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Effects of stocking rate, season, and changes in fungal endophyte level on performance of steers grazing tall fescue

Mark A. Marsalis

To the Graduate Council:

I am submitting herewith a thesis written by Mark A. Marsalis entitled "Effects of stocking rate, season, and changes in fungal endophyte level on performance of steers grazing tall fescue." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant, Soil and Environmental Sciences.

Henry A. Fribourg, Major Professor

We have read this thesis and recommend its acceptance:

John C. Waller, John H. Reynolds

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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EFFECTS OF STOCKING RATE, SEASON, AND CHANGES IN FUNGAL ENDOPHYTE LEVEL ON PERFORMANCE OF STEERS GRAZING TALL FESCUE

A Thesis
Presented for the
Master of Science
Degree
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Mark Alan Marsalis
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Abstract

A study was conducted to evaluate steer performance on tall fescue (*Festuca arundinacea* Schreb.) pastures with different *Neotyphodium coenophialum* [(Morgan-Jones and Gams) Glenn, Bacon, and Hanlin] endophyte infestation levels ranging from < 5 to > 80% and three stocking densities (SD). Eighteen 1.2-ha pastures were grazed with newly weaned Angus (*Bos taurus* L.) and Angus cross steers. Each pasture was assigned one of four initial endophyte (E+) levels of about 0, 20, 40, and 80% infestation. Steer and available forage weights were measured at 21-d intervals. Average daily gain (ADG) and beef production for each season were 335 g d^{-1} and 100 kg ha^{-1} for fall/winter, 480 g d^{-1} and 155 kg ha^{-1} for spring, and 260 g d^{-1} and 38 kg ha^{-1} for summer, respectively. Seasonal ADG for fall/winter, spring, and summer were 475, 556, and 297 for low SD; 286, 496, and 317 for medium SD; 242, 389, and 166 g d^{-1} for high SD. Increasing SD from low to high resulted in decreased ADG due to low amounts of available forage at high SD. Decreases in ADG and beef production per unit of E+ level increase were largest at high SD, implying that E+ tall fescue plants increased in number more at high SD than at low or medium SD, and that reduced gains may have been due to increased proportions of E+ tall fescue ingested by steers as a result of E+ level shift. Performance of steers grazing tall fescue pastures changing in E+ level over four years was affected by SD and season interactions and varied with treatment combinations.

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I. Introduction

Tall fescue is the predominant and most important cool-season grass in Tennessee and grows in what is known as the 'fescue belt', between the northern and southern regions of the United States (Figure 1). This transition zone is located in the eastern U.S., from southern Illinois and Ohio south to northern Mississippi and Georgia and from eastern Oklahoma east to the Virginia Piedmont and the Carolinas (Fribourg et al., 1991b). Tall fescue is desirable because it is easily established, well adapted to a wide range of soils and pH (4.5-8.0) and tolerates some pests and grazing abuse. The most widely grown cultivar of tall fescue in the Southeast is ‘Kentucky 31’ (Hoveland et al., 1980). Most of the approximately 1.5 million ha of tall fescue in Tennessee are used for cattle grazing and non-agricultural purposes such as ornamental lawn and roadside vegetative cover, recreational turf, and as a soil erosion preventative (Fribourg et al., 1988). The majority of tall fescue grown in the Southeast is infected with the endophytic fungus *Neotyphodium coenophialum*. This endophyte is of interest to farmers and researchers for many reasons, particularly the benefits it provides to the host tall fescue plant and the harm it causes to cattle and horses grazing the infected fescue. Endophyte infected tall fescue causes a condition in cattle known as tall fescue toxicosis (Paterson et al., 1995). The signs of tall fescue toxicosis in cattle include decreased body weight gains, milk production, conception rates, serum prolactin and ability to dissipate body heat. This condition causes severe harm, not only to the cattle, but to the

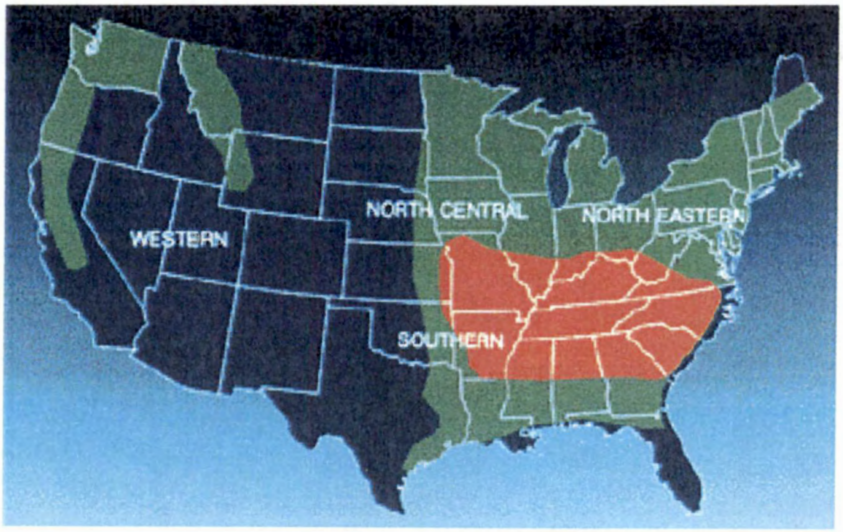

Figure 1. Adaptation of tall fescue in the United States.
(Adapted from: Fribourg et al., 1991. Tenn. Farm Home Sci. 160.)

- ■ Well adapted, area of major use
- ■ Generally adapted, little use
- ■ Generally not adapted

agricultural economy. Estimated annual losses average about $1 billion in the U.S. and about $100 million in Tennessee alone (Fribourg et al., 1997). The *N. coenophialum* endophyte causes the tall fescue toxicosis via ergot-like alkaloids, resulting in alterations of physiological mechanisms within the animal, most of which are still unknown. In addition to tall fescue toxicosis, the conditions known as fescue foot, bovine fat necrosis, and agalactia in horses are also associated with the *N. coenophialum* fungus.

There are beneficial contributions of the endophyte to the host tall fescue plant. The endophyte provides insect and disease tolerance, as well as tolerance to soil nematodes, livestock overgrazing (Hill et al., 1991) and drought conditions. Drought tolerance is especially important in the southern region where dry, hot conditions are frequent in summer. The physiological mechanisms by which the endophyte contributes to these tolerances and to plant persistence are unknown. It is known, however, that absence of the endophyte from tall fescue results in decreased plant performance and productivity (Bouton et al., 1993).

Much time and effort have gone into tall fescue research with respect to its relationship with the endophytic fungus, problems associated with tall fescue toxicosis and the benefits it provides to the plant. Research has been limited in the area of stocking densities of cattle on tall fescue pastures with different levels of endophyte infestation. Studies involving the shift of endophyte infestation incidence over time have also been very limited. Preliminary data have yielded promising results. If it can be shown that varying SD and levels of endophyte infestation affect steer performance and forage productivity consistently, then scientists can begin to understand better the mechanisms by which *N. coenophialum* interacts with its surrounding community.

II. Literature Review

Plant Morphology and Characteristics

Tall fescue is a perennial bunch grass belonging to the *Bovinae* section of the *Festuca* genus of the *Poaceae* family. It grows in a tufted type pattern and has tillers used for lateral expansion and the formation of new plants (Brock et al., 1997). Tall fescue can form rhizomes for additional plant expansion, which result in positioning to a better, more favorable area for growth. Rhizomes tend to be numerous in the winter, decline in spring and increase in summer/autumn. The rise and peak seasons of summer and autumn are the two that exert the most stress on the plant, indicating that rhizomes are longer-lived and can survive adverse conditions. Survivability of rhizomes is critical to plant expansion (Brock et al., 1997). Rhizomes can be influenced by season and grazing management. Several perennial species, including tall fescue, keep the apical bud close to or below the soil surface, thus minimizing grazing damage. Extensive grazing can reduce the number and ability of dormant buds to develop into rhizomes. Greater numbers of rhizomes lead to increased plant competitiveness and stand persistence. On the other hand, severe basal shading and overcrowding reduce tillering activity. Thus, moderate grazing promotes tillering and lateral expansion and leads to a thick, full stand. Infected tall fescue is known to produce more plant material than its endophyte free (E-) counterpart under drought conditions. Endophyte infected tall fescue has deeper growing points (Hill et al., 1991), greater tillering activity in certain months of the year, and will yield more dry matter (DM) than E- tall fescue, particularly in the fall months after a severe summer drought (Bouton et al., 1993). Since environmental conditions in the mid-South are normally an alternation of periods of abundant rainfall and droughts, the drought tolerance contribution from the endophyte is especially important (Gwinn et al., 1998). Tall fescue can be stockpiled in late August through October and grazed from November to late January (Fribourg et al., 1991b) and is well adapted to the upper South; E+ tall fescue is somewhat adapted to the extreme boundaries of the lower South. However, tall fescue stands infested at low incidence rates and E- tall fescue stands may not be well adapted to the lower South, as was shown by Gates and Wyatt (1989) who compared the adaptability of 'AU-Triumph' tall fescue to 'Marshall' annual ryegrass (*Lolium multiflorum* Lam.). Ryegrass outperformed the tall fescue, suggesting that low endophyte tall fescue was not a profitable alternative to other E- strains on the extreme edge of the 'fescue belt'. In marginal areas, such as west central Alabama, E- tall fescue has been adequate at providing much better stands than less well adapted species, such as AP-2 Phalaris (*Phalaris aquatica* L.) (Hoveland et al., 1980). The E- Kentucky 31 cultivar sustained higher stocking rates, animal grazing days, gain ha^{-1} and average daily gain than did phalaris. The phalaris pastures contained more weeds and were more summer dormant than the tall fescue pastures. Another study, by Pederson and Joost (1989) in Louisiana, showed that highly infested KY-31 and Georgia Jesup had great seedling vigor the first three months after seeding, a characteristic which is important for initial establishment of the plant. However, only the Jesup exhibited better long-term persistence with highly infested levels (89% stand) than with lower levels of infestation (< 50% stand). These different studies indicate that the range of adaptability of tall fescue varies greatly and is dependent upon environmental conditions and the level of endophyte infestation that is associated with the pasture.

In one experiment, cows grazing E- tall fescue/legume mixtures had greater body weight gains and better body condition scores than those consuming such forages as smooth bromegrass (*Bromus inermis* L.)/red clover (*Trifolium pratense* L.) pasture mixtures or corn (*Zea mays* L.) crop residues. This showed that E- tall fescue was a good quality forage, not only when compared to E+ tall fescue, but to other forages/feeds as well (Hitz and Russell, 1998). Forage quality tests indicate that tall fescue at an early growth stage is an excellent source of digestible DM, crude protein (CP) and minerals for grazing animals. Endophyte-free tall fescue maintains adequate nutrients throughout the grazing season, but nutrients may be highest at early maturity (McCracken et al., 1993). Although percent bound N increases as the plant matures, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) increase as the season progresses. In-vitro organic matter digestibility (IVOMD) decreases with forage maturity. Nutrient composition of the plant can vary between E- and E+ cultivars. Tall fescue containing the endophyte tends to yield more DM than E- plants, especially during the fall (Hill et al., 1991). On the other hand, Aldrich et al. (1993) reported that E+ plants had lower DM and organic matter digestibility, less CP and higher concentrations of NDF than did E- plants. Nevertheless, E+ tall fescue contains adequate nutrients for grazing cattle. Unfortunately, the good quality of tall fescue is overshadowed by the problems associated with the mutualistic *N. coenophialum* fungus (Schmidt and Osborn, 1993).

Neotyphodium coenophialum and Ergopeptide Alkaloids

There are no outwardly visible signs of the *N. coenophialum* endophyte on the tall fescue plant (Fribourg et al., 1988). The endophyte mycelia occur between the plant cell walls and utilize nutrients taken up through the roots of the plant (Fribourg et al., 1991b). It is possible that the endophyte must obtain the nutrients via plant cell leakage and/or freeing of the nutrients from the cell wall (Siegel, 1993). The endophyte uses the plant nutrients to synthesize secondary metabolites known as alkaloids, which may help provide stress tolerances to the plant. *Neotyphodium coenophialum* provides beneficial qualities to the plant, especially in areas where tall fescue sustainability is marginal (Fribourg et al., 1988). Ergovaline is the predominant ergopeptine alkaloid found in infected tall fescue and is found at biologically active levels (Garner et al., 1993). Support for the theory that ergopeptine alkaloids are the causative agents of tall fescue toxicosis began as early as 1977 when Bacon et al. (1977) proposed that the endophyte *Epichloë typhina* (Fries) Tulasne, later reclassified as *Acremonium coenophialum* Morgan-Jones and Gams (1982), was related to tall fescue toxicosis. The endophyte was later reclassified as *Neotyphodium coenophialum* (Glenn et al., 1996). Porter et al. (1979) isolated and identified ergot alkaloids from the endophyte, suggesting that these alkaloids may have been the causative agents of tall fescue toxicosis. The findings of Yates et al. (1985) of high levels of ergopeptine alkaloids in E+ tall fescue extracts from pastures with cattle suffering from fescue foot further supported the idea that these alkaloids may be responsible for tall fescue toxicosis. The predominant alkaloid was ergovaline. Ergovaline represents 84-97% of the total ergopeptine alkaloids present in E+ tall fescue (Lyons et al., 1986). Endophyte free tall fescue contains none of these alkaloids. Unfortunately, availability of ergovaline is very limited on a commercial basis and it is expensive; until it can be produced economically so that its effects can be studied directly, the role of this compound in tall fescue toxicosis remains uncertain. Ergotamine tartrate can be used to simulate ergovaline responses in cattle, and it is cheaper and more readily available than ergovaline. Ergopeptine alkaloids are biosynthesized via acetate metabolism. Acetate metabolism yields dimethylallylpyrophosphate, which combines with tryptophan and yields lysergic acid. Lysergic acid, when combined with amides and an amino group, produces ergopeptine (Garner et al., 1993). It has been suggested that tryptophan, inorganic phosphorus and oxygen may be regulators of ergot alkaloid synthesis. Since these findings, many additional studies have further determined the role of ergopeptine as well as that of other alkaloids in stress tolerance of plants and tall fescue toxicosis. The alkaloid lysergamide (LAA) is also present in tall fescue in high quantities (~ 45% of that of ergovaline) and has been shown to cause vasoconstriction, one of the effects of tall fescue toxicosis in cattle (Oliver et al., 1993). It is suggested that loline alkaloids and peramine are associated with deterrence of insects, whereas ergopeptine alkaloids are related to tall fescue toxicosis. This may not be completely accurate considering that many alkaloids may work in conjunction to result in specific properties (Porter, 1995). Any one endophyte strain may contain several genotypes with different compounds, which may impart varying methods of resistance. A strain, for example, may be able to provide stress tolerance but without the toxic compounds associated with toxicosis (Latch, 1993). Prestidge et al. (1982) found that endophytes could impart stress tolerances to plants and that the *Acremonium lolii* endophyte in perennial ryegrass (*Lolium perenne* L.) could protect the plant from the Argentine stem weevil (*Listronotus bonariensis* Kuschel) attack. Toxins and deterrents, such as peramine and ergopeptine alkaloids, were produced and reduced insect herbivory. Other compounds are known to cause insects to die prematurely or affect the insect at different stages of its life cycle and its eating habits (Latch, 1993).

Concentrations of alkaloids can vary with changing environmental conditions such as season, fertilizer application and temperatures. The pyrrolizidine alkaloids N-acetyl and N-formyl loline were found in tall fescue at greater concentrations in late spring and early summer, when tall fescue toxicosis occurs most frequently, than in winter (Kennedy and Bush 1983). It is suggested that the possible causes of concentration fluctuations are factors such as soil moisture and fertility, air temperature and grazing pressure. The *Festuca*

X *Lolium* hybrid used by Kennedy and Bush (1983) had an endophyte infestation of $>$ 99%. Moderate temperatures resulted in the highest alkaloid concentration, whereas both high and low temperatures caused a decrease in concentration. This suggested that soil moisture played a more vital role in alkaloid accumulation than temperature, especially since DM accumulation decreased and both N-acetyl and N-formyl loline concentrations increased with moisture deficit (Kennedy and Bush 1983). In addition, Lyons et al. (1986) showed that plants grown with higher N fertilization rates contained higher ergopeptine alkaloid concentrations than those without fertilization.

Effects of the Endophyte on Cattle Performance

The endophytic fungus *N. coenophialum* in tall fescue is associated with the condition in cattle known as tall fescue toxicosis. The endophyte is also responsible for conditions known as fescue foot and bovine fat necrosis (Schmidt and Osborn, 1993). Fescue foot occurs during cold weather and is characterized by a gangrenous infection of the feet and tail, and may lead to eventual sloughing of the hooves or loss of the tail. Fescue foot can be induced with only a few days of grazing E+ tall fescue and exposure to cold weather. Bovine fat necrosis (liptomatosis) is characterized by masses of hard, necrotic fat in adipose tissue of the abdominal cavity and can lead to digestive disorders in cattle. Townsend et al. (1987) found that steers grazing high E+ tall fescue tended to convert more fatty acids into saturated fatty acids than did steers grazing low E+ tall fescue.

Neither bovine fat necrosis nor fescue foot is as detrimental to the cattle industry as tall fescue toxicosis. The effects of the latter are spread out over a larger area and the impact on agriculture is felt on a much larger scale than the two former conditions. An example of this impact is the perception that Southeastern cattle are inferior to cattle from other areas of the U.S. when sent to the marketplace. Buyers discriminate against these cattle mostly because of their rough haircoat, poor appearance and lowered weights and weight gains (Schmidt and Osborn, 1993). Other signs of tall fescue toxicosis are decreased prolactin concentrations in blood serum, conception rates, feed intake and beef production, and increased respiration rates, vasoconstriction, urination and salivation. Signs of tall fescue toxicosis have been observed with as little as 20% endophyte infestation (Gwinn et al., 1998).

A. Feed Intake by Cattle

It is hypothesized that reduced performance is linked to decreased DM intake. Osborn et al. (1992) showed that the main factor affecting weight loss on E+ pastures was reduced intake, yet at the same time some animals consuming E+ tall fescue had intake values above maintenance level; however, the animals lost weight, suggesting that the fungus contributed to weight loss due to effects other than intake. Grazing steers on E- KY 31 pastures consumed about 20% more forage than on E+ pastures (Fribourg et al., 1991b). Goetsch et al. (1987) found that DM intake decreased linearly as a percent of body weight, about a

0.0055% decrease for each 1% increase in infestation. Cattle grazing E+ pastures have been observed in the warm season to graze more during cooler evenings and night hours rather than daytime hours, when they spent most of the time standing in water or in the shade (Schmidt and Osborn, 1993). Often, the result of this inactivity was lowered overall intake of forage.

B. Average Daily Gain

Average daily gain values are decreased as a result of the endophytic fungus in a manner parallel to effects on intake. One study by Crawford et al. (1989) resulted in a negative linear relationship between ADG (0.09 kg d^{-1}) and percent endophyte infestation (per 10%). These values were close to those Stuedemann et al. (1988) reported as a 0.05 kg d^{-1} decrease per 10% infestation increase. Schmidt and Osborn (1993), while studying replacement heifer ADG, obtained results similar to those of Stuedemann et al. (1988), a 0.045-0.05 kg decrease in ADG per 10% increase in infestation. These studies suggested that the relationship between ADG and percent infestation was linear. In contrast, Fribourg et al. (1991a) reported that the relationship was not linear and that lower levels of infestation had a greater effect on ADG per unit increase than higher levels, suggesting a possible critical level for tall fescue toxicosis. All of these studies have led to a general 'rule of thumb' of about 0.05 kg decrease in ADG per 10% increase in infestation or about 0.6-0.8 kg d^{-1} and 0.4-0.5 kg d^{-1} gain on E- and E+ pastures, respectively.

C. Beef Production

Since ADG, combined with SD, results in a value for beef production, it is fitting to discuss the two aspects of ADG and SD together. It is known that weight gains per animal and per ha are lower on E+ than on E- pastures (Read and Camp, 1986). Lowered ADG leads to decreased overall production per unit of land. Hoveland et al. (1981) measured beef production of about 415 kg ha^{-1} and ADG was about 0.5 kg d^{-1} on highly infected pastures; these were low values for spring. The effect of beef production decrease is more severe in summer, particularly at high SD (Degroote and Lefebvre, 1996) and is decreased further by increasing levels of endophyte. Several E- cultivars such as E- KY 31, Johnstone, Kenhy and AU-Triumph will yield adequate production per ha and ADG values for steers grazing them as long as the grass stand can withstand the grazing pressure (Fribourg et al., 1991b).

D. Prolactin/Milk Production

Prolactin is a hormone responsible for milk production in cows and it can be significantly reduced by tall fescue toxicosis. Increasing endophyte infestation will decrease blood serum prolactin levels considerably, indicating a linear effect with E+ levels increasing from as low as 22% (Fribourg et al., 1991b). The reduction of prolactin can affect the overall performance of the animal. Since pituitary concentrations of prolactin are not reduced, it is thought that the toxic compounds (alkaloids) produced by the endophyte act as an agonist to the dopaminergic suppression of prolactin secretion at the D2-dopamine receptor (Strickland et al., 1993). In addition, dopamine changes gut motility or alters ruminal motility and suppresses blood flow to various organs, this leading to decreased digestion and nutrient availability (Strickland et al., 1993; Thompson and Stuedemann, 1993). Dopamine antagonists have increased prolactin levels in cattle (Paterson et al., 1995). It is also thought that temperature has an effect on prolactin levels, with reduced levels at higher temperatures. However, plasma prolactin levels have been shown to decline regardless of environmental conditions in tall fescue toxicosis situations (Aldrich et al., 1993).

E. Reproduction

Tall fescue toxicosis affects reproduction in cattle grazing E+ tall fescue. Schmidt et al. (1986) reported that beef heifers raised on E- tall fescue had calving rates of 96%, whereas those on E+ pastures had only 55% calving rates. For each 10% increase in infestation, conception rates decreased 3.5% and daily milk production decreased 1.05 kg. Another study indicated that calf crop decreased from 80% (E-) to 50% (E+) and pre-weaning gains were significantly lower on E+ than on E- pastures (Fribourg et al., 1991b).

