7	41.0	3.0	84.8	437	40.8	37.3
	NI	14.8	51.2	4.6	84.3	401	53.1	36.5
25	EI	11.6	29.5	2.4	76.4	359	41.0	33.5
	NI	18.5	31.7	3.9	73.9	247	59.1	40.3
27	EI	10.2	31.7	1.8	60.8	638	28.5	38.0
	NI	15.6	40.4	3.0	68.5	988	37.6	48.7
28	EI	8.5	25.4	2.4	66.4	272	28.9	23.6
	NI	15.4	26.8	2.8	71.9	294	35.1	33.4
32	EI	16.0	40.1	3.7	61.3	890	43.6	55.6
	NI	16.8	33.8	3.0	65.5	687	45.2	56.0
33	EI	11.4	21.1	2.9	60.2	532	23.0	47.2
	NI	19.6	18.6	3.0	74.9	238	36.7	42.1

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