F. Respiration Rate/ Heart Rate/ Vasoconstriction

The alkaloid lysergamide has vasoconstrictive properties and has a greater effect on veins than arteries (Oliver et al., 1993). Steers in a hot environment normally have high peripheral temperatures due to vasodilatation and the dissipation of elevated core body heat. When steers have infected tall fescue in their diet, the peripheral temperatures decrease, indicating a vasoconstrictive activity and lack of heat dissipation to the extremities (Osborn et al., 1992). High rectal temperatures and respiration rates are further evidence that body temperatures are not being dissipated. Both are significantly high in steers consuming infected tall fescue. Heart rates are commonly depressed in cattle grazing E+ tall fescue and may be due to pressure exerted from vasoconstriction. In contrast, Osborn et al. (1992) reported that in a controlled environment, rectal temperatures and respiration rates were similar in steers consuming both E- and E+ tall fescue. This study suggested a diet-environment interaction and that high temperatures may have compounded the effects of the endophyte.

Environmental Effects

Factors such as ambient temperature and drought play a major role in compounding the effects of tall fescue toxicosis. Heat is probably the most influential factor on tall fescue toxicosis. Animal performance reduction is greater in spring and summer than in fall and winter (Fribourg et al., 1991b). Since summer is usually hot and dry, cattle already experience some stress and tall fescue toxicosis only worsens the situation. Steers kept in a controlled environment of 21°C were reported to have significantly higher feed intake, lower rectal temperatures and almost half the respiration rate of those kept in a hot environment of 32°C (Osborn et al., 1992). Decreases in gain (ADG and beef production) are more prominent in warmer months (April to June) and are magnified because of the potential for greater gains, especially in spring, when there are larger quantities of available forage. Hyperthermia, panting, salivation and an inability to adapt to high temperatures can be attributed to tall fescue toxicosis (Schmidt and Osborn, 1993). This becomes even more important when moving cattle from cool environments to areas of high temperatures when they are not adapted to such locations, e.g., Western feedlots.

Droughts will cause great stress to tall fescue plants and the *N. coenophialum* endophyte will help to alleviate this stress. Since alkaloid accumulation is increased with drought stress, it can be hypothesized that this increase in alkaloid concentration contributes to more intense tall fescue toxicosis effects on cattle. Other environmental factors, such as acid rainfall and greenhouse gases, are becoming a popular concern and may affect plant tolerances. Since precipitation with pH less than 4.5 has been reported throughout the eastern U.S. and acidic soils are common, the need for an acid tolerant species such as tall fescue is warranted. Reynolds and Wolt (1989) showed that orchardgrass (*Dactylis glomerata* L.) plants were affected more by acidic rainfall than were tall fescue plants; and orchardgrass leaves were damaged at a higher pH than tall fescue leaves. These results suggested that tall fescue may have been more tolerant to adverse environmental conditions than other species.

Grazing and Stocking Rates

Stocking density (grazing pressure) can have an effect on plant and animal performance in tall fescue pasture systems. In many situations grazing pressure is thought to be the main stress factor when environmental conditions are mild (Gwinn et al., 1998). As stated earlier, grazing tall fescue will help produce a thick, full stand by promoting tillering activity and lateral expansion as long as the stand is not overgrazed (Brock et al., 1997). Moderate grazing is usually preferred because it allows for stand expansion and limits mature, poor quality grass from developing, but does not put too much stress on the grass. Although old stems tend to be more resistant than young stems to microbial degradation because of their high C:N ratio (Hume and Brock, 1997), it is this aspect of mature growth that can limit animal performance. The manner in which grazing pressure is related to forage availability and animal production is shown in Figure 2 (Blaser et al., 1986). Bransby et al. (1988) observed that heavy grazing of infected tall fescue yielded higher gains per ha than moderate or light SD. This may have been due to the fact that lower SD allow for mature growth and increased seedhead production. Seeds are known to have higher

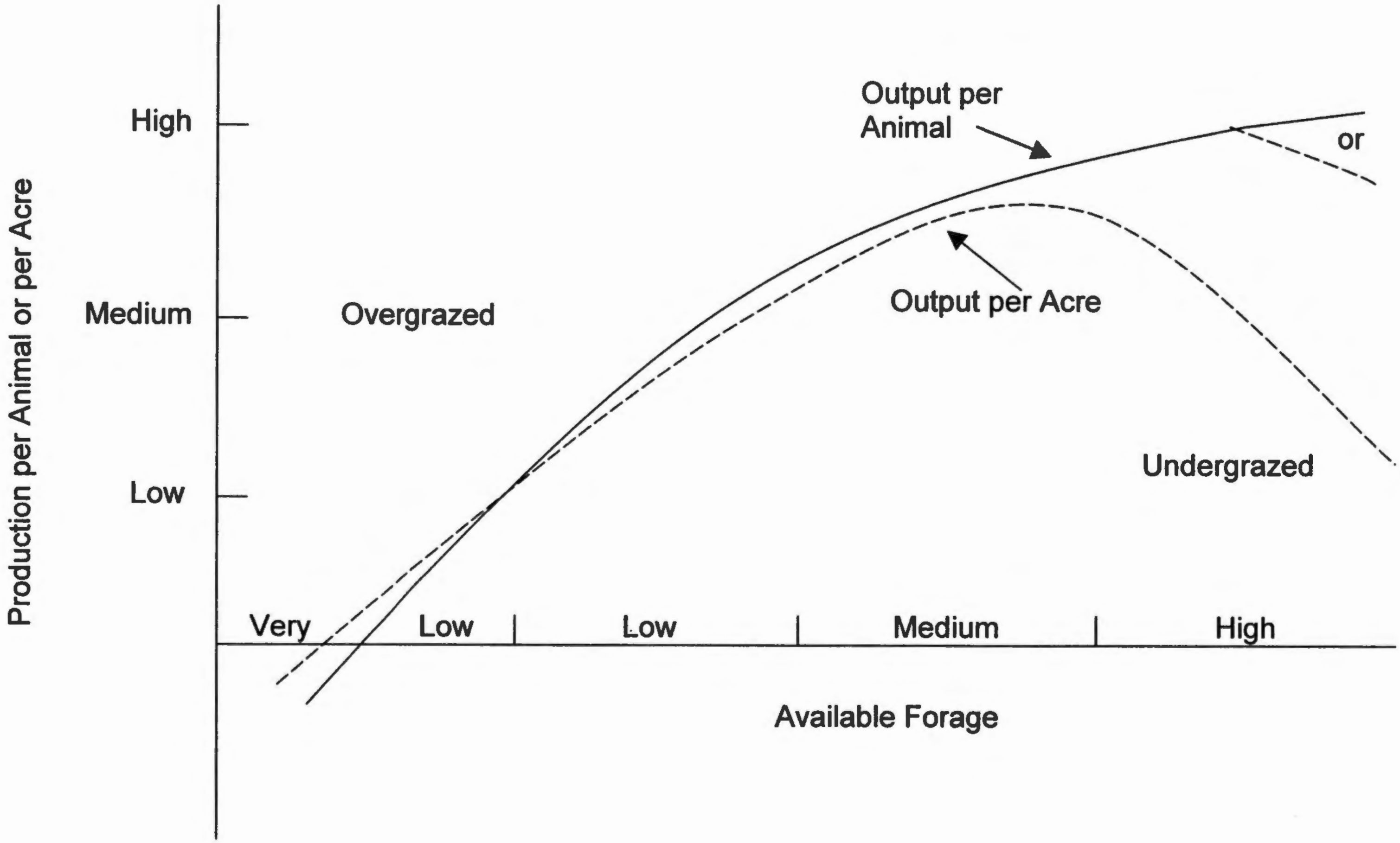

Figure 2. Influence of available forage on production per animal and per acre of pasture.
(Adapted from: R.E. Blaser. 1986. Virginia Agric. Exp. Stn. Bull. 86-7.)

concentrations of the endophytic fungus than leaves. When coupled with poor quality mature growth, ingestion of the seedheads will limit animal production. The study reported by Bransby et al. (1988) was isolated and involved a short study period, only an 84-d spring period at the southern extreme of the tall fescue range. Effects across all seasons should be evaluated to determine which SD is best. Other studies have shown that cattle grazing E+ tall fescue at a high SD had reduced ADG (Hoveland et al., 1997) and decreased overall performance. Tall fescue persistence can be adversely affected by increasing SD (Gates and Wyatt, 1989), especially during extreme drought. Careful attention must be given to pastures during summer and on the regional edges of tall fescue adaptability. It is suggested that low-to-moderate SD is best on the extreme southern edge of tall fescue adaptation. Anything higher than moderate SD may damage the stand of grass and in turn, overall production. Continuous grazing has been shown to yield more tillers and plants on a mean density basis, whereas rotational grazing results in larger individual plants with more DM per plant than plants continuously grazed (Hume and Brock, 1997). Total DM is lower in continuously grazed pastures than in rotationally grazed pastures. It is thought that livestock grazing creates a mosaic of areas with varying degrees of utilization. Cid and Brizuela (1998) showed that areas grazed heavily contained less biomass per unit surface, were more dense and had higher N concentrations than lightly utilized patches. It seemed that cattle gained nutritional benefits from heavily utilized patches. Regrowth was of better quality than mature growth and heavily utilized patches had less live and dead biomass than lightly utilized patches, indicating that grazing prevented residue cluttering. Nitrogen concentrations were 26% higher in heavily utilized patches than in lightly utilized patches (Cid and Brizuela, 1998) due to the fact that grazing maintained short and young vegetation that contained low C:N ratios. Defoliation may have initiated N uptake and translocation to damaged leaves, leading to a better quality plant.

Endophyte Shift

There is some speculation over whether or not the *N. coenophialum* endophyte infestation level increases over time. Crawford et al. (1989) reported little or no change in E+ level over a 2- to 3-year period. Other studies (Read and Camp, 1986; Shelby and Dalrymple, 1993) observed a shift in E+ levels, more prominent at low levels of initial infestation. Over a 4-year period, initial levels of 27% had increased to 84% and initial levels of 58% had increased to 92%. It was calculated that the average annual increase in E+ level was about 4.1% for all plots. Initially low E+ pastures may become highly infested due to a high mortality rate for E- tillers or plants, and a high E+ survival rate. Endophyte free and high E+ levels did not significantly change over time. Stands with zero percent initial levels began to show detectable levels, but this was attributed to contamination by seeds from adjacent pastures (Shelby and Dalrymple, 1993). Endophyte free stands became very sparse and needed to be reseeded. Regarding the effects of mowing on the endophyte shift, mowing can be compared to grazing in that it prevents seedhead formation and promotes the spread of the plant (increased number of plants). It was found that mowing plots yielded more plants per m^2 than unmown plots. Mowing and grazing promote tillering activity and the E+ plants can fill the gaps left behind by the dead E- plants. This was one possible explanation of how the endophyte may have spread. Infestation level changes may be due to enhanced survival of the E+ plants where competition for moisture during drought is intense (Shelby and Dalrymple, 1993) and grazing pressure is high. Since E+ plants are more drought tolerant than E- plants, lack of rainfall can also contribute to endophyte shift. At the Highland Rim Experiment Station, Springfield, TN, Thompson et al. (1989) showed that high E+ levels (60-75%) had not changed much over 2 years, but low levels of around 30% had doubled and 15% infestation had tripled, again emphasizing that low levels of E+ were more apt to change over time than high levels. Fribourg et al. (1991a) found that moderate-to-high SD affected infestation levels, particularly at medium (25-60% E+) initial endophyte levels. Little or no change in E+ level was detected when intermediate E+ level pastures were grazed at low pressure or in initially high (80% E+) level pastures at all SD (Gwinn et al., 1998). In general, pastures of 60% E+ or higher do not change as dramatically as intermediate E+ level pastures and highest percent change will result with medium to high SD. Few significant changes have been observed at infestation levels of 35, 60, and 81% (Fribourg et al., 1991a). Clearly, it is recognizable that there is a relationship between SD and infestation rate on E+ pastures.

The *N. coenophialum* endophyte is very competitive, is incorporated into the sexual reproductive cycle of the plant and is disseminated via the seed. The endophyte is spread through the seed of the tall fescue plant and will lead to infestation level increase if pastures are allowed to reach reproductive stages of growth. In addition, cattle are vectors of the endophyte through their feces, which contain infected seeds when the cattle are moved from E+ to E- pastures (Shelby and Schmidt, 1991). It is suggested that a 3-d quarantine period be implemented for an adequate removal of E+ seed from the animal digestive tract. One steer is estimated to pass > 3,000 viable E+ seeds within 3 d of removal from an E+ pasture (Shelby and Schmidt, 1991).

Preference

Another area of uncertainty is the issue of whether or not cattle prefer certain types of forage to others, and whether they can select between E+ and E- plants. Fisher et al. (1999) indicated that cattle prefer hays cut later in the day to those cut in the morning hours. This preference was attributed to the fact that PM hays contained lower levels of fiber (ADF, NDF, cellulose and lignin) and higher concentrations of total nonstructural carbohydrates (TNC), monosaccharides and disaccharides than did AM hays. These carbohydrates tended to accumulate throughout the day, and decreased fiber concentrations. This was one example of how nutritive value may have influenced grazing habits. Another example was when cattle frequently grazed the previously mentioned heavily utilized patches

(Cid and Brizuela, 1998). Since these patches were of better nutritive value, the cattle may have been able to sense the difference and preferred the patches. If cattle do show preference to specific grass growth stages, then it is possible that they may prefer E- to E+ tall fescue. This preference would lead to an decrease in clover and E- plants in E-/E+-clover pastures and an opportunity for E+ plants to spread (vanSanten, 1992). Fribourg et al. (1991a) reported this to be the case when clover populations decreased as percent infestation increased, and suggested the cattle grazed more of the clover as E- plants decreased. Research by vanSanten et al. (1991) showed similar results that supported this theory.

Analysis Techniques

There are several different methods for the isolation of the ergopeptine alkaloids, including colorimetric analysis, tandem mass spectrometry (MS/MS), high-pressure liquid chromatography and enzyme immunoassays (Garner et al., 1993). Immunoassays are widely accepted as highly sensitive indicators of ergopeptine alkaloids and allow for rapid analysis of many samples at one time. Endophyte infestation levels can be determined by enzyme linked immunosorbent assay (ELISA) techniques by using about 12 tiller samples per ha at any season of the year (Fribourg et al., 1991b; Savary et al., 1990). Sampling results of winter, spring, summer and fall are not different for detection of E+ incidence in tall fescue (Thompson et al., 1989).

Breeds of Cattle

Certain species of cattle are better adapted to heat conditions than others and may be less affected by the problems associated with tall fescue toxicosis. Brahman-Angus (*Bos indicus-Bos taurus*) crosses have been shown to suffer less of a decrease in ADG than the Angus breed, with ADG decreases of 14% and 38%, respectively (McMurphy et al., 1990). When grazing E+ tall fescue in Oklahoma, Brahman cross steers had no rise in rectal temperatures and gained weight faster than Angus steers. Goetsch et al. (1988) observed similar results with British cross (34% decrease in ADG) and Brahman cross cattle (21% decrease in ADG). Another study (Mezzadra et al., 1992) indicated that breed performance varied with SD. Average daily gain was higher for Limousin (*Bos taurus*) crosses than for Angus cattle at a low SD, but the opposite was true at a high SD. Beef production was highest at a SD of ~3.5 animals ha^{-1} for Angus, but was highest at a SD of ~2.8 animals ha^{-1} for Limousin cattle. This study suggested that smaller animals produced more meat under limited forage conditions and that larger animals were more affected by declining forage availability than smaller animals.

Management

Beef cattle production in the southeastern U.S. is inefficient because cattle are grazing land that is not of prime grain crop growing status (Hoveland, 1986).

Production could be improved by better management, improved forages, animal health programs and controlled breeding seasons. Better management can improve several aspects of beef-forage systems such as cow-calf and stocker operations. A timed, controlled breeding season will help to utilize available forage during seasons of high forage production. Improved health care with elaborate records may help minimize potential losses at all production levels. Economical forage production calls for the selection of the proper species of grass to match environmental conditions as well as the soil. Better fertilization practices than those commonly used today and supplemental forage efficiency improvement are also necessary for economical production. Even though E- tall fescue requires a higher level of management than E+ tall fescue, it leads to improved gains. The individual producer must weigh all of these options and determine what is best for the situation at hand. The costs need to be spread over a broad economic base by not putting too much emphasis on individual animal needs at one level, but focusing on the systems that affect costs and returns presently and in the future of the product lifetime. By incorporating several systems together, the producer can substantially improve profitability. Regarding pasture improvement, or renovation with legumes, Fribourg et al. (1988) suggested that pastures with E+ levels less than 30% can be substantially improved by the addition of clover (*Trifolium* sp.) and that this clover can offset the effects of the endophyte and lead to satisfactory animal performance. It is suggested that long-term ingestion of low levels of ergopeptide alkaloids causes less acute effects of tall fescue toxicosis than do high levels of alkaloids. This, coupled with clover diet dilution effects, can lead to considerably improved animal performance. In contrast, pastures with 30% infestation or more should be considered for reestablishment (Fribourg et al., 1988) since the effects of clover are not as significant as those observed in pastures with < 30% infestation. Nevertheless, grazing of E- tall fescue is the best method for avoiding tall fescue toxicosis.

Adding alfalfa (*Medicago sativa* L.) in mixtures may decrease the effects of tall fescue toxicosis some, but not as much as the use of E- tall fescue (Hoveland et al., 1997). Taking into account that most pastures in the Southeast are 60-90% infested and an infestation of 65% may cause as much as a 45-kg loss over 150 d, reestablishment and renovation are very necessary if production is to be improved. Ongoing research has shown that E- tall fescue can survive and continue to produce under certain management conditions. Reestablishment can be very difficult and costly, but long-term benefits and returns could outweigh the initial costs. Hoveland et al. (1981) found that SD could be increased when cattle grazed E+ tall fescue with the addition of Regal ladino white clover (*T. repens*, L.), birdsfoot trefoil (*Lotus corniculatus*, L.) or N application. Increasing the SD improved beef production and was highest (653 kg ha^{-1}) on tall fescue-clover pastures and lowest (420 kg ha^{-1}) on tall fescue-N pastures with tall fescue-trefoil pastures being intermediate (447 kg ha^{-1}). Average daily gain followed the same pattern, with tall fescue-clover being the highest (0.68 kg d^{-1}) and tall fescue-N the lowest (0.48 kg d^{-1}). It seemed that clovers and legumes replaced some E+ tall fescue intake of cattle and thus reduced the effects of the endophyte. These results exemplify the benefits of legume addition to the diet and how renovation is superior to N fertilization alone.

New stocking practices are becoming popular in various parts of the U.S. Intensive-early-stocking (IES), for example, is thought to be more profitable than season-long-stocking (SLS). Some studies (McCracken et al., 1993; Huffaker and Wilen, 1991) showed that forage quality declined throughout the grazing season and IES utilized the good quality forage early and heavily. The pastures were then rested later in the season for regrowth. When nutrient decline was high (mid-May to June) due to adverse environmental factors, IES was best, but when nutrient decline was low (April to mid-May), SLS was a better way to maintain profitability. Ruminal fermentation rate was high early in the tall fescue-growing season and microbial growth was more than optimal at this time (McCracken et al., 1993). This model did not take into account factors such as the endophyte-tall fescue toxicosis interaction or many other variables. All variables must be considered when adopting a grazing management strategy.

Future strategies for improved production and the elimination of tall fescue toxicosis are promising. Novel endophytes are currently being produced which provide benefits to the host tall fescue plant, but without the adverse effects on cattle associated with tall fescue toxicosis. Rumen microflora manipulation is an area of interest that attempts to detoxify or reduce harmful alkaloids. Finally, the use of antidotes, vaccines and supplements provides promise in future elimination of the tall fescue toxicosis. It was shown that chemicals such as estradiol-17 β had a positive effect on ADG in summer months (Davenport et al., 1993). Mean yearly changes in endophyte infestation levels of about 14% (Gwinn et al., 1998) indicate the urgency of improved management practices needed to maintain E- pastures and help with the problem of inferior cattle performance until the physiological mechanism(s) of the endophytic fungus and its ergot alkaloids are understood (Davenport et al., 1993). Future practices must be altered so that they incorporate new techniques to help curb the deleterious effects of tall fescue toxicosis and the spread of the causative endophyte. Much work is needed if the southeastern U.S. is going to improve its beef production systems and eliminate the detrimental effects of the *N. coenophialum* endophyte.

III. Materials and Methods

General Procedures

This study was conducted from fall 1993 to summer 1997 at Ames Plantation, Grand Junction, TN (35°6′ N, 89°13′ W) specifically the Hancock Place, and at the Departments of Plant and Soil Sciences, Entomology and Plant Pathology, and Animal Science, University of Tennessee, Knoxville, TN. At Ames Plantation, eighteen 1.2-ha pastures on a Memphis silt loam soil (fine-silty, mixed, thermic, Typic Hapludalf) were assigned one of four initial tall fescue endophyte infestation level treatments of about 0, 20, 40, and 80%. The pastures were assigned also a low, medium or high SD, which were adjusted from season to season each year depending on forage availability (Table 1). Six pastures of each of the three SD were used. Each SD had at least one of each of the four initial endophyte level treatments, except for the high SD (Figure 3). The 0% initial E+ level was not planned to contain the high SD treatment, but was assigned the high SD treatment after seeding mixtures were switched unintentionally.

Table 1. Stocking densities in animals per 1.2 ha pasture, 1993-1997.

Grazing Season	Low	Medium	High
Fall/Winter	2-3	3-4	4-5
Spring	3	4-5	6-7
Summer	2-3	3-4	5-6

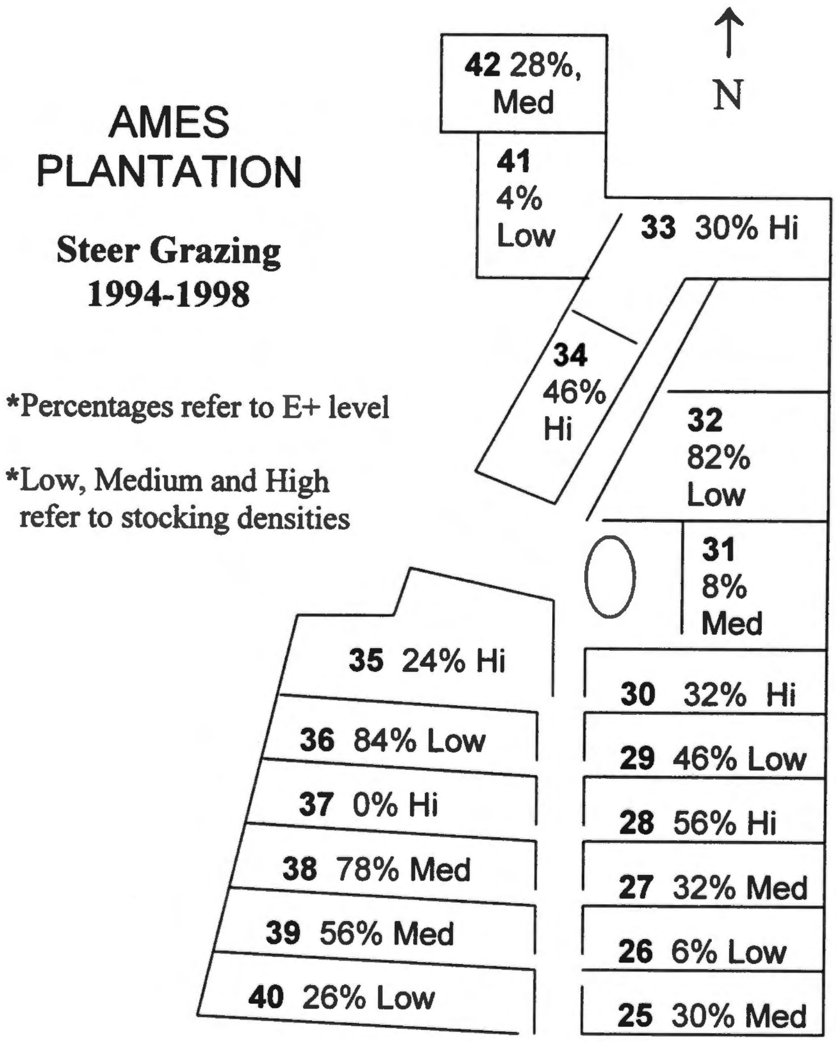

Figure 3. Map of experimental pastures.

Pastures of different endophyte infestation levels within a specific SD were stocked with equal numbers of animals, regardless of pasture condition. Initial treatments to specific pastures are shown on Figure 3.

Pasture Establishment

Prior to seeding, all vegetation was killed in 1990 by using 1.12 kg ha^{-1} of glyphosate [*N*-(phosphonomethyl)-glycine] and disking after the herbicide had taken effect. 'Tifleaf II' pearlmillet [*Pennisetum americanum* (L.) Leeke] was then seeded in 15-cm rows at 30 kg ha^{-1} (Gwinn et al., 1998). After the pearlmillet was grazed and hay was cut, the crop was removed and replaced in October by winter wheat [*Triticum aestivum* (L.) emend. Thell.] seeded in 15-cm rows at 134 kg ha^{-1}. The winter wheat was grazed in the fall and winter, and grain was harvested in June 1991. A soybean [*Glycine max* (L.) Merr.] crop (Maturity Group III) was used the following summer and then harvested for grain. Weather conditions in 1990 resulted in less than desirable stands of 1990-91 winter wheat and 1991 soybeans. Therefore, the process was repeated in 1991-92. Another winter wheat crop was planted in the fall of 1991 and harvested in June 1992. Prior to tall fescue seeding in the fall of 1992, the pastures were disked lightly and sprayed with glyphosate in July. Although the two-year smother crop process was not conventional practice, it was necessary to ensure that there were no remaining tall fescue plants from previous stands.

Soil samples were taken from all potential pasture areas in fall 1992. Agricultural limestone was applied when soil pH was less than 5.5. Fertilizer was applied when soil tests showed low P or K levels. These fertilizers were applied each winter and spring to provide medium levels of fertility. Pastures were also fertilized with about 56 kg N ha^{-1} in early September and in the spring of each year.

Pastures were seeded in September 1992 in 20-cm rows at about 17 kg ha^{-1} using a no-till drill. All seed used was of the same genotype of 'Kentucky 31' tall fescue grown at the Plant Sciences Unit of the Knoxville Experiment Station, Knoxville, TN. Endophyte free and 80% E+ or greater pastures were seeded using only E- seed and E+ seed, respectively. The two intermediate infestation levels were obtained by using appropriate ratios by weight, adjusted for germination. Uniform pasture stands were established by spring 1993 and were grazed from March to September. Pastures were fertilized with 56 kg N ha^{-1} and stockpiled (fertilized and allowed to accumulate growth) from early September to early November.

Animal Management

The animals used in this experiment were mostly from the Ames Plantation herd or purchased from privately owned herds having characteristics (e.g., age, breed, and weight) similar to those of Ames Plantation. Ninety-six steers that initially weighed between 227 and 273 kg were used each year.

Animals were put on pasture in early November at the appropriate fall/winter SD (low = 2 to 3; medium = 3 to 4; high = 4 to 5). When insufficient forage existed in fall/winter and summer seasons, the SD were reduced (low = 2; medium = 3; high = 4). Cattle were fed range cubes (Co-Op Range Cubes, Tennessee Farmers Cooperative, Lavergne, TN 37086) to provide maintenance during times of insufficient or unavailable forage and to maintain appropriate SD. Grazing was terminated when mean standing forage height in a specific treatment was less than 2.5 cm. Forage heights ranged from about 13 to 46 cm, 10 to 20 cm, and 2.5 to 10 cm for low, medium, and high SD, respectively. All extra animals needed to increase SD in spring were kept on separate tall fescue pastures and maintained through fall/winter at performance levels similar to those of steers on experimental pastures. Steers used in the fall/winter period were the same ones used the following spring and summer periods. Extra steers were added to the fall/winter animals to adjust for increasing SD in spring (low=3; medium=4 to 5; high=6 to 7). Spring SD were reduced when the summer grazing season began in late June/early July. All extra animals were fed a diet other than tall fescue for at least 3 d prior to being added to experimental pastures to prevent seed contamination from non-experimental pastures.

At the end of the summer period, steers were removed from the experimental pastures. Pastures were stockpiled during the early fall of every year to provide adequate available forage for the fall/winter grazing season.

All pastures were inspected daily to assure animals were in proper pastures and to check the health of all animals. In addition, every pasture had a windbreak available for the fall/winter season and shade for the spring and summer seasons. Adequate water, salt and minerals were available for all animals.

Data Collection

Steers were weighed at the beginning and end of each season and at 21-d intervals during each season. Fall/winter grazing began as close as possible to November 1 and continued until March. Spring season began in mid-March and ended about July 1. Summer grazing lasted from July 1 to the end of August each year.

Data were collected at 21-d intervals and included steer weights, strip clippings and pasture scores. Weighing dates coincided with strip clippings and pasture scoring dates during spring and summer. Strip clippings were used to measure available forage and nine clippings were taken per 1.2-ha pasture. A stratified random sampling method (Thompson et al., 1989) was used with 3.048 X 0.508 m strips and at a stubble height of 2.54 cm. Forage clippings were put into bags for drying and were weighed when dry. Available forage (kg DM ha^{-1}) was calculated by taking the difference between the final bag weight and the initial bag weight for a period. The mean weight of the nine strip clippings was converted from kg per m^2 to represent kg ha^{-1}. Pasture scores were taken by two trained individuals at the beginning and end of the fall/winter period and at 21-d intervals during the spring and summer. Pasture scoring included minimum, maximum and mean tall fescue heights, species composition and percent ground cover. The minimum amount of available forage required for the grazing animals was about 500 kg ha^{-1} (Degroote and Lefebvre, 1996).

Blood samples were collected on weighing dates closest to July 1. The blood serum was analyzed for prolactin concentrations. Protocol for radioimmunoassy analysis of prolactin is presented in Appendix E.

Weather data were recorded daily and included precipitation and minimum and maximum air temperatures. These weather data were used to correlate with animal performances and forage availability.

Neotyphodium coenophialum infestation levels were measured with the PAS-ELISA procedure (Thompson et al., 1989) (Appendix D). Prior to seeding in fall 1992, endophyte levels of the two seed lots (0 and 80%) to be mixed were determined. Beginning in June 1993, tillers were collected by a stratified random sampling method (Thompson et al., 1989) utilizing 50 sampling spots per pasture, with one tiller from each sampling spot. Tiller samples were collected only once a year, during summer (June to August), and frozen immediately in liquid N and stored frozen until PAS-ELISA analysis.

Forage quality data were obtained by using near-infared reflectance (NIR) technology (FOSS NIRSystems, Model 5000, Silver Spring, MD 20904) and were analyzed for CP, ADF, NDF, and DM. Blood serum analyses, ELISA and NIR were conducted in the laboratories of the Animal Science, Entomology and Plant Pathology, and Plant and Soil Sciences departments, University of Tennessee, Knoxville.

Statistical Analysis

Since all treatments were not assigned to every block, data were analyzed using the Mixed Procedure of SAS (SAS, 1999). A randomized block design (RBD) with a factorial arrangement was used. Average daily gain, beef production and forage quality least squares means were generated, having SD and season as treatments, year as a random block effect and E+ level as a continuous regression within the model. An additional blocking factor was assigned to groups of pastures based on previous knowledge of similar characteristics such as soil fertility, drainage and previous production potential. Least squares means were analyzed for differences within interaction combinations of SD and season. Mean separation was obtained by Fisher's protected LSD (Steel and Torrie, 1980).

Since E+ levels were determined only once per year in early summer, fall/winter and spring levels had to be estimated. This was done by plotting the summer test levels for a particular pasture and fitting a regression line to the E+ level points for the four years. An equation was produced from the line and used to generate the estimated level for the pasture. A newly estimated E+ level for a particular summer was assumed to remain at that level through the fall/winter and spring seasons until a change in level was detected the following summer. In addition, E+ level slope values were used to determine the effect of E+ levels on ADG and beef production.

IV. Results and Discussion

Climate

The varying climatic conditions from 1993 to 1997 influenced the management of the experimental pastures. During periods of low precipitation and limited available forage, SD were reduced, but pastures were never terminated completely on any pasture except in early 1995 and late spring 1997. High SD pastures were terminated in 1995 and 1997 because of excessively low available forage, inadequate stands, and the inability to maintain the steers appropriately within the experimental protocol. Climatic data are presented in Appendix A beginning with 1993 because the first fall/winter grazing season of the experiment began in the fall of 1993.

The overall 4-year climatic conditions of precipitation and temperature (maximum and minimum) were similar to the 30-year normal averages for Bolivar, TN (35°16′ N, 89°59′ W). Average monthly precipitation totals (Appendix A1) from 1993 to 1997 were at or above 30-year average totals except for the months of February, April, August, November, and December. The largest amounts of precipitation occurred during the spring months and averaged about 140 mm. When 30-year normal temperatures were compared to the 4-year experimental period, both the mean maximum and minimum daily averages were similar to the normal and only minor differences occurred (Appendices A2, A3).

During the 1993 calendar year there were precipitation deficits during the spring and summer seasons that continued into the fall (Appendix A4). Although the precipitation was below average, adequate tall fescue stands were maintained and ready for experimental animals in November. Maximum daily temperatures were similar to 30-year normals, but were above normal for July and August (Appendix A5). Minimum temperatures were above normal in July and August as well (Appendix A6).

In 1994, the months of January through July received more than adequate amounts of rainfall and were almost twice as much as 30-year normals in June and July. In contrast, August to December precipitation amounts were considerably lower than average (Appendix A7). Nevertheless, available forage for 1994 was maintained as adequate, except for the high SD and medium SD in winter and early spring, where values dropped below 500 kg ha^{-1} (Appendix B1). Maximum and minimum temperatures averaged above normal, particularly in November and December (Appendices A8, A9).

The 1995 year exhibited several periods of precipitation deficit, including January to early spring, June, September, October and December (Appendix A10). As a result, available forage dropped considerably from January to early spring, particularly at the high SD (Appendix B2). Since rainfall was large in July and August, available forage was maintained above adequate levels throughout the remainder of the year. Maximum and minimum temperatures were also high for August, but low in November and December (Appendices A11, A12). The low temperatures in these two months may have caused the very small amounts of available forage for the 1995 winter period (Appendix B3).

In 1996, again there were precipitation deficits early in the year (Appendix A13). This deficit from February to April, combined with below normal maximum and minimum temperatures (Appendix A14, A15), caused a considerable reduction in available forage (Appendix B3). As precipitation levels increased from May to August, available forage increased.

The study was ended after the summer grazing season in 1997. Rainfall was adequate through the first half of the year, but was deficient in July. March, June and September had very large precipitation amounts (Appendix A16). Temperatures were similar to normals and slightly cool in April and May (Appendices A17, A18).

Although there were no extended periods of drought or extreme hot or cold temperatures during the study, temporary fluctuations in climatic conditions affected overall management of the pastures. Occasionally, tall fescue availability was reduced and SD had to be adjusted accordingly, but no stands were damaged irreparably because of drought or extreme temperatures except when subjected to high SD.

Available Forage

Forage availability was analyzed as total DM at a particular sampling date during all grazing seasons and over all SD. The quantities of forage are shown in

Appendices B1, B2, B3, and B4, for all years. The general pattern was that the low SD resulted in the largest available forage, followed by the medium SD, and the high SD resulted in the smallest amount of available forage. Overall, fall/winter and summer seasons contained similar amounts of average available forage, but the spring season amounts were much smaller, except for 1993. For the low and medium SD, the spring season amounts were considerably smaller than fall/winter and summer, but at the high SD, the difference was not as great.

The reason available forage quantities were so much smaller in spring than in the other two grazing seasons was because more animals were put on pasture to utilize the good quality and potentially abundant growth from April to June. In addition, the spring grazing period contained the month of March, which was not very favorable for tall fescue growth. Since the animals were eating the forage, when the March weather was unfavorable, the resultant spring total availability was decreased. Stocking rates were reduced in summer and fall/winter seasons and as a result, available forage increased.

The 1993-1994 available forage (Appendix B1) shows that forage quantity decreased as the fall/winter season progressed (from November to February) into months with less favorable climatic conditions. High and medium SD values indicate that available forage was below 500 kg ha^{-1} for several months throughout the year. As climatic conditions became more favorable for growth (April to early-June), forage growth increased. Rainfall was very high during the early summer months, and resulted in adequate forage growth in the late summer.

Due to the period of non-grazing (stockpiling of pastures) from late September to mid-November, available forage increased to very large levels for the fall/winter season of the 1994-1995 grazing year (Appendix B2). Low SD pastures averaged about 4000 kg ha^{-1} at the beginning of the fall/winter season. Although there was low rainfall for the season, warm temperatures during the winter resulted in excellent tall fescue growth. High SD pastures dropped well below 500 kg ha^{-1} into negligible amounts. This decrease was amplified by low rainfall from January to April. From April to December, high SD pastures averaged more available forage than medium and sometimes, low SD pastures. This was a result of not grazing high SD pastures from January to the end of March due to inadequate amounts of forage.

The forage quantities were expected to be large in the fall/winter season of the 1995-1996 grazing year due to stockpiling, but were much smaller than in the previous summer (Appendix B3). Although the pastures were not grazed from the end of August to late December, the amount of forage was quite small for the beginning of the new fall/winter grazing season. This could be attributed to the high temperatures of the previous summer and the low temperatures during November. Again, forage quantity decreased as the fall/winter season progressed, and the decrease was amplified by below average precipitation and temperatures from February to April, as well as increasing SD. As precipitation levels increased from May to December, available forage increased.

The 1996-1997 grazing year (Appendix B4) followed similar fluctuating patterns, but had larger fall/winter forage values than 1995-1996 due to warm winter temperatures and adequate precipitation. High SD pastures were terminated in the spring of 1997 because of inadequate available forage and decreased stands. These pastures were not reincorporated into the remainder of the experiment.

Average Daily Gain

When all SD were averaged over all seasons, the low SD resulted in the largest ADG, medium SD was intermediate and high SD resulted in the smallest ADG (Table 2). These results were all significantly different ($P < 0.01$). When seasons were averaged over all SD, spring ADG was larger than that of fall/winter and summer (Table 3). Seasonal differences were significant ($P < 0.01$). The interaction between SD and season showed that during the spring, low SD differed significantly from the high SD, but not from the medium SD (Table 4). Fall/winter ADG for low SD was significantly different from both the medium and high SD. During summer, only the high SD was significantly different from the other two SD. At the low SD, spring was significantly larger than summer, but not fall/winter. Medium and high SD results showed that spring ADG was significantly larger than both fall/winter and summer (Figure 4). All SD by season interaction means were different at $P < 0.01$.

Table 2. Average daily gain least squares means of steers at three stocking densities averaged over all seasons, 1993-1997.

Stocking Density	ADG
	g hd^{-1} d^{-1}
Low	443[a]
Medium	367[b]
High	266[c]

Stocking densities not sharing the same superscripts are significantly different at $P < 0.01$.
n = 72

Table 3. Average daily gain least squares means of steers during three seasons averaged over all stocking densities, 1993-1997.

Season	ADG
	g hd^{-1} d^{-1}
Fall/Winter	335[b]
Spring	480[a]
Summer	260[c]

Seasons not sharing the same superscripts are significantly different at $P < 0.01$.
n = 72

Table 4. Average daily gain least squares means of steers at three stocking densities during three seasons, over all E+ levels, 1993-1997.

Season	Stocking Density		
	Low	Medium	High
		g hd^{-1} d^{-1}	
Fall/Winter	475[ab]	286[cd]	242[de]
Spring	556[a]	496[ab]	389[bc]
Summer	297[cd]	317[cd]	166[e]

Seasons and SD not sharing the same superscripts are significantly different at $P < 0.01$.
n = 72 for each SD and season

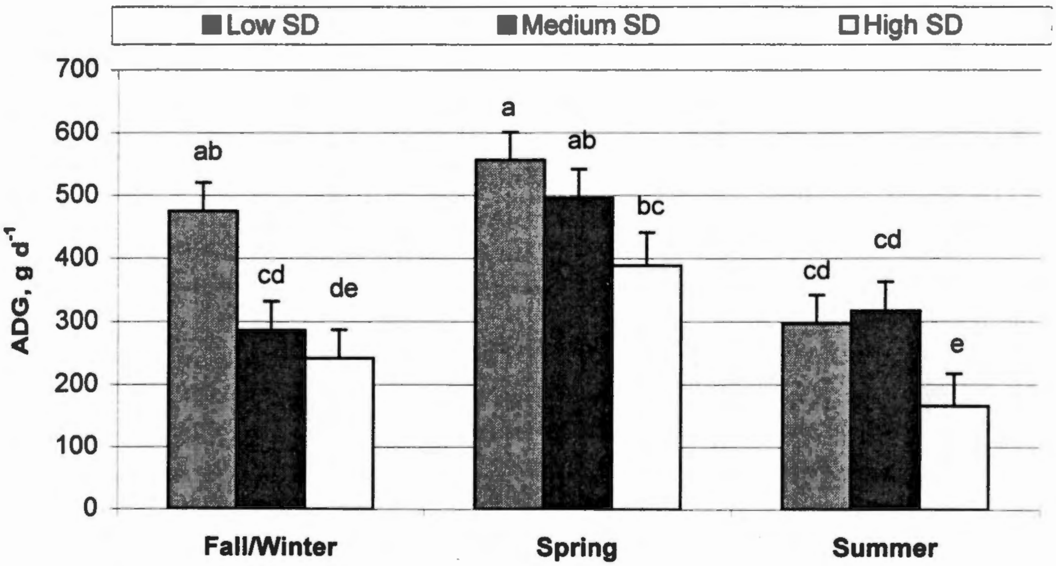

Figure 4. Average daily gain least squares means of steers at three stocking densities over all E+ levels and seasons, 1993-1997.

Seasons and SD not sharing the same superscripts are significantly different at $P < 0.01$.

n = 72 for each season

In general, as SD increased, ADG decreased. This decrease was attributed to small amounts of available forage at the medium and high SD. The general pattern indicated that as available forage decreased, ADG decreased also. Decline of ADG in times of adequate forage (e.g. summer) may have been attributed to heat stress or the reduced intake by the steers caused by the endophyte and associated alkaloids.

Based on the regression analysis of ADG over all years and seasons (Figure 5), a one-unit increase in E+ level would result in a mean decrease in ADG of 0.44 g d^{-1}, or 4.4 g d^{-1} per 10% increase in infestation at medium SD. This decrease in ADG was amplified to 40 g d^{-1} for every 10% increase in E+ level at the high SD. The ADG decrease for low SD was 14 g d^{-1} per 10% increase in E+ level. The high SD value of 40 g d^{-1} was similar to results reported by Stuedemann and Hoveland (1988) and Schmidt et al. (1993) of 40 to 50 g d^{-1} decrease in ADG per 10% increase in endophyte infestation. Crawford et al. (1989) reported decreases in ADG of about 90 g d^{-1} per 10% E+ level increase.

Overall results for the four years indicated that the medium SD had less of an effect on ADG decrease per unit of infestation than did the low SD (Figure 5), although the low SD resulted in the largest ADG averaged over all seasons. Since the low stocking density ADG were large, the seasonal changes (decreases) that occurred from spring to summer were greater for the low SD than for the medium SD. The large reduction in ADG at the high SD may have been due to the changes in E+ level that occurred in the high SD pastures.

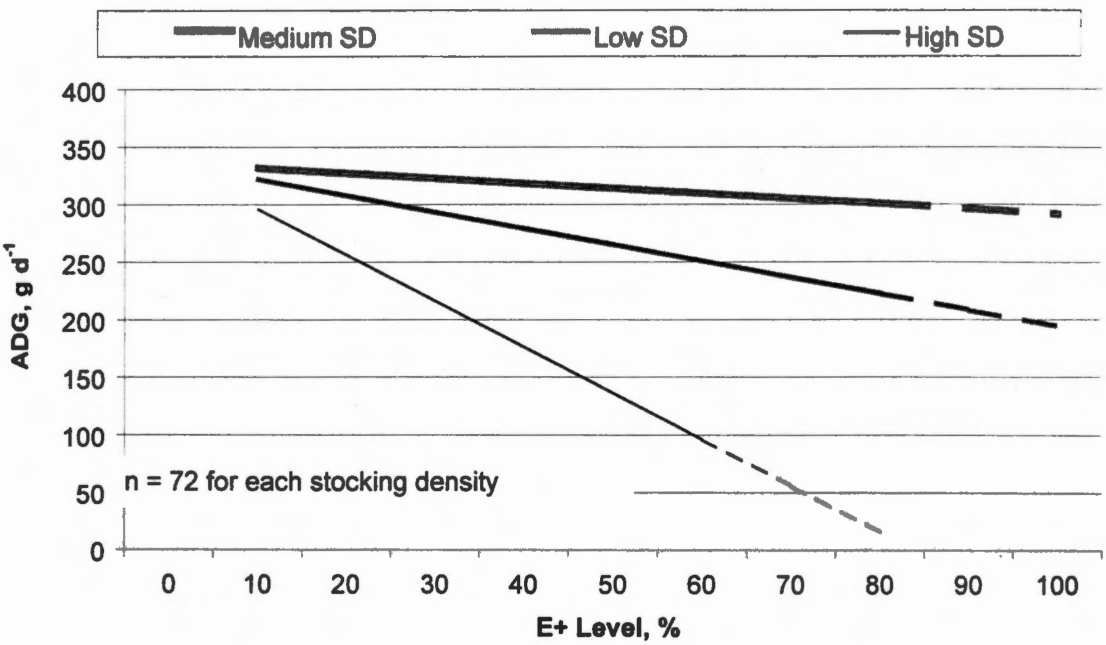

Figure 5. Computed effect of E+ level on average daily gain at three stocking densities over all seasons, 1993-1997.

Endophyte level increased rapidly at the high SD, which could have resulted in greater amounts of E+ plants and associated toxins ingested by the animal.

Over all seasons and pastures, medium SD was comparable to the low SD for ADG for the entire grazing year. Low SD gain was greater than medium SD in the spring, but was smaller than medium SD in the summer. These differences were not significantly different ($P < 0.01$), indicating that low and medium SD had similar effects on overall ADG during the spring and summer seasons.

There were differences in ADG between low SD and medium SD for the fall/winter season. When the animals consumed the stockpiled tall fescue, the regrowth was less abundant in the fall/winter season (due to unfavorable climate in winter) than in spring. Forage availability decreased as the fall/winter season progressed. Increased SD (from low to medium) may have magnified the forage decline in fall/winter and resulted in small ADG. In contrast, as the spring season progressed, the forage availability increased. As a result, more animals per ha were not as damaging to the tall fescue plants in the spring.

The high SD resulted consistently in small ADG in all seasons when averaged over all pastures and years, probably because of less available forage. High SD average daily gains were particularly small in the fall/winter and summer seasons when tall fescue productivity was limited.

Beef Production

Beef production for a season was calculated by multiplying the ADG by the number of animal grazing days (number of animals*number of days in the season) of the pasture. Animal grazing days depended on SD and were thereby different when SD were adjusted periodically for season and forage availability changes.

When averaged over all seasons (Table 5), beef production was greatest for the medium SD, intermediate for low SD and smallest for high SD ($P < 0.05$). Seasonal beef production across all SD (Table 6) resulted in spring having the largest beef production, followed by fall/winter and summer ($P < 0.05$). Seasonal and SD interaction analysis indicated that although the average high SD over all seasons was smallest in beef production (Table 7), the spring high SD yielded the largest amount and was significantly greater than the low SD (Figure 6). This was expected since more animals were put on pasture in the spring than in fall/winter or summer and in larger increments for the high SD (4-5 increased to 6-7) than for the low (2-3 increased to 3) or medium SD (3-4 increased to 4-5). Bertelsen et al. (1993) reported that beef production per ha increased as stocking rate increased within a rotational grazing system, without sacrificing gain per animal. Within the summer treatment, there were no significant differences among SD.

Table 5. Beef production least squares means of steers at three stocking densities averaged over all seasons, 1993-1997.

Stocking Density	Beef Production
	kg ha^{-1}
Low	97^{b}
Medium	108^{a}
High	89^{c}

Stocking densities not sharing the same superscripts are significantly different at $P < 0.05$.
n= 72

Table 6. Beef Production least squares means of steers during three seasons averaged over all stocking densities, 1993-1997.

Season	Beef Production
	kg ha^{-1}
Fall/Winter	101^{b}
Spring	155^{a}
Summer	38^{c}

Seasons not sharing the same superscripts are significantly different at $P < 0.05$.
n = 72

Table 7. Beef production least squares means of steers at three stocking densities during three seasons, over all E+ levels, 1993-1997.

Season	Stocking Density		
	Low	Medium	High
		kg ha^{-1}	
Fall/Winter	131^{b}	109^{b}	63^{c}
Spring	127^{b}	164^{a}	174^{a}
Summer	33^{d}	52^{cd}	30^{d}

Seasons and SD not sharing the same superscripts are significantly different at $P < 0.05$.
n = 72 for each SD and season

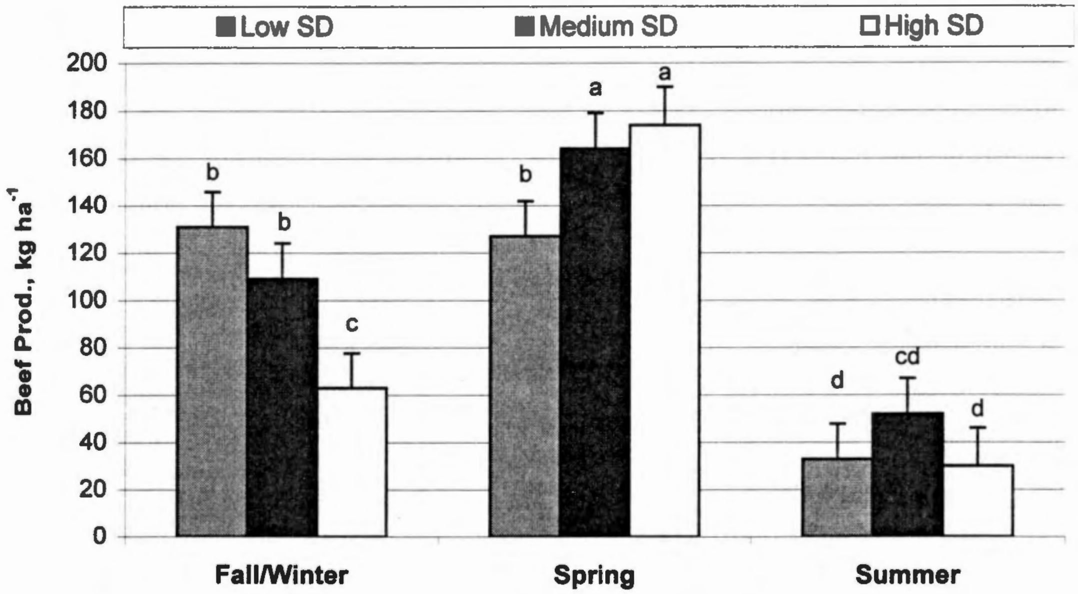

Figure 6. Beef production least squares means of steers at three stocking densities over all E+ levels and seasons, 1993-1997.

Seasons and SD not sharing the same superscripts are significantly different at $P < 0.05$.

n = 72 for each season

The high SD resulted in the smallest beef production in fall/winter. The low SD had the largest beef production. These results were opposite of those obtained in spring. Since the increments between the SD were not as large in the fall/winter as they were in spring, the high SD may not have had such a large positive effect on beef production. This could explain why increasing SD had an overall negative effect on beef production, particularly when combined with lower CP concentrations within the forage in the fall/winter compared to that of spring.

Regression analysis of beef production over all years indicated that a one-unit increase in E+ level resulted in a mean decrease in beef production of 0.21 kg ha^{-1}, or a 2.1 kg ha^{-1} reduction per 10% increase in E+ level at the medium SD (Figure 7). The high SD magnified the decrease to a 10.2 kg ha^{-1} decline per 10% E+ level increase. Low SD resulted in about a 3.3 kg ha^{-1} decrease in beef production.

The effect of increasing endophyte infestation on beef production was similar to that of ADG. At the high SD, the decrease in beef production per unit of E+ level increase was the greatest, but was the lowest at the medium SD. This effect may be explained by the results of the fall/winter season. Since the beef production for high SD in spring is so much larger than in fall/winter, the greatest declining effect would be shifted toward the high SD. The opposite trend patterns presented in Figure 6 for fall/winter and spring show how beef production was largest in spring, but smallest in fall/winter. This change exemplifies the significant decline within the high SD. The regressions were run over all seasons, therefore changes between any two seasons would be reflected in the regression

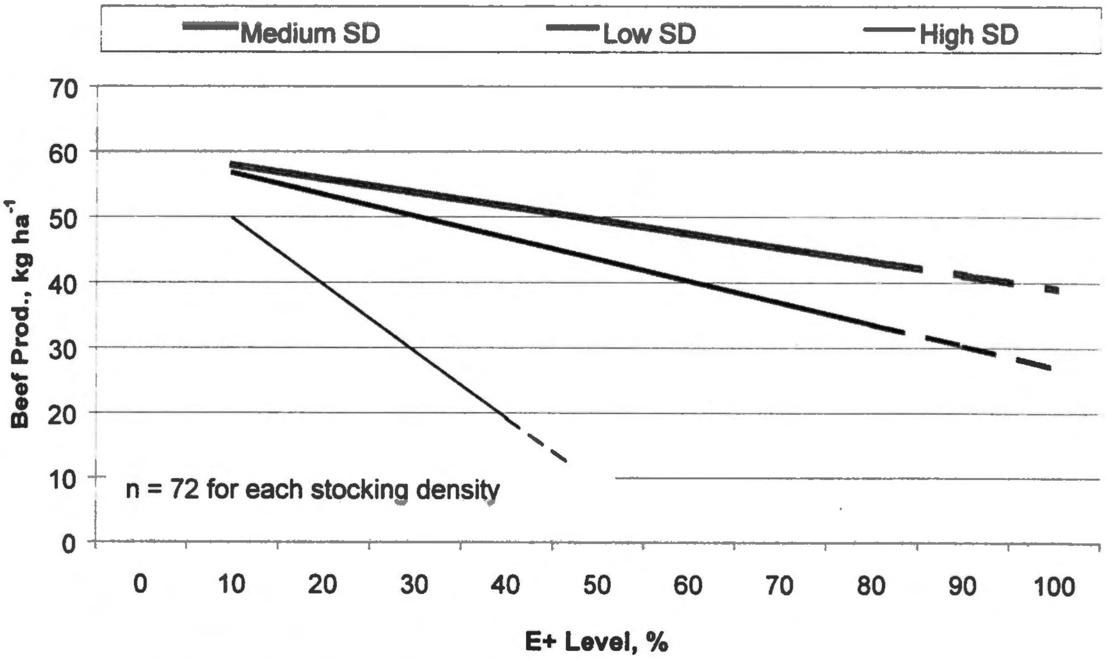

Figure 7. Computed effect of E+ level on beef production at three stocking densities over all seasons, 1993-1997.

slopes. Spring beef production was far superior to that of fall/winter and summer due to increased numbers of animals within each SD and high forage production rates. Although the forage availability analyses indicated that spring had the smallest amounts of available forage, it was due to increased animal numbers and increased forage consumption. Total live weights of steers on pasture are presented in Appendices C1, C2, C3, and C4 and tended to be largest during the spring months and at the high SD because of increased numbers of steers on pasture.

Forage Quality

Acid detergent fiber and NDF were expected to decrease within forage samples in the spring because vegetative growth was abundant. Fiber concentrations were smallest in the spring and largest in the summer. When averaged over all SD, ADF concentrations were smallest in spring, intermediate in fall/winter and highest in summer; these values were all different at $P < 0.01$ (Table 8). Neutral detergent fiber concentrations were similar among seasons with the smallest for spring, intermediate for fall/winter and largest for summer ($P < 0.01$). Interaction comparisons for SD and season are shown in Figures 8 and 9 for ADF and NDF, respectively. Stocking density had no significant effect on fiber concentrations. Fiber content within the tall fescue plants was expected to decline in spring due to increases in immature vegetative regrowth and fiber was expected to increase in fall/winter and summer. Turner et al. (1996) reported

Table 8. Acid detergent fiber, neutral detergent fiber, and crude protein least squares means of tall fescue during three seasons averaged over all stocking densities and E+ levels, 1993-1997.

Season	ADF	NDF	CP
		%	
Fall/Winter	39.7[b]	71.1[b]	11.8[b]
Spring	38.5[c]	69.5[c]	14.9[a]
Summer	40.6[a]	73.6[a]	10.8[c]

Seasons within a column not sharing the same superscript are significantly different at $P < 0.01$.
n = 72 for each forage component.

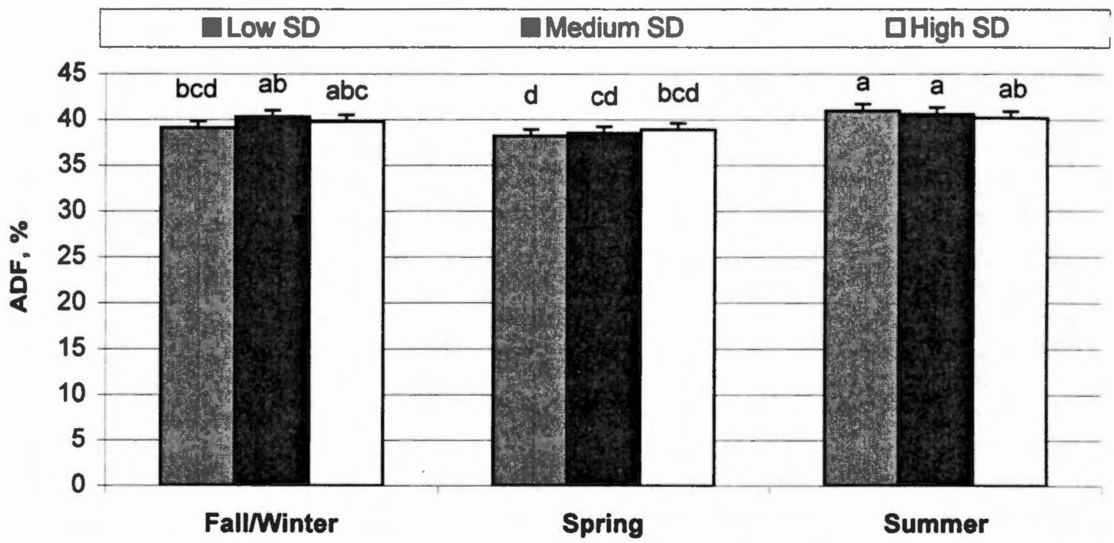

Figure 8. Acid detergent fiber (ADF) least squares means of tall fescue at three stocking densities over all E+ levels and seasons, 1993-1997.

Seasons and SD not sharing the same superscripts are significantly different at P < 0.01.

n = 72 for each season

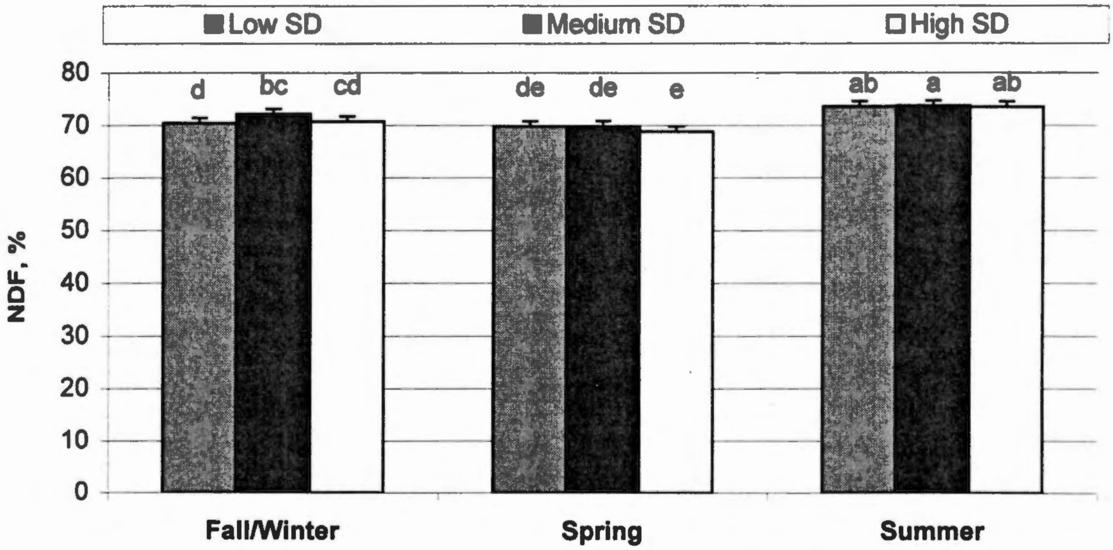

Figure 9. Neutral detergent fiber (NDF) least squares means of tall fescue at three stocking densities over all E+ levels and seasons, 1993-1997.

Seasons and SD not sharing the same superscripts are significantly different at $P < 0.01$.

n = 72 for each season

decreasing fiber levels in tall fescue as the growing season progressed toward spring. This was observed in our study for ADF and NDF, but the differences were neither significant nor meaningful in relation to having an effect on forage digestibility or animal performance.

The concentrations of CP were expected to increase tall fescue with grazing pressure because of low C:N ratios (Cid and Brizuela, 1998). Crude protein did increase to significantly high levels as SD increased in the fall/winter and spring seasons (Figure 10). Averaged over all SD, the spring contained the largest CP levels, followed by fall/winter and summer (Table 8). When averaged over all seasons, only the high SD was different ($P < 0.01$).

All the forage quality analyses from this study indicated that spring was the superior season for good tall fescue growth. Since season was a much greater factor than SD in determining the overall quality of the plant, more emphasis was put on season than on SD when relating forage quality to ADG or beef production.

Prolactin

Prolactin concentrations were analyzed for only two years (1996 & 1997) of the study because samples for the other years were lost due to a freezer breakdown. Over these two years SD did not have an effect on prolactin concentrations in the blood of steers grazing E+ tall fescue. In contrast, increasing endophyte infestation levels did have an effect on prolactin concentrations in July 1996. July 1997 results indicated no such relationship

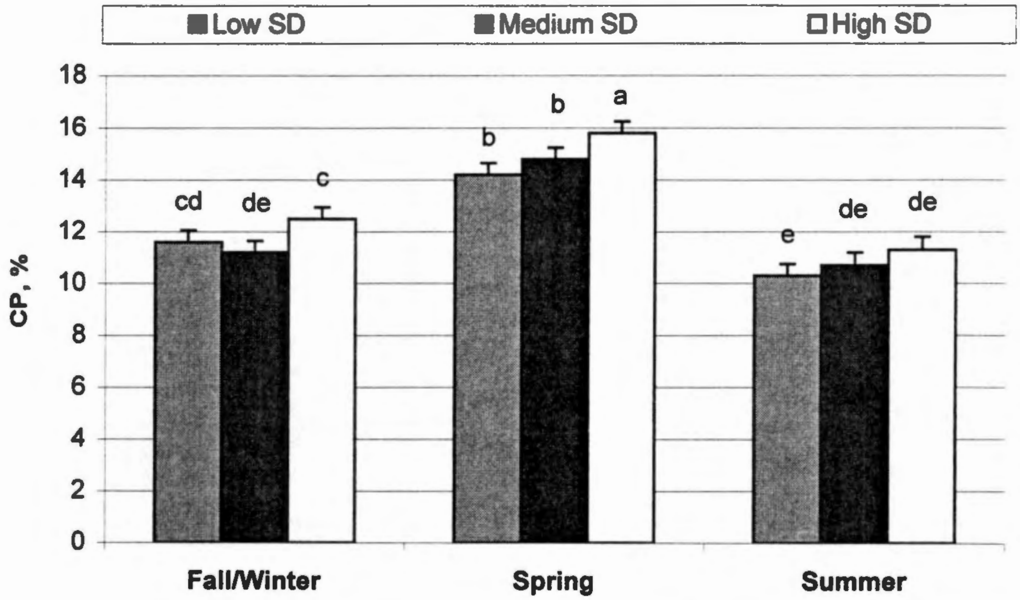

Figure 10. Crude protein (CP) least squares means of tall fescue at three stocking densities over all E+ levels and seasons, 1993-1997.

Seasons and SD not sharing the same superscripts are significantly different at P < 0.01.

n = 72 for each season

(Appendices E1, E2). Decreases in prolactin such as those shown in 1996 could become quite significant at endophyte infestation levels of 80-100%. Goetsch et al. (1987) reported that prolactin decreased linearly (15, 11.2, 0.1, 0.8, and 0.0 ng mL^{-1}) as endophyte infestation in tall fescue hay increased from 0 to 25, 50, 75, and 100% in the diet of dairy steers. Stamm et al. (1994) reported similar results with increasing concentrations of ergovaline in the diet of beef steers.

Endophyte Level Shift

Since endophyte infestation levels changed over the 3-year period, they were considered results rather than planned methodology. It was necessary to analyze E+ levels separately because of this. All pastures (except one) with initial E+ levels of 8% or greater increased in percent infestation. The E+ level shift analysis was used to define the change as it related to initial infestation and SD. Endophyte level shifts were analyzed using the Mixed Procedure of SAS, but differently from the previously mentioned variables (ADG, beef production, and forage quality). Since E+ level shifts were dependent upon initial endophyte infestation levels, original (seeded) E+ levels (0-8%, 24-32%, 46-56%, and 78-84%) were entered into the model with SD as treatments. Year was no longer a block, but was a repeated measures effect, making all individual pastures identical across years. Means of the different groupings of original E+ level and

SD treatment interactions were analyzed for significant changes over all years. Since 1994 E+ test levels were the same as those at seeding, any E+ level change from 1994 was considered to be a change from the initial seeding level. The endophyte shift analysis was based on estimates of E+ levels for individual pastures and years derived from regression line equations for each pasture.

Pastures were grouped according to initial E+ levels and analyzed statistically. Results are presented as percentages of E+ level infestation (Figure 11). The 1994 E+ levels were the same as seeded levels and were considered the basis for E+ level changes.

It should be noted that all high SD and one medium SD-category combinations contained only two years of data due to the previously described pasture terminations.

The category A (0-8% E+) of initial endophyte level did not change much over the 3-year period. The average increase over all SD was about 7% for all three years or < 3% per year. These changes are considered negligible because the PAS-ELISA procedure is accurate only within ± 5%. The medium SD yielded the highest percent increase, which was due to an initial E+ level of 8%. The low and high SD initial E+ levels were 0% and did not change at all.

The category B (24-32% E+) infestation level changes were significant for low and high SD and were 23 and 28% for 3 and 2 years, respectively. The medium SD resulted in an 11% increase. Category B averaged about an 8.5% change per year over all SD. The reason why the medium SD resulted in a lower E+ level shift than the low SD was because one pasture with a medium SD

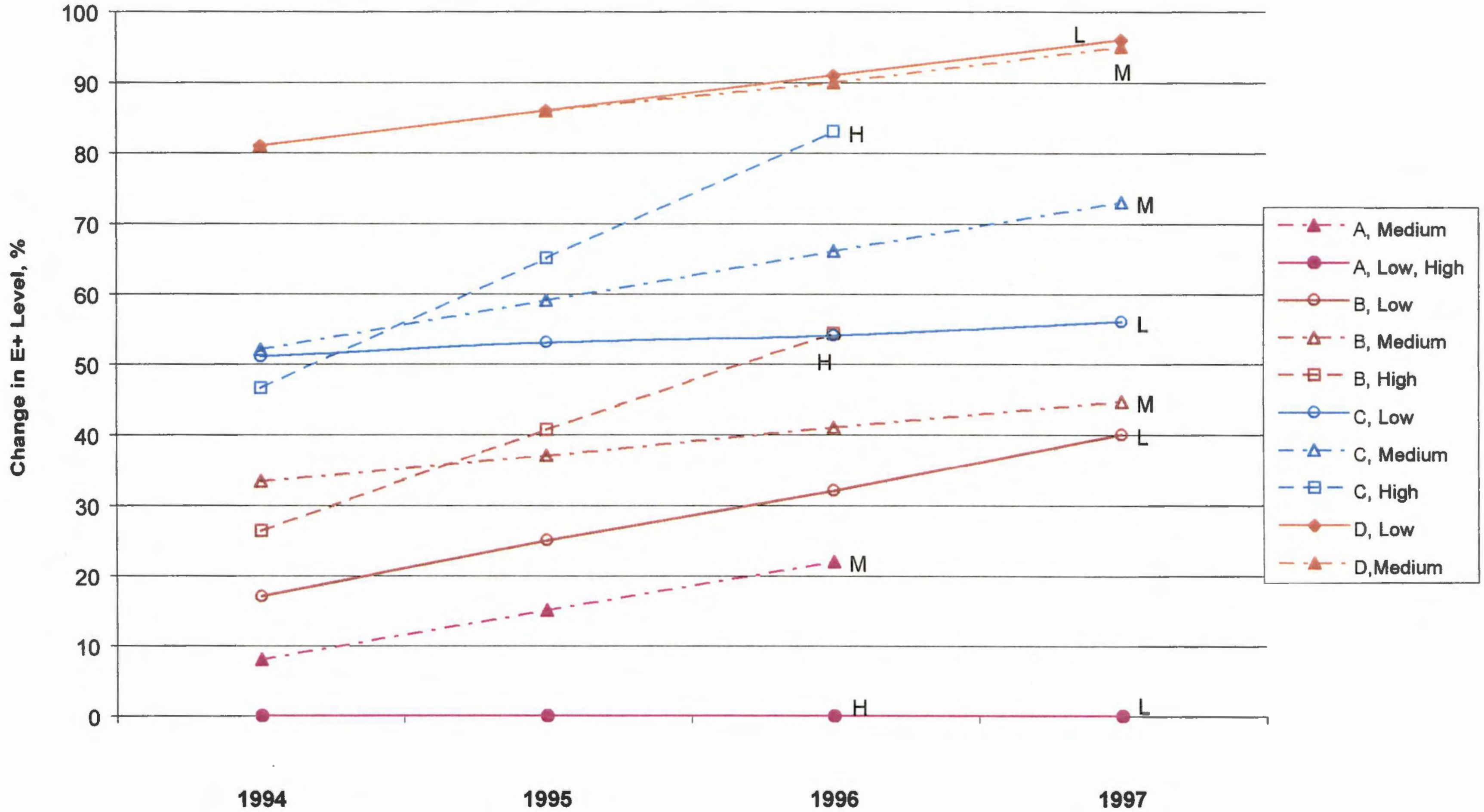

Figure 11. Changes in E+ level, 1994-1997, by initial categories and stocking densities.

A,B,C,and D refer to initial E+ categories; and Low, Medium, and High refer to stocking densities.

treatment decreased in apparent infestation level and thus decreased the average for the medium SD.

The category C (46-56% E+) shifts were evident and were the largest among all categories. The low, medium and high SD yielded 5%, 21%, and 37% increases over 3, 3, and 2 years, respectively. Averaged over all SD, the increase was about 9% per year. The category C performed similarly to the category B, but was affected more by the high SD than was the category B.

The category D (78-84% E+) was not complete in that there was no high SD for this grouping. Originally, there was not supposed to be a high SD in the category A but, during seeding, seed mixtures were switched and the 0% E+ level was put into a high SD pasture. This left the category D without a high SD treatment. Medium and low SD resulted in similar E+ level shifts to one another, and increased 14 and 15% over 3 years, respectively. The three-year average total was about 9.7% for both SD, or 4.8% per year.

Overall results for E+ level shifts were consistent with previous research. The study by Gwinn et al. (1998) used the same pastures and experimental procedures as our study, but consisted of only the first two years of data (from 1994 to 1996). Our values are slightly different from those of Gwinn et al., but our values take into account one additional year (1997), when significant E+ level changes occurred. Shelby and Dalrymple (1993) reported changes of about 4% per year. Our results indicated an 8-9% increase per year in E+ level when pastures had initial infestation levels ranging from 24-56%, and little or no change at E+ levels of 0 or > 80%. Thompson et al. (1989) indicated that 65-90%

E+ levels did not change much over 2 years, but 30% and 15% initial E+ levels had doubled and tripled, respectively. In contrast, Crawford et al. (1989) reported that little or no change occurred in most of their endophyte infested pastures over 2 years. Our study indicated significant changes, some of which occurred within the third year of the study. This indicates that more than 2 years of data may be needed to assess accurately the E+ level shifts that occur in pastures grazed by cattle.

This study indicated that cattle grazing pressure resulting from increasing SD, and E+ tall fescue plant persistence were the causes of E+ level shifts. Pastures were not allowed to mature to seed head growth stage, and animals were fed non-fescue forages for 3 d prior to being switched from E+ to E- pastures to prevent fecal E+ seed contamination (Gwinn et al. 1998). In addition, prior to seeding the experimental pastures, all previous tall fescue growth had been eliminated thoroughly. Although there were periods of temporary drought, there were no extended periods of severe environmental stress on the pastures. All of these reasons would lead to the belief that the E+ level shifts were caused by factors other than environmental stress or seed contamination. This study indicated that increasing SD had a significant effect on increasing E+ levels and the effect was greatest at high stocking rates and for intermediate initial E+ levels. The grazing pressure may have eliminated less adapted E- plants and thereby allowed the E+ plants to invade the empty spaces left by dead E- plants.

V. Conclusions

Climatic conditions affected the management of pastures from year to year, particularly in times of drought or below or above normal temperatures. Stocking densities were adjusted when tall fescue availability was low and some pastures were temporarily withheld from grazing so that available forage could increase to minimum requirement levels. No experimental pastures were damaged permanently or terminated until the final spring and summer seasons, when the high SD pastures were unable to produce enough forage to maintain the grazing steers. Total forage quantities were never as large as they were the first year of the experiment; and they progressively declined with each following year. These results indicate that continuous grazing can damage tall fescue stands permanently, particularly on E- and low E+ pastures and during periods of drought.

Increasing SD from low to high resulted in decreased ADG. This was attributed to decreased amounts of available forage at the high SD. In addition, the decrease in ADG per unit of E+ level increase was largest at the high SD, implying that E+ tall fescue plants increased in number more at the high SD than at the low and medium SD. This may have caused reduced gains due to increased amounts of E+ tall fescue ingested by the animals at the high SD. Spring resulted in the largest ADG, followed by fall/winter and summer, and was a result of increased forage production during spring with favorable climatic conditions for tall fescue growth. In spring and summer seasons, the medium SD average daily gains were comparable to those of the low SD. Only during fall/winter were the low SD average daily gains significantly larger than those of medium SD. This difference was attributed to the decline of good quality stockpiled tall fescue during the fall/winter at medium and high SD. The low SD resulted in the largest amounts of available forage and thus, the largest ADG.

Beef production was largest during the spring season and at the medium SD overall. As SD increased, beef production increased, except during fall/winter. Having large amounts of stockpiled tall fescue for fall/winter at the low SD produced the largest amounts of beef ha^{-1}. Only during the spring was the high SD the best method for beef production. Fall/winter and summer were not good seasons for the high SD treatment due to rapidly decreased available forage and these combinations resulted in the smallest amount of beef ha^{-1}. Similar to the ADG results, the beef production decrease per unit increase in E+ level was greatest at the high SD. This may have been also attributed to a more significant shift in E+ level at the high SD than at the low and medium SD, or because of the significant decline in beef production from spring to summer and fall/winter.

Forage quality was affected by season and was consistent with that reported in the literature. Both ADF and NDF concentrations were largest in summer, intermediate in fall/winter and smallest in spring. Although these differences were significant statistically, they were small and not thought to have had much of an effect on forage digestibility or animal performance in this study. Crude protein was affected by both season and SD. Spring resulted in the largest concentrations of CP and summer resulted in the lowest. These data supported the concept that quality of tall fescue declined as the growing season progressed, even though the plants were not permitted to develop to reproductive stage. Since the high SD contained the largest concentrations of CP during spring and fall/winter, this concept was further supported and implied that since grazing pressure was increased, mature growth was inhibited greatly.

Prolactin concentrations were affected by increasing E+ levels and resulted in an inverse relationship in 1996. Although these results were limited to only two years of the study, they indicated that prolactin may have been a useful marker in detecting the effects of *N. coenophialum* and the associated alkaloid, ergovaline, on grazing cattle. Since prolactin concentrations are highly variable and dependent upon many factors, more than one sampling per year is required to assess accurately the effects of E+ level on hormonal concentrations in cattle grazing E+ tall fescue.

Increasing SD significantly shifted E+ levels from initial (seeding) levels to more highly infested levels over the 3+-year period. Intermediate initial E+ level pastures (24-56%) were affected the most by increasing SD, and 0 and > 80% E+ level pastures were affected very little. These results indicate the urgency of proper management of tall fescue pastures and suggest that pastures containing E+ tall fescue may have different E+ levels from year to year, resulting in altered cattle performance.

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Literature Cited

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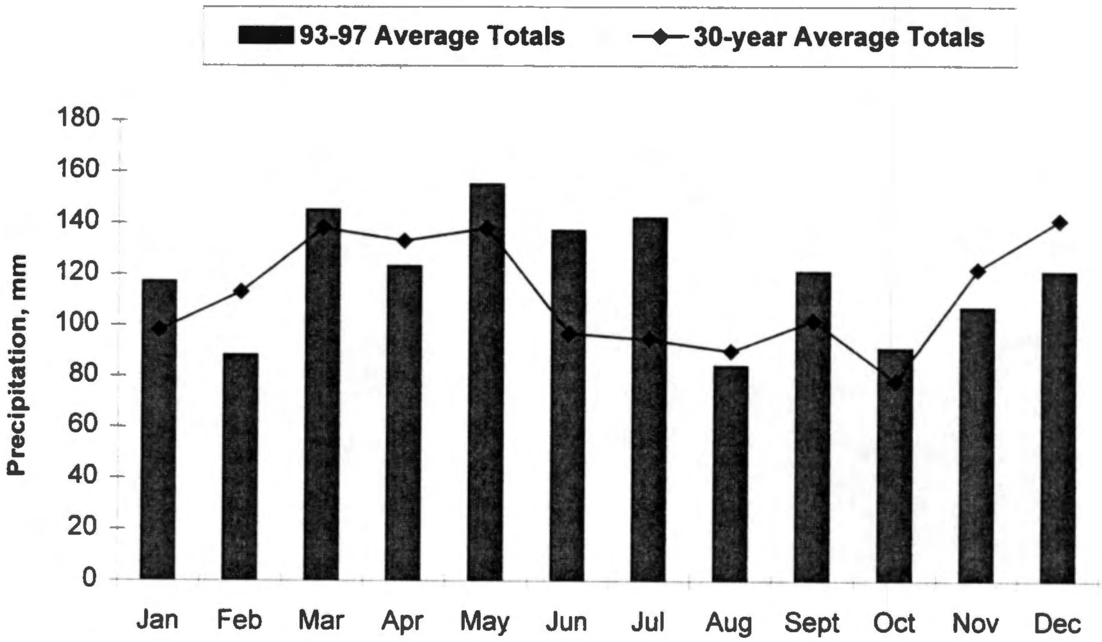
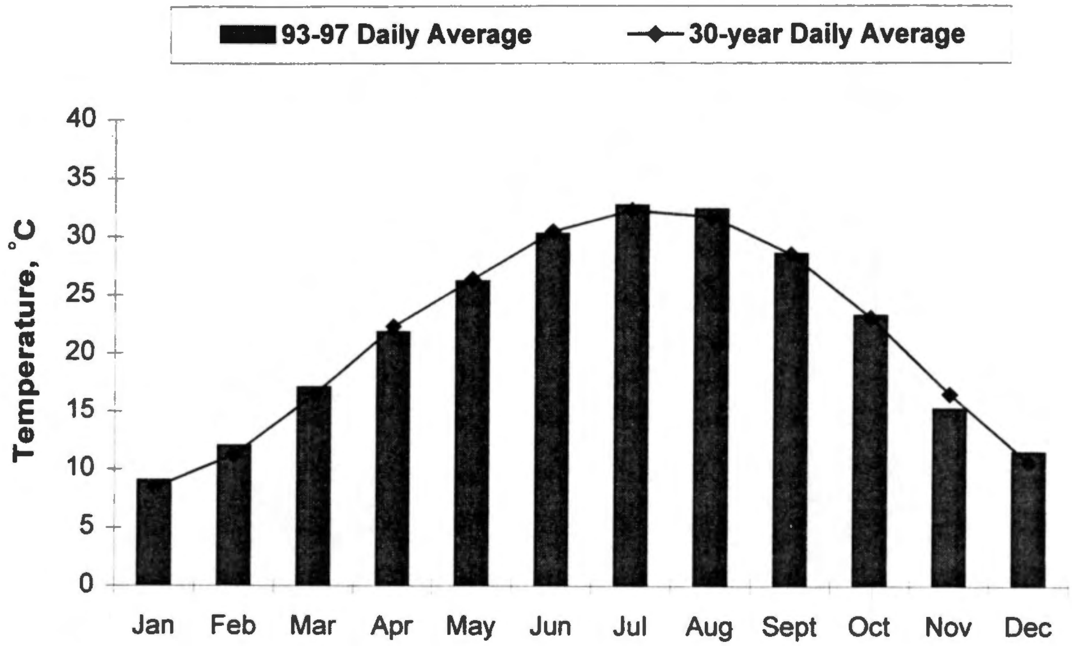
Appendices

Appendix A

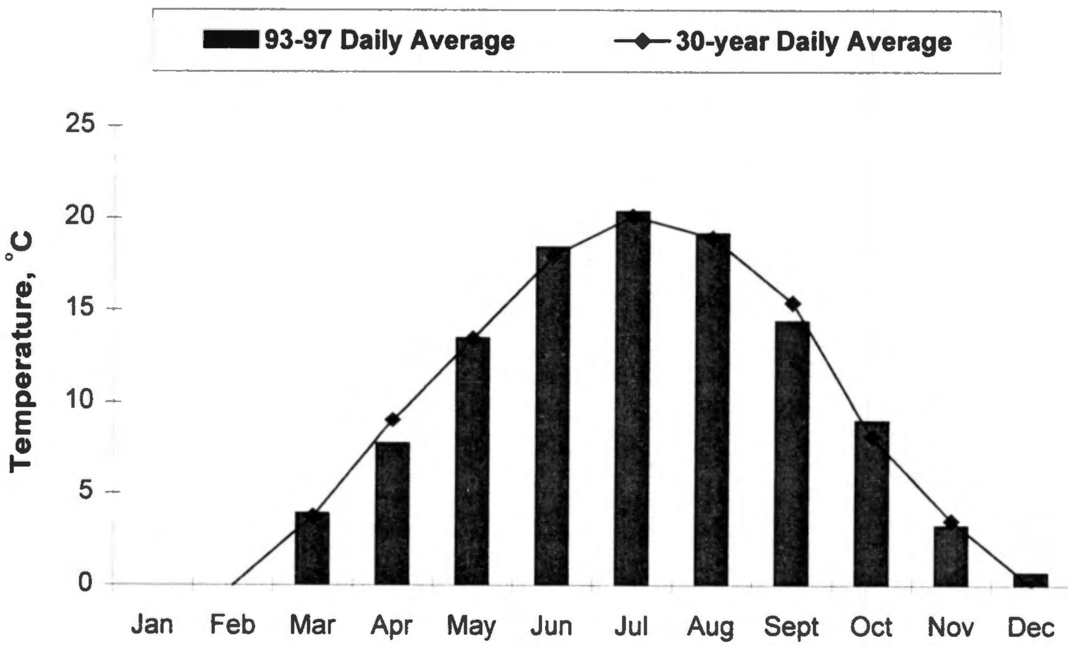
Climatic Conditions



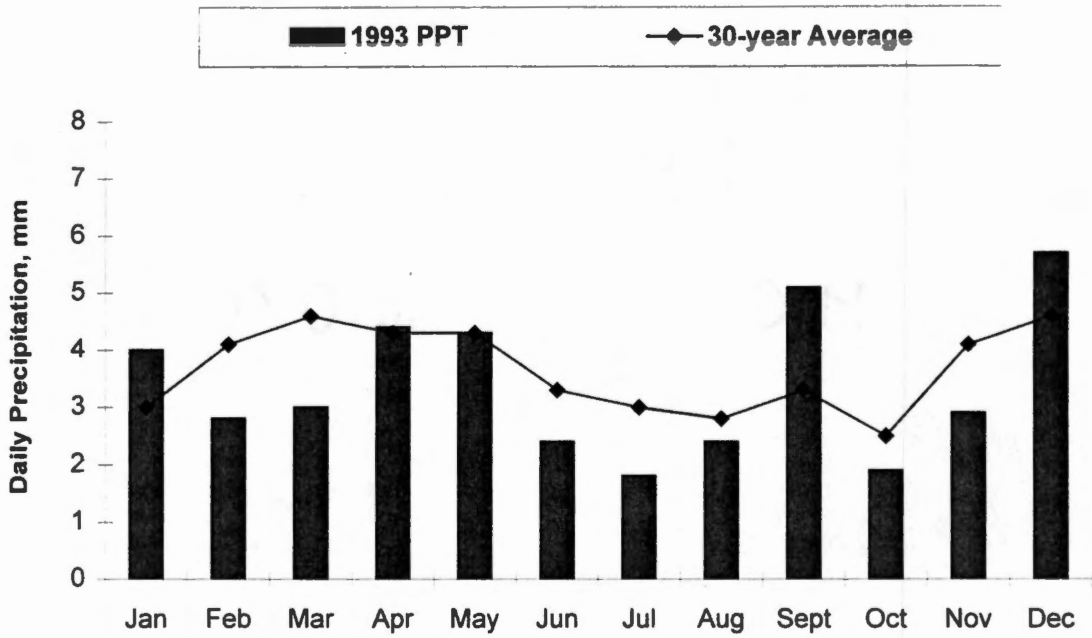
A-1. Thirty-year normal precipitation compared to 1993-1997 monthly average totals.



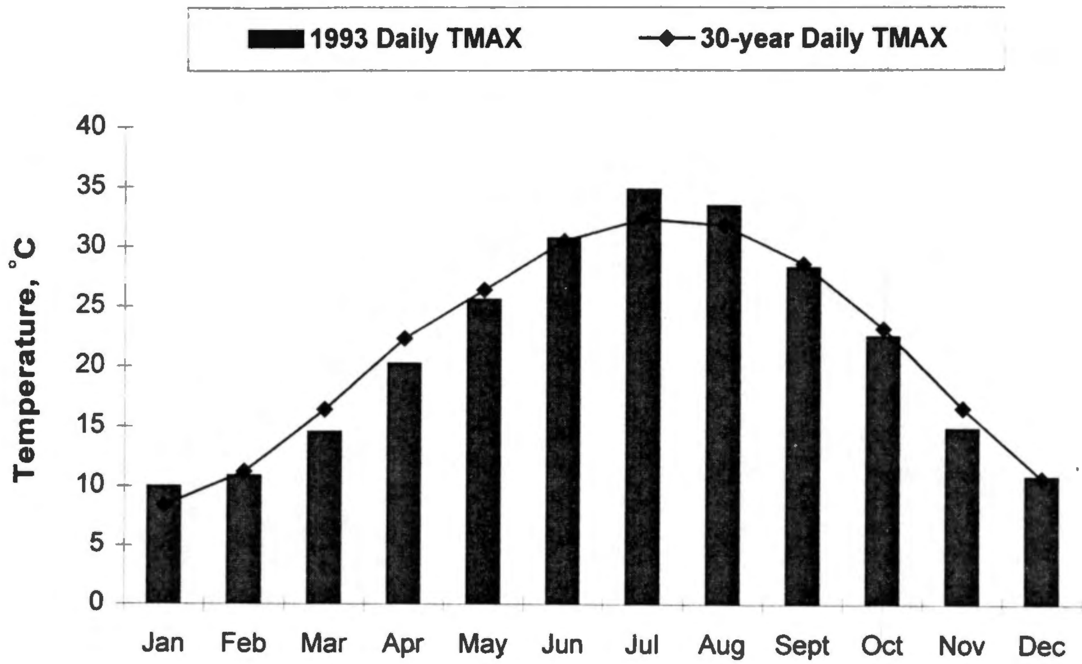
A-2. Thirty-year normal maximum temperatures compared to 1993-1997 monthly maximum temperatures.



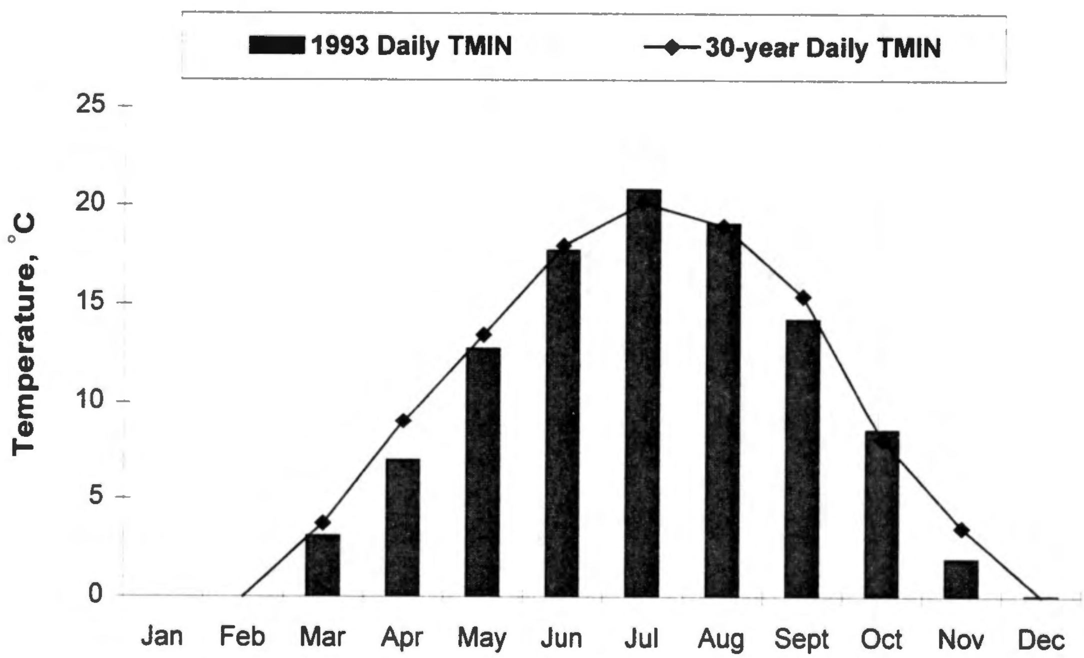
A-3. Thirty-year normal minimum temperatures compared to 1993-1997 monthly minimum temperatures.



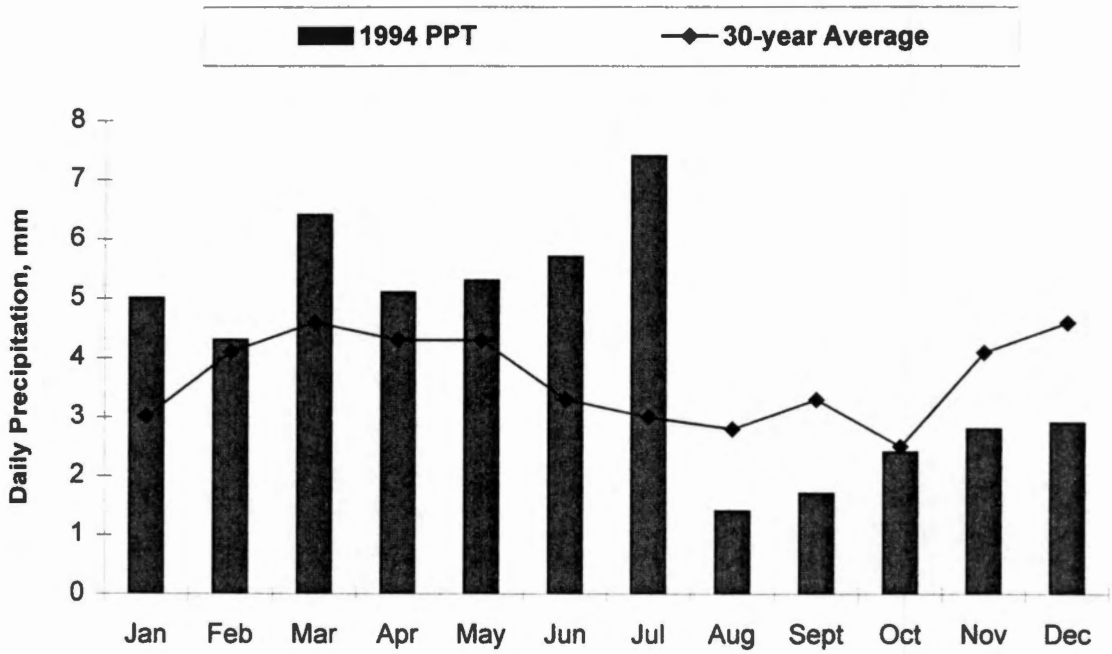
A-4. Thirty-year normal daily average precipitation compared to Ames-1993 daily averages.



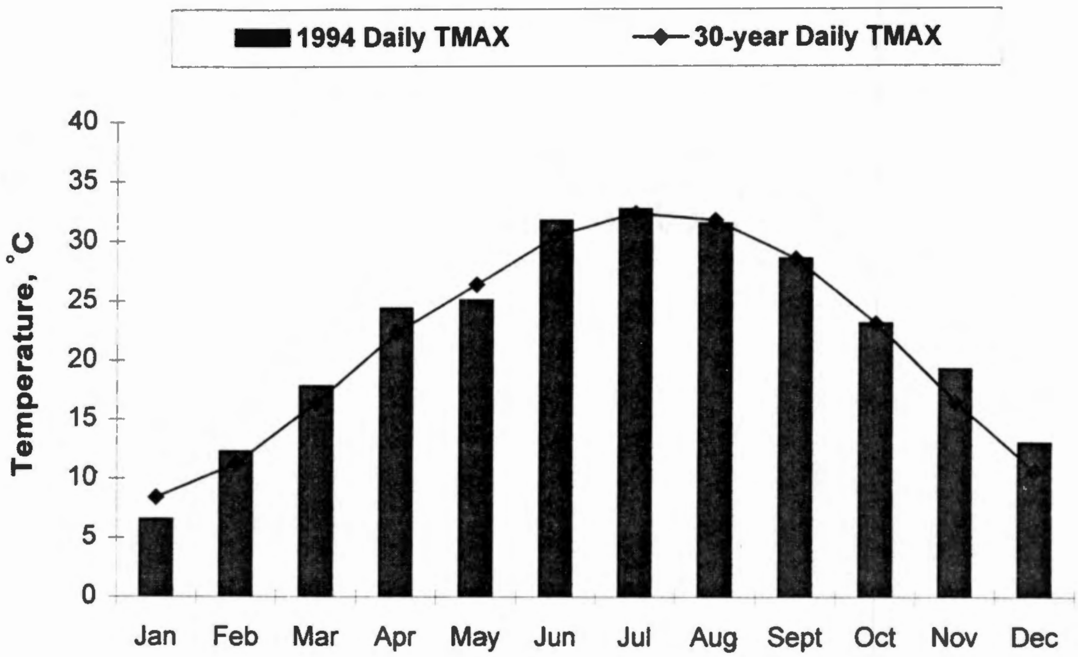
A-5. Thirty-year normal maximum temperatures compared to Ames-1993 daily maximum temperatures.



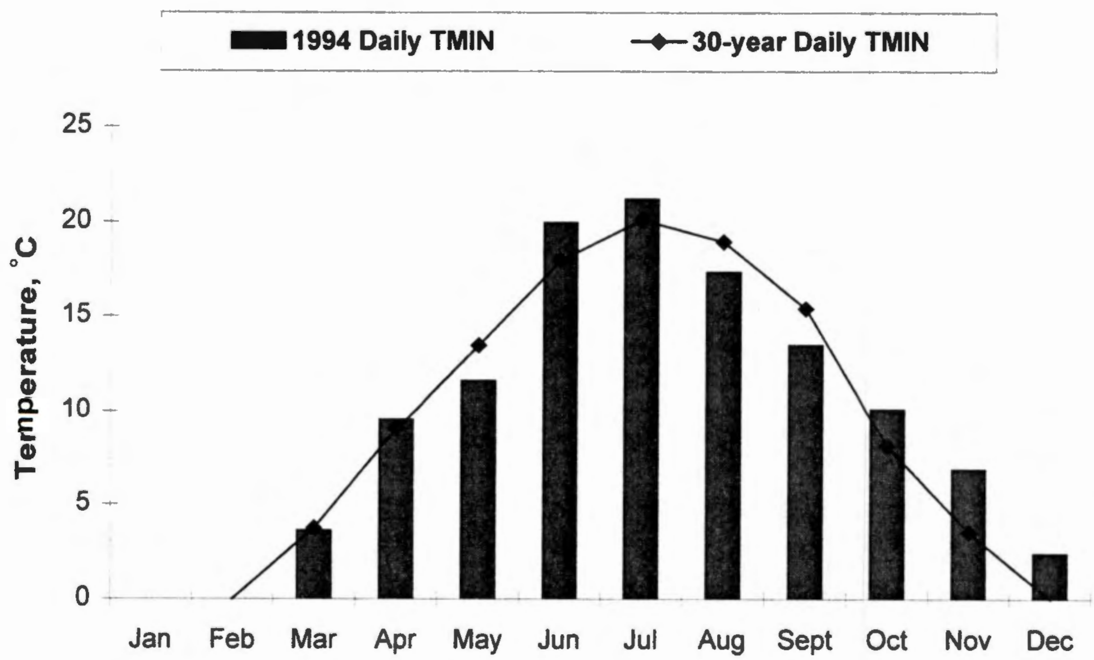
A-6. Thirty-year normal minimum temperatures compared to Ames-1993 daily minimum temperatures.



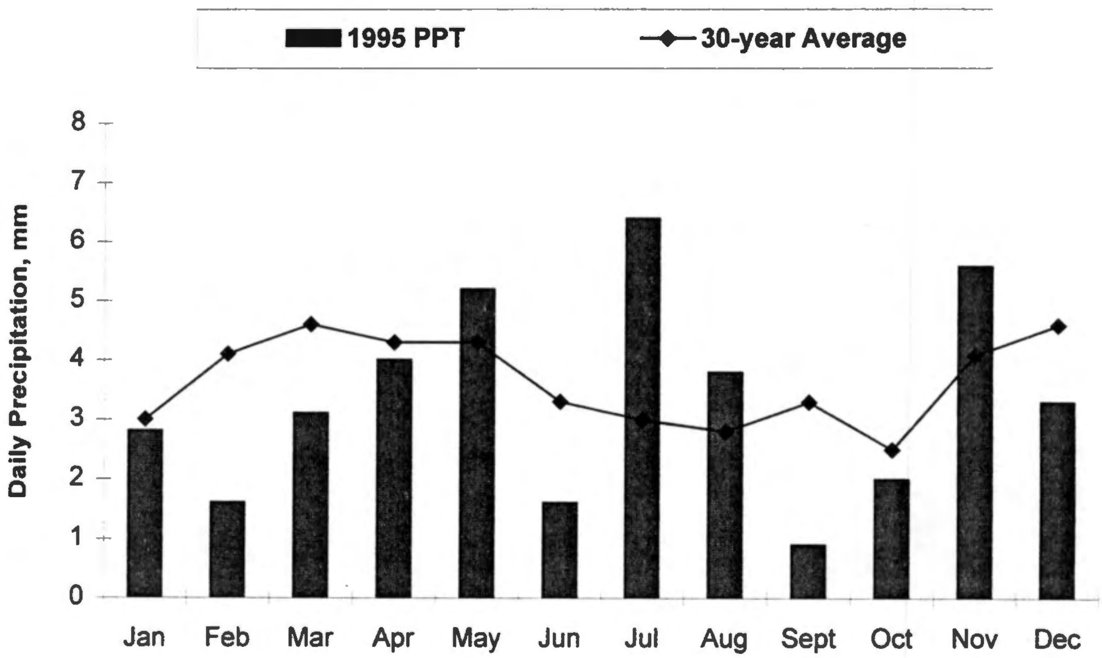
A-7. Thirty-year normal daily average precipitation compared to Ames-1994 daily averages.



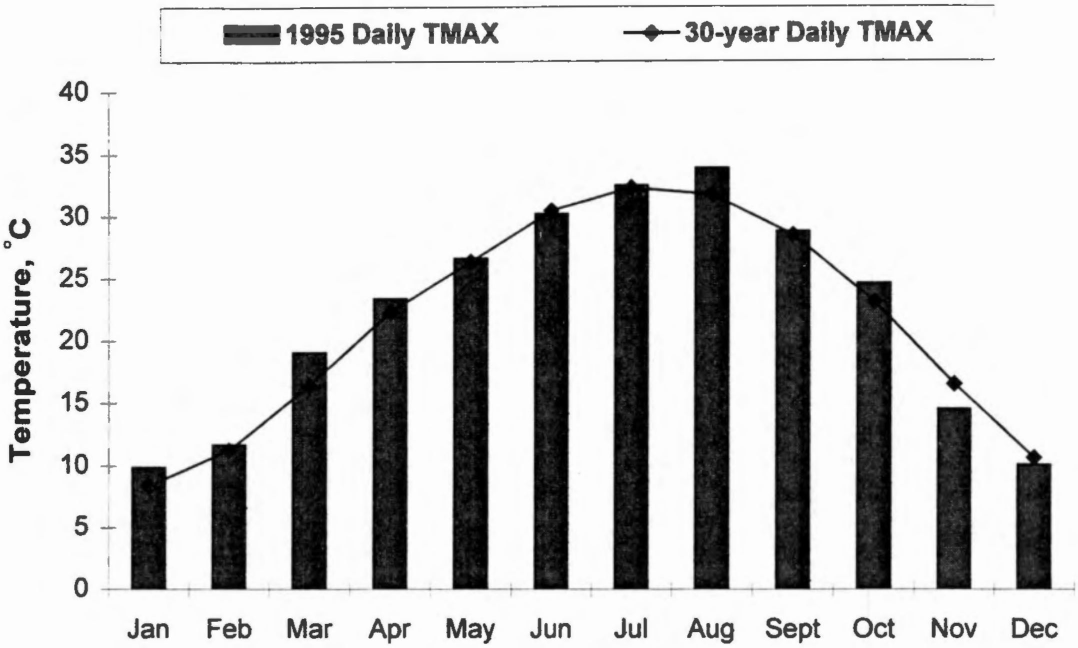
A-8. Thirty-year normal maximum temperatures compared to Ames-1994 daily maximum temperatures.



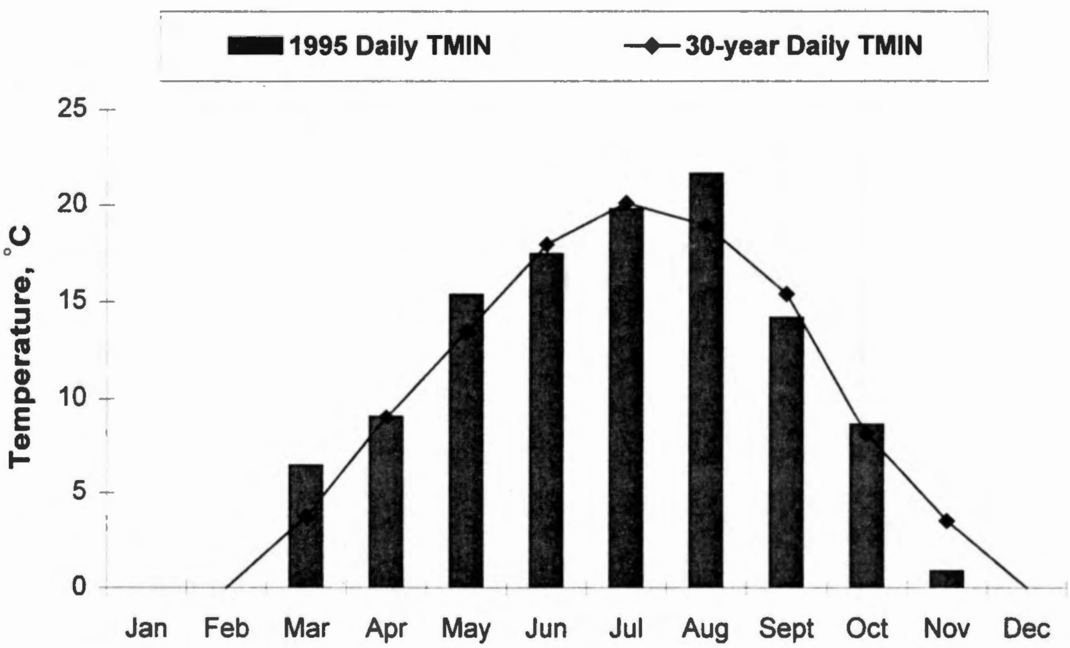
A-9. Thirty-year normal minimum temperatures compared to Ames-1994 daily minimum temperatures.



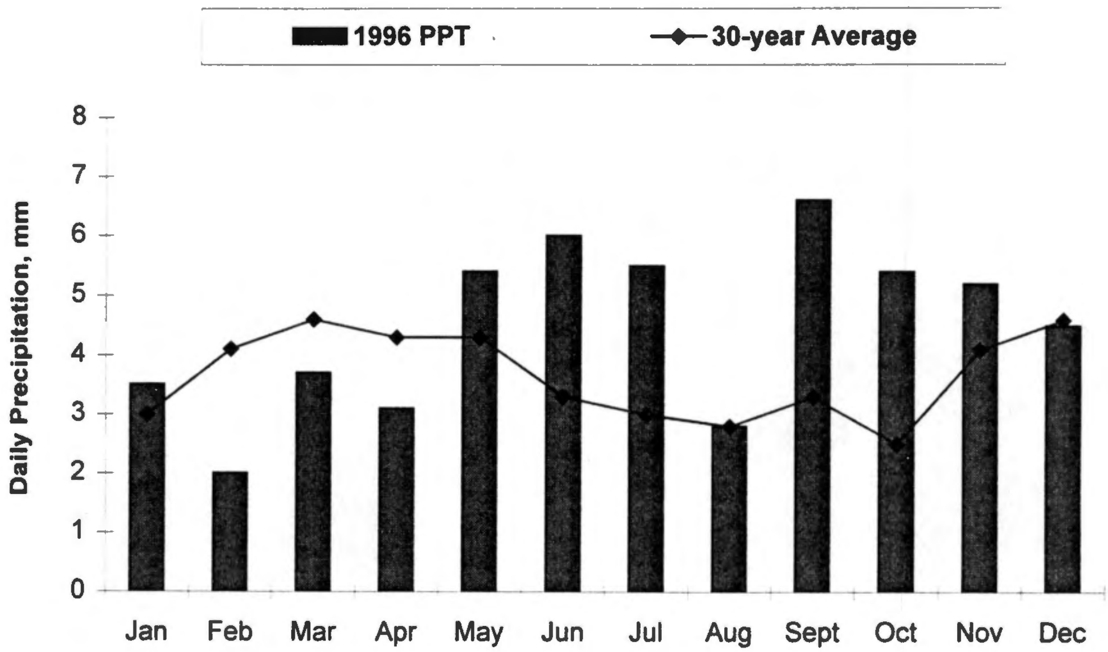
A-10. Thirty-year normal daily average precipitation compared to Ames-1995 daily averages.



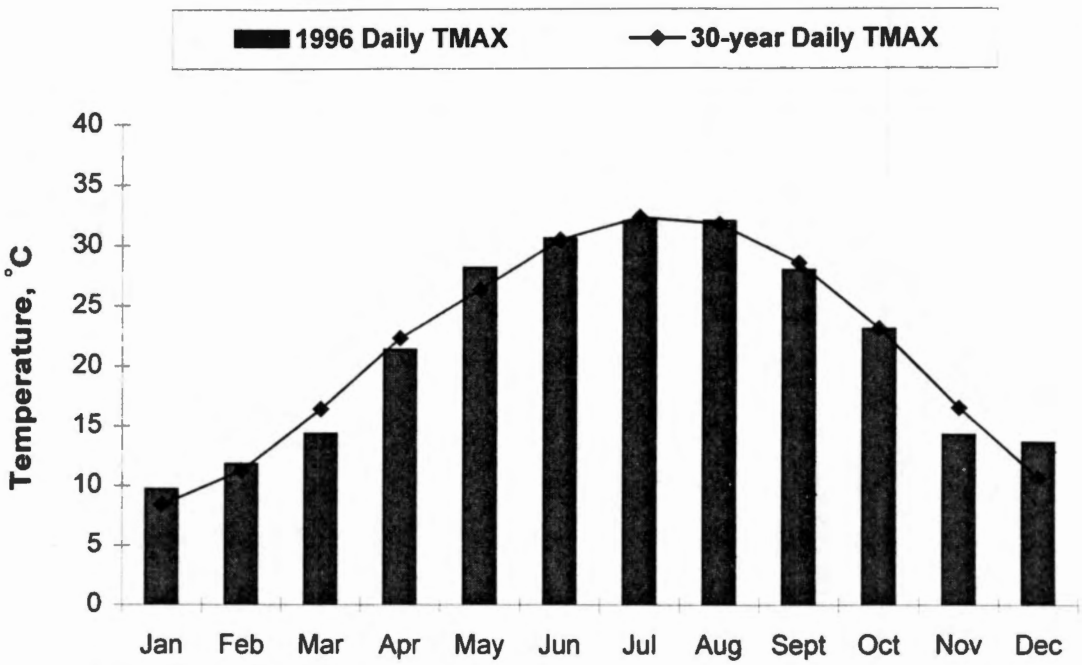
A-11. Thirty-year normal maximum temperatures compared to Ames-1995 daily maximum temperatures.



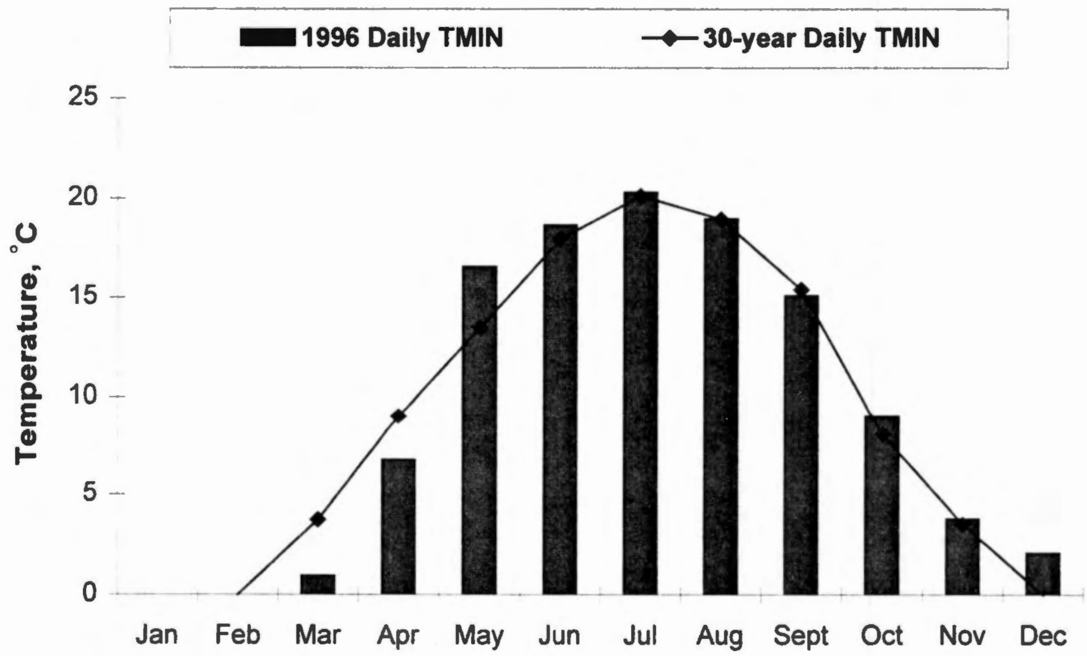
A-12. Thirty-year normal minimum temperatures compared to Ames-1995 daily minimum temperatures.



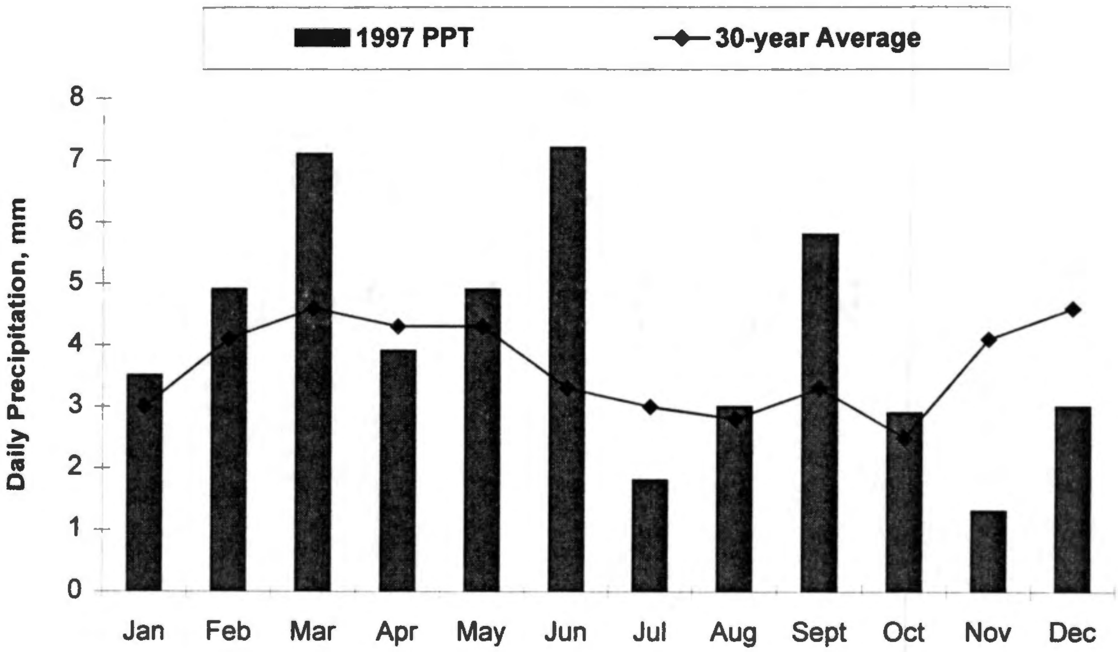
A-13. Thirty-year normal daily average precipitation compared to Ames-1996 daily averages.



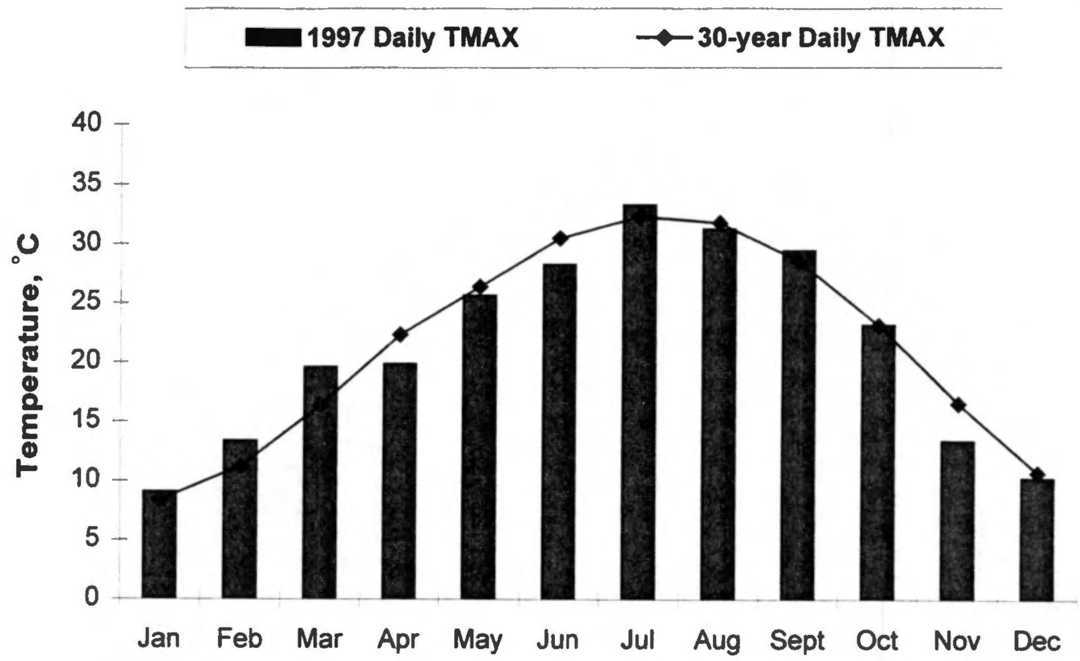
A-14. Thirty-year normal maximum temperatures compared to Ames-1996 daily maximum temperatures.



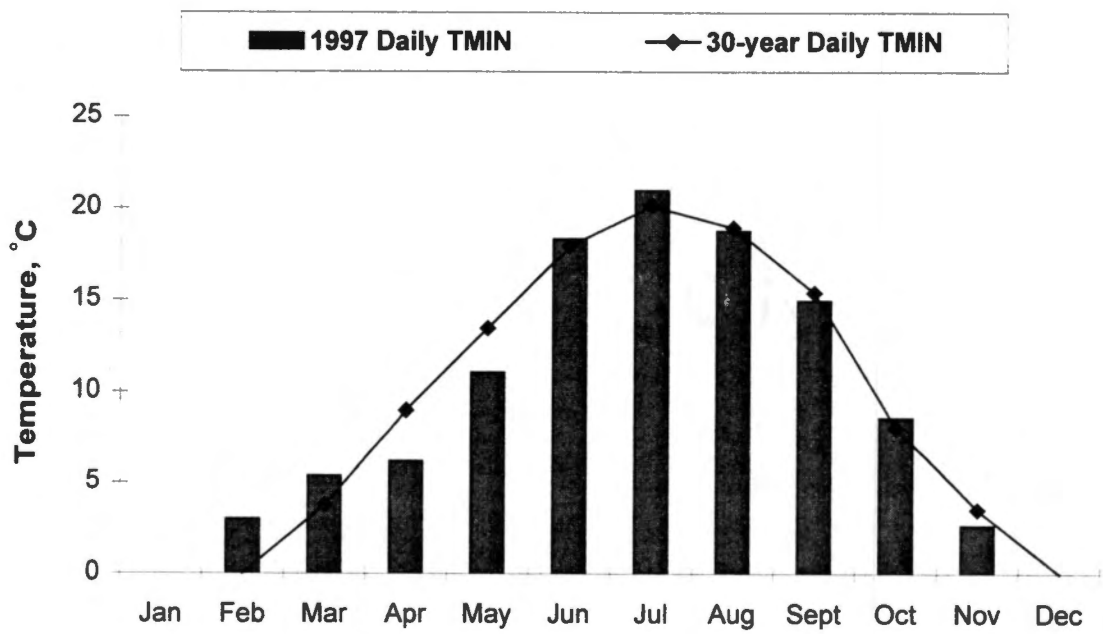
A-15. Thirty-year normal minimum temperatures compared to Ames-1996 daily minimum temperatures.



A-16. Thirty-year normal daily average precipitation compared to Ames-1997 daily averages.



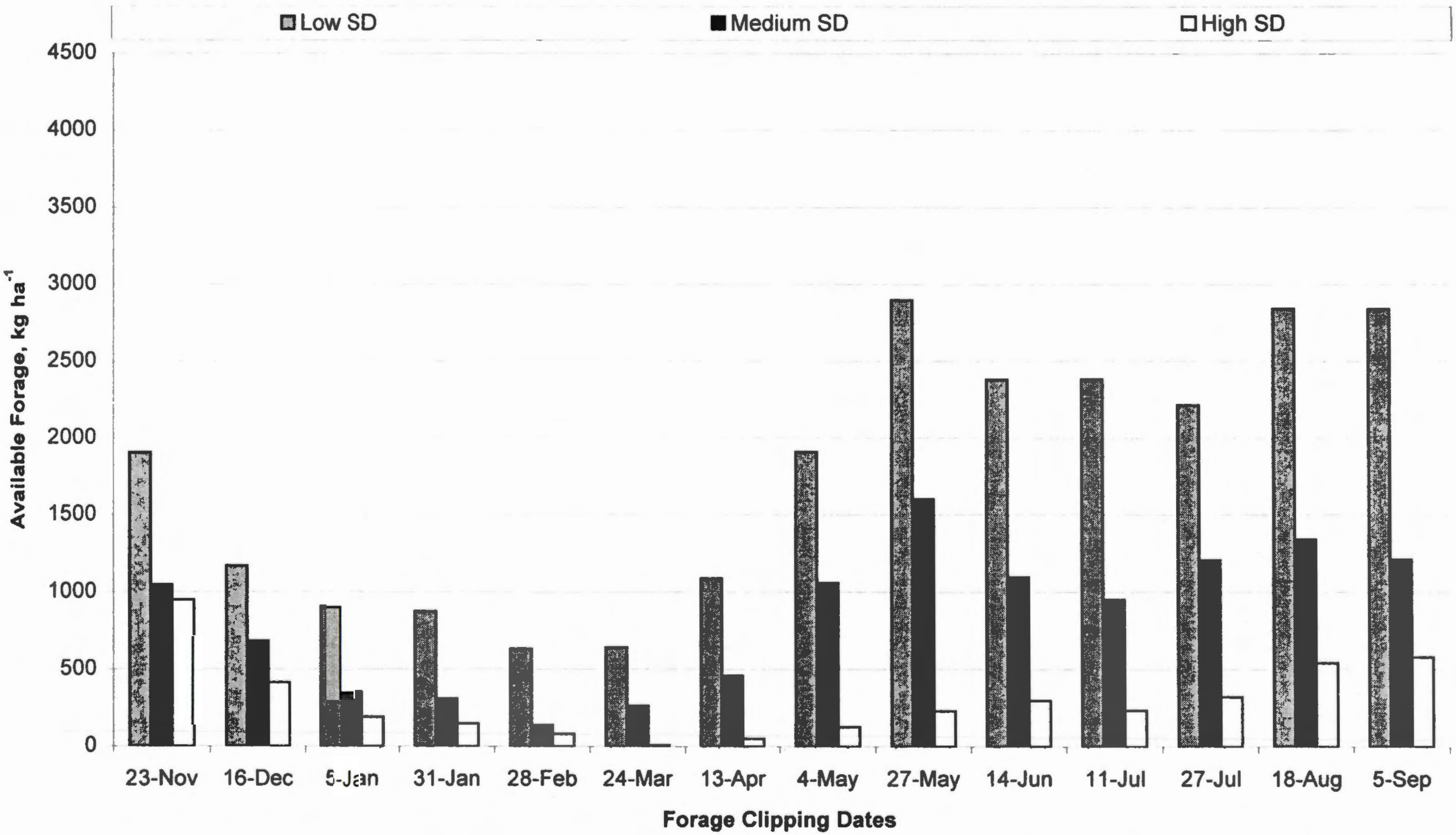
A-17. Thirty-year normal maximum temperatures compared to Ames-1997 daily maximum temperatures.



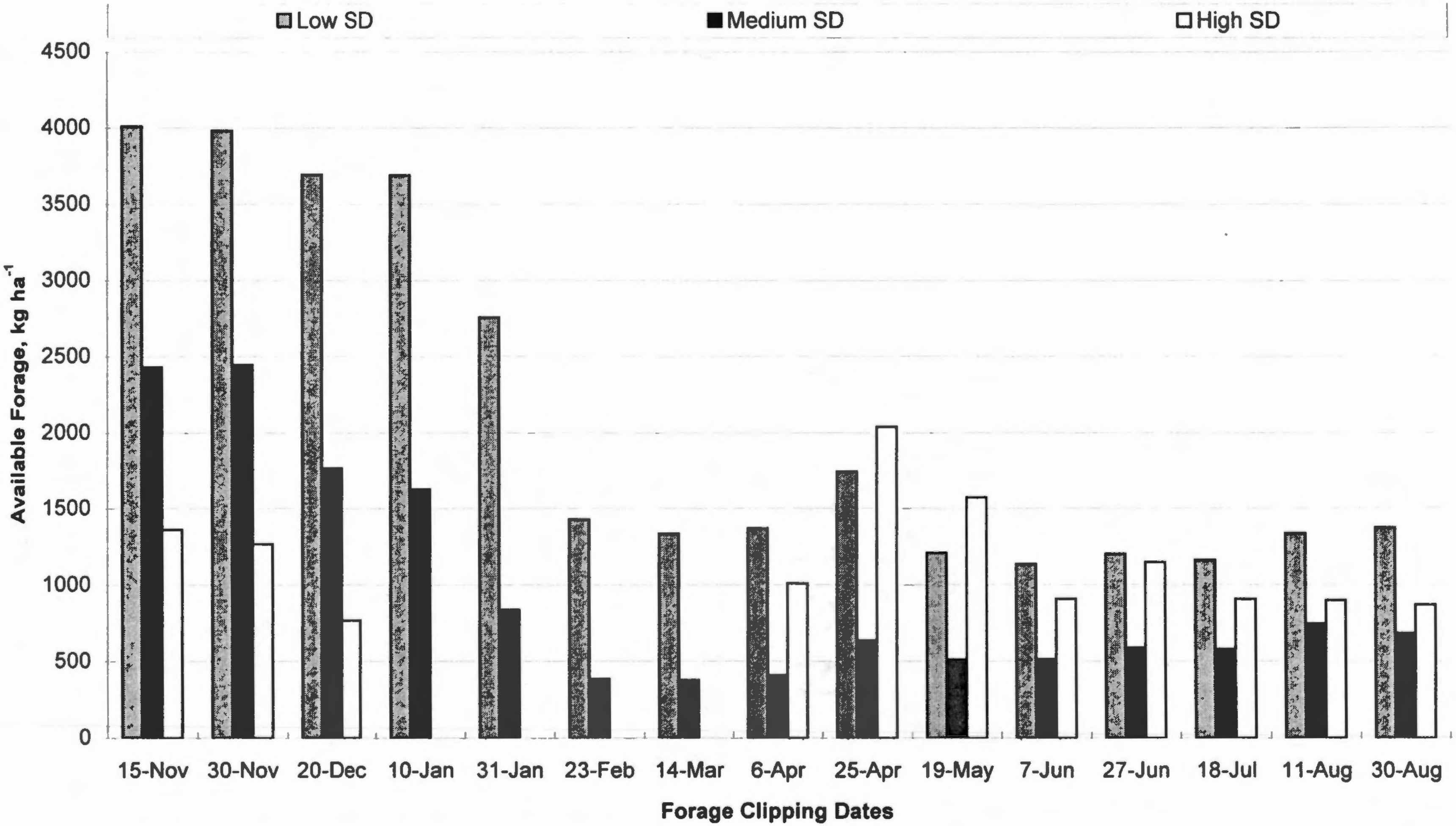
A-18. Thirty-year normal minimum temperatures compared to Ames-1997 daily minimum temperatures.

Appendix B

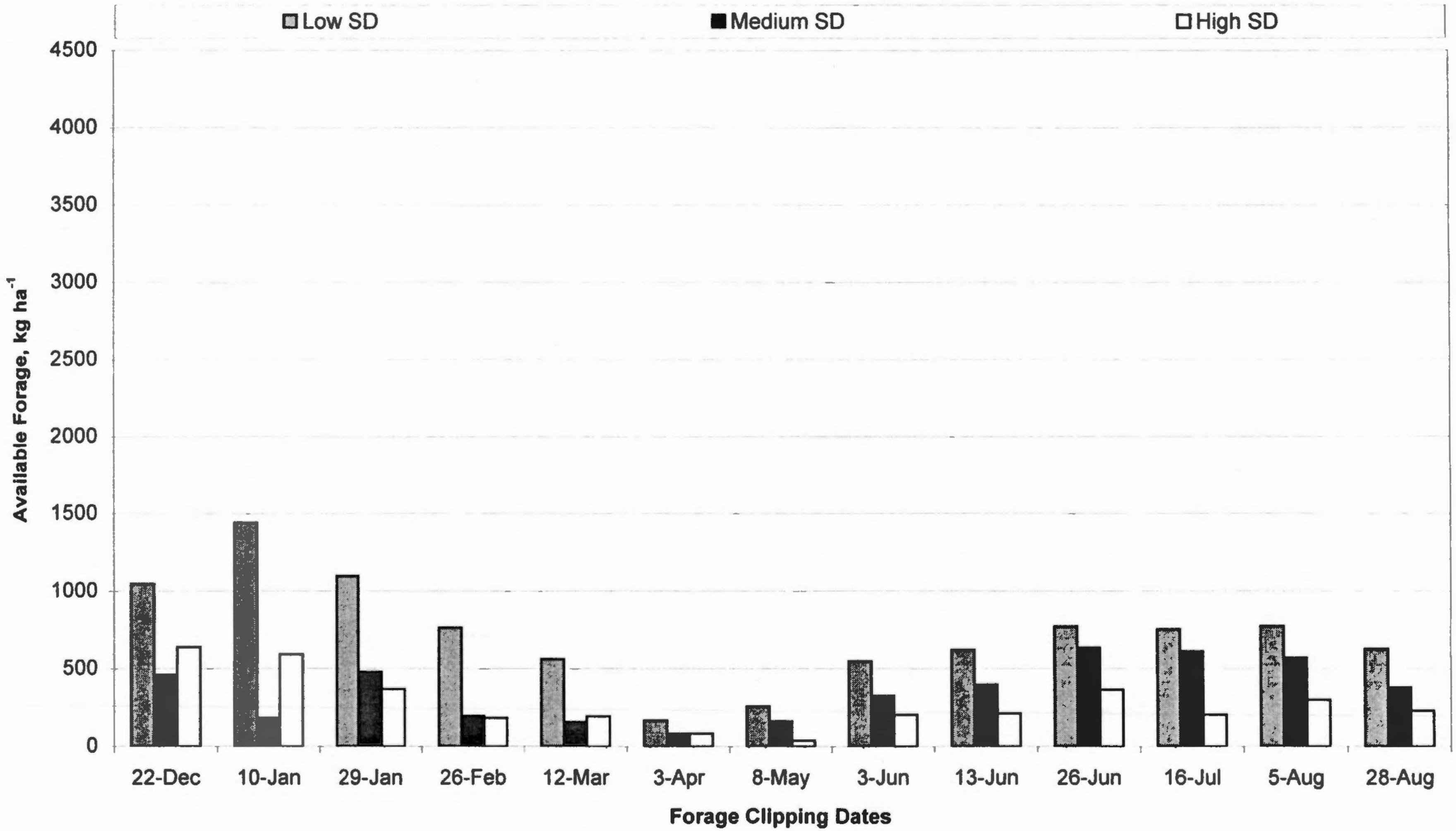
Available Forage



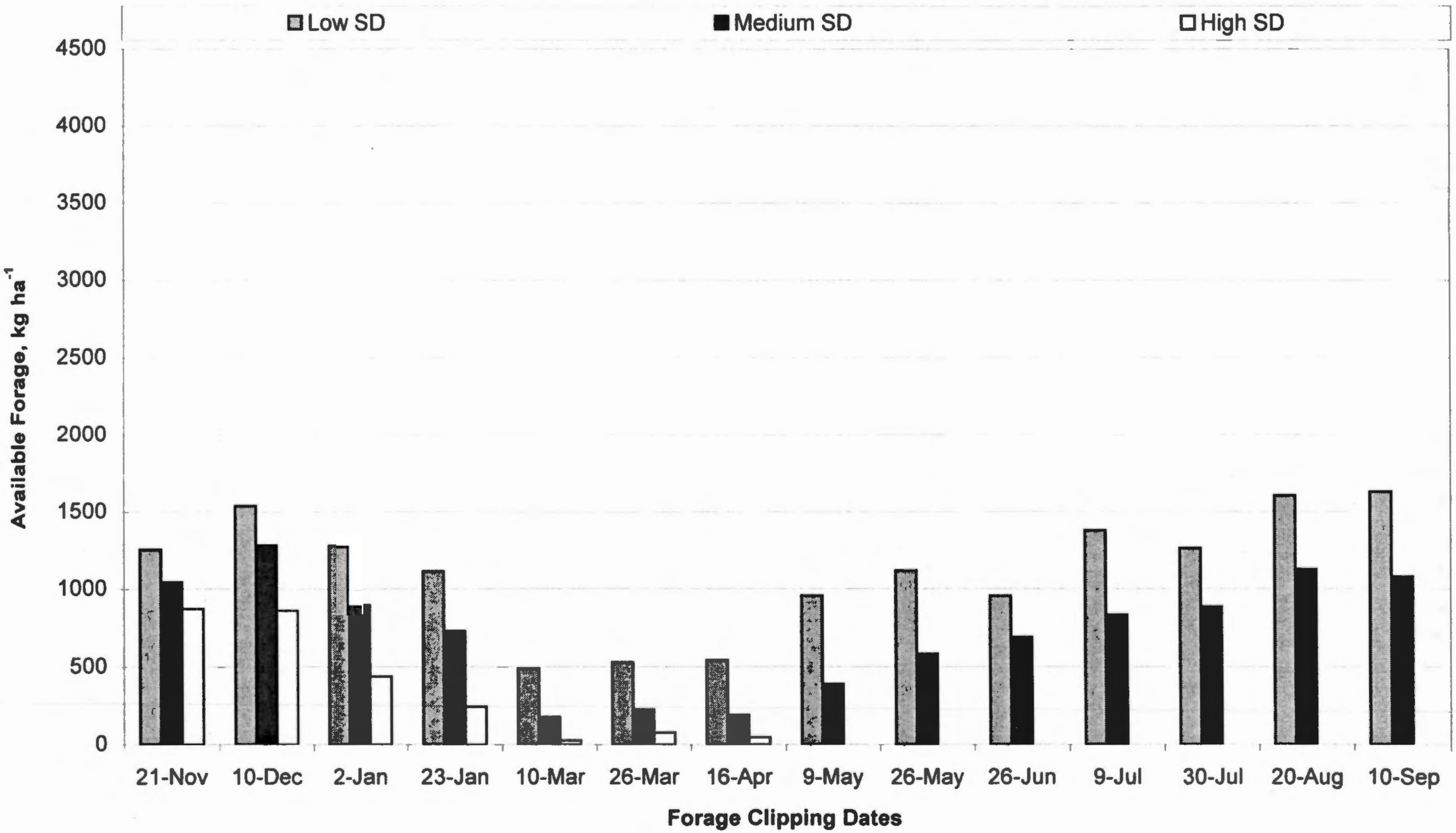
B-1. Available forage for all stocking densities, averaged over all E+ levels, 1993-1994.



B-2. Available forage for all stocking densities, averaged over all E+ levels, 1994-1995.



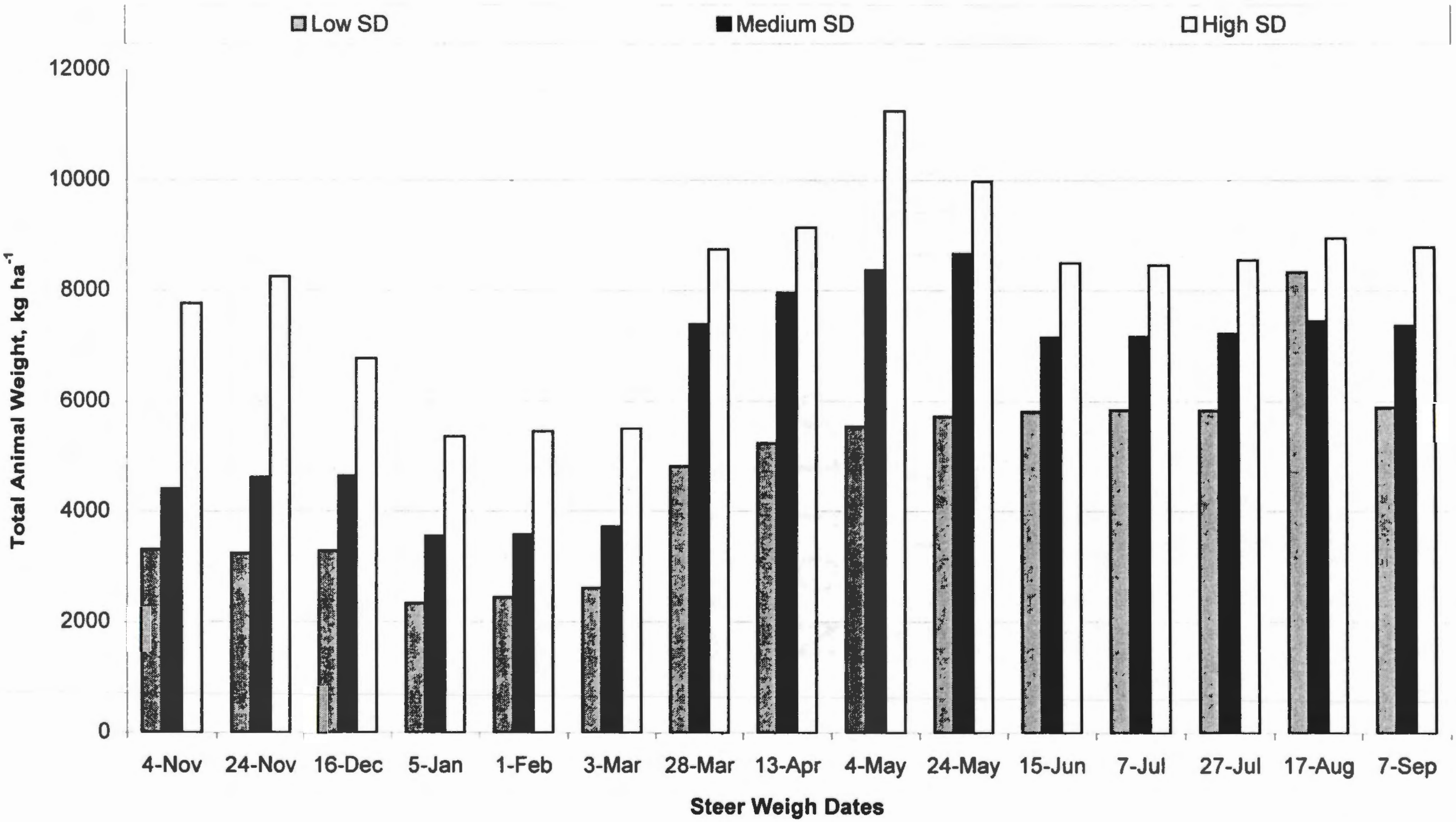
B-3. Available forage for all stocking densities, averaged over all E+ levels, 1995-1996.



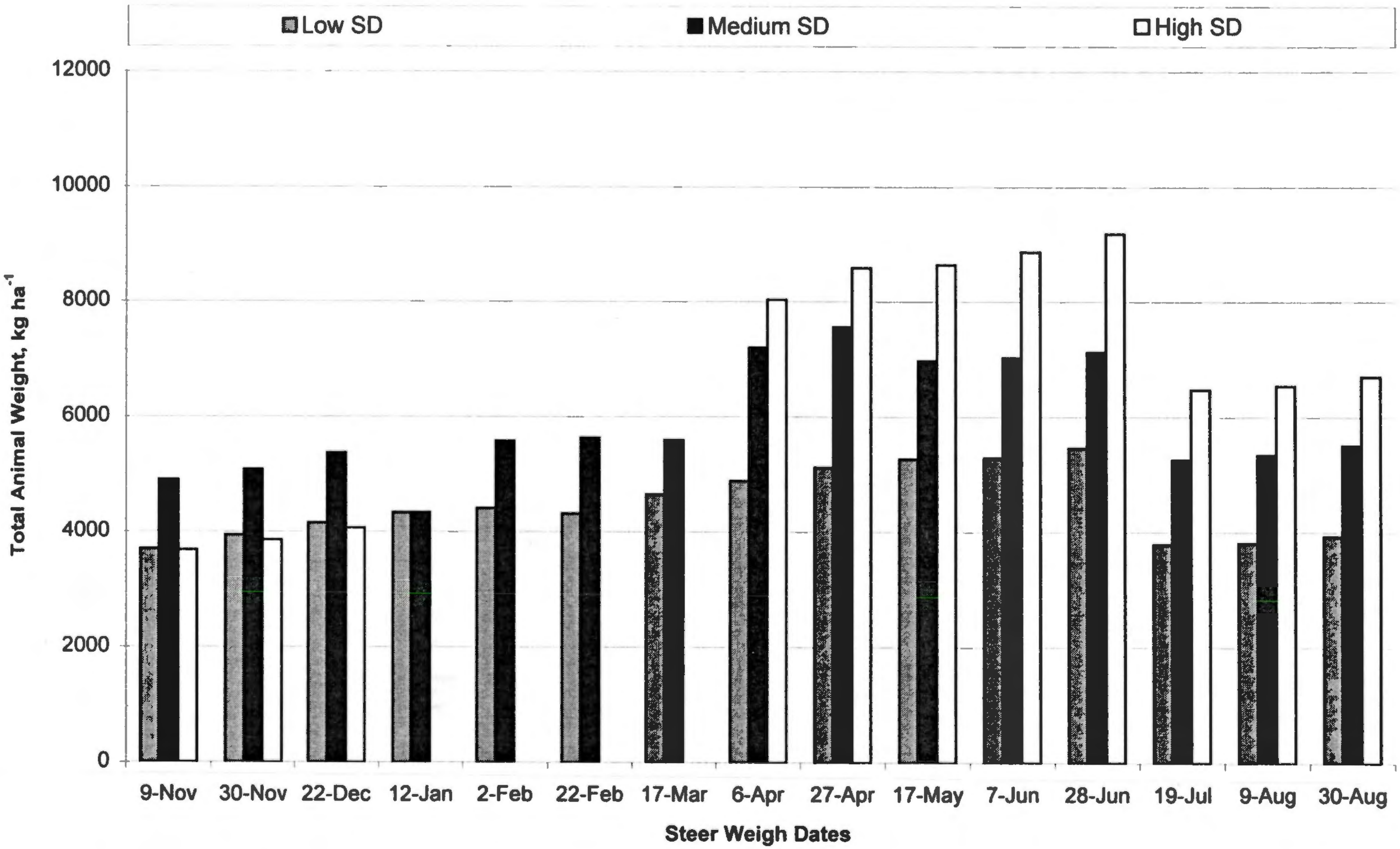
B-4. Available forage for all stocking densities, averaged over all E+ levels, 1996-1997.

Appendix C

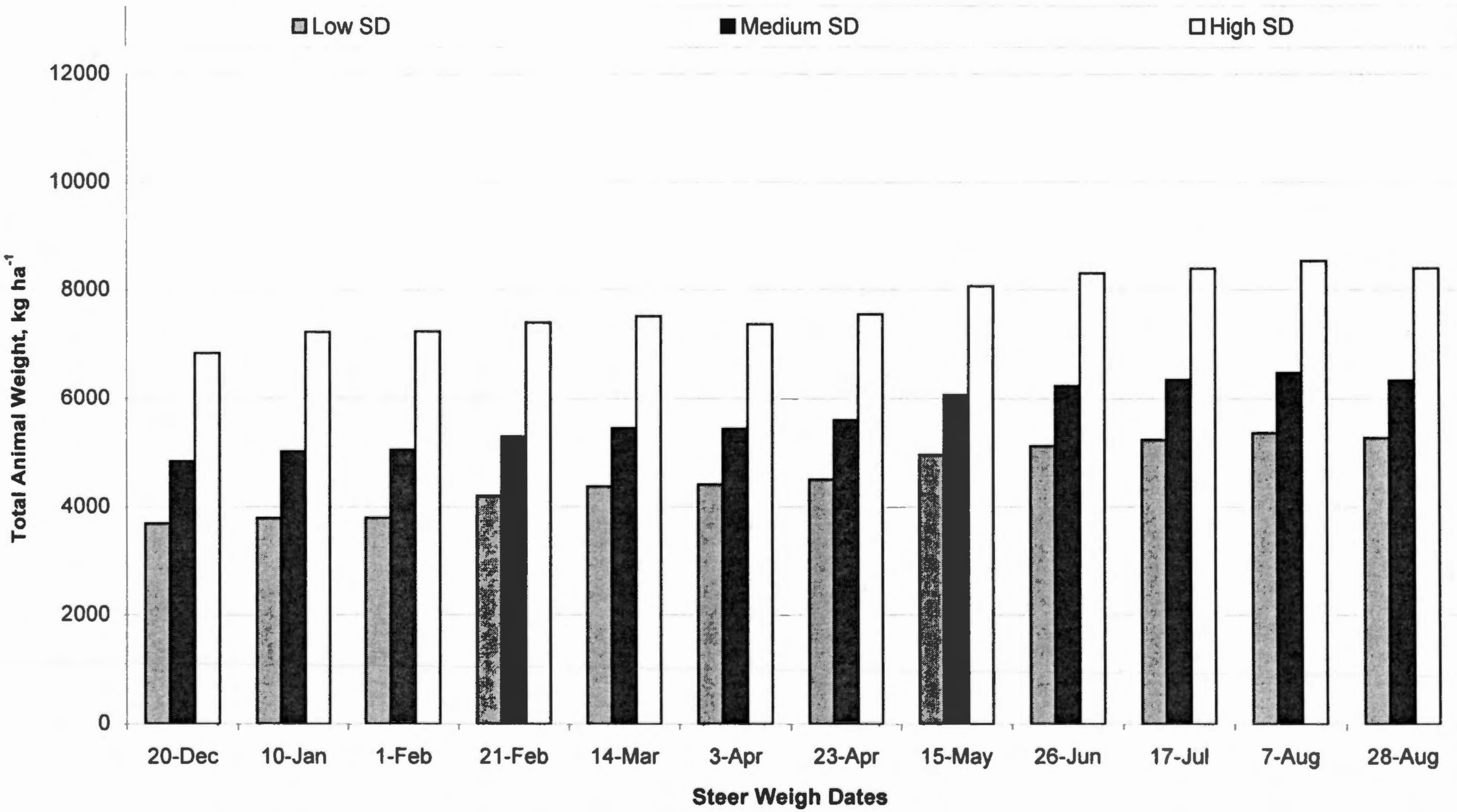
Total Live Weight



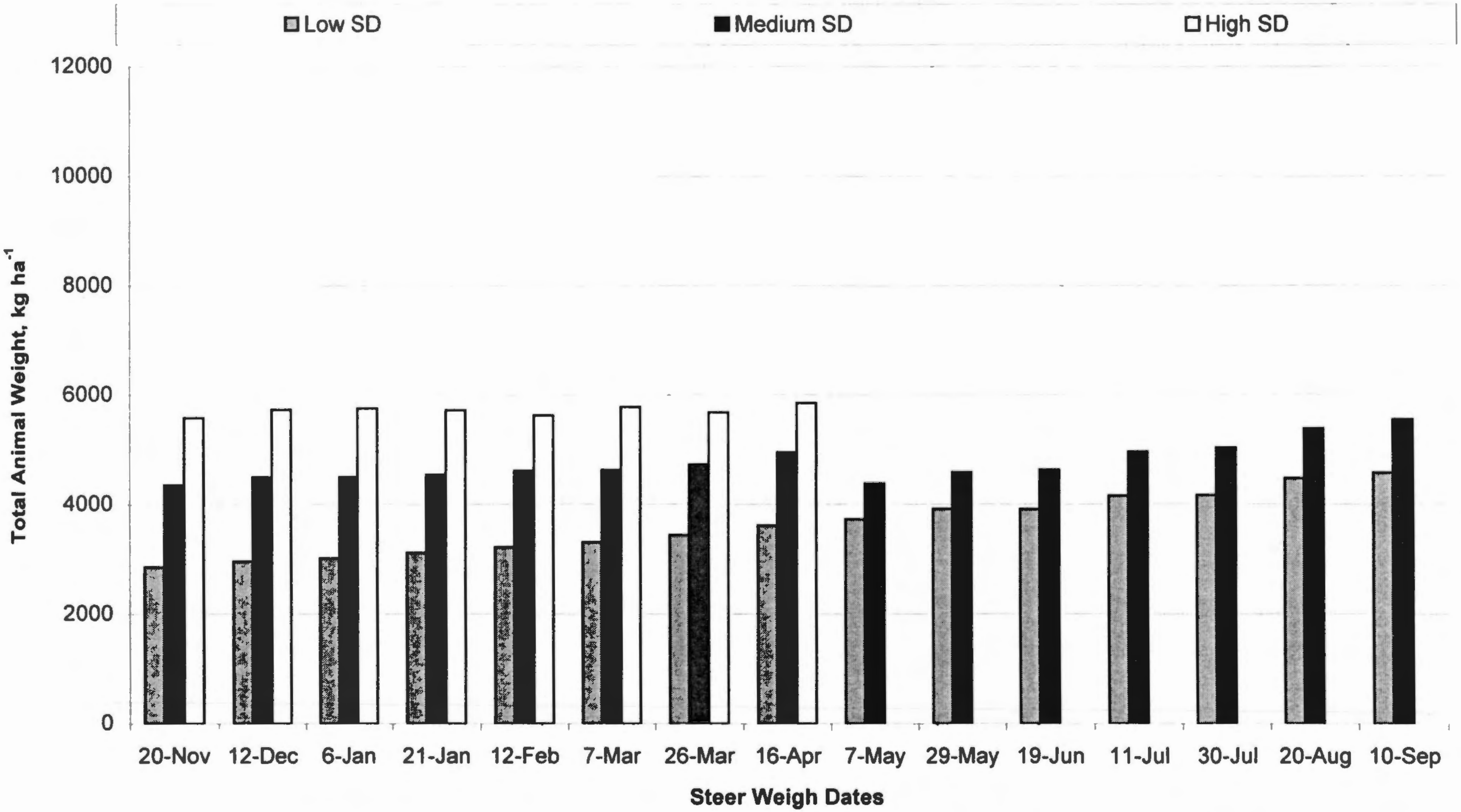
C-1. Total live weight of all steers at three stocking densities, averaged over all E+ levels, 1993-1994.



C-2. Total live weight of all steers at three stocking densities, averaged over all E+ levels, 1994-1995.



C-3. Total live weight of all steers at three stocking densities, averaged over all E+ levels, 1995-1996.



C-4. Total live weight of all steers at three stocking densities, averaged over all E+ levels, 1996-1997.

Appendix D

PAS-ELISA Procedure

PAS ELISA Procedure

Day 1:

I. Protein A

1. ELISA plates are Plastic Injectors' Code-A-Well 96-well plates.
2. Mix 20 µL of Protein A (in freezer) with 20 mL of Carbonate-Coating Buffer (CCB) for each plate used. Wash the protein A from each tube by adding some of the CCB with a Pasteur pipette, rinsing this in and out, then back to the 20 mL CCB. Repeat this procedure twice to be sure all the Protein A is in the CCB.
3. Using a multi-channel pipettor, add 200 µL of the Protein A-CCB solution to each well.
4. Cover the plate(s) and incubate at 30ºC for 2 hours.

II. Antisera 1

1. Mix 20 µL antisera (in freezer) with 20 mL of Phosphate Buffered Saline (PBS)-Tween per plate.
2. Remove plate(s) from incubator. Wash 3X with PBS-Tween, using a squeeze bottle (or plate washer). Be sure to rinse all wells.
3. Add 200 µL of antisera solution to each well.
4. Cover plate(s) and return to 30ºC for 2 hours.

III. Sample

1. Collect E+ and E- sample tillers from Greenhouse for use as controls.
2. Prepare unknown samples for grinding and set up leaf squeezer and sink area for grinding.
3. Prepare ELISA setup 'map' to record the layout of each plate.
   (a.) Run E- control tillers through the leaf squeezer. For each tiller squeezed, wash with 1 mL PBS-Tween and collect the liquid. 1.2 mL of sample is needed for each plate; grind sufficient number of tillers to produce the necessary amount.
   (b.) Repeat step 3a. using E+ control tillers. 0.8 ml of sample is needed for each plate.
4. Same as II.2. If using more than one plate, wash one, and load it with samples before washing subsequent plates.
5. Add 200 µL PBS-Tween as blank to wells A1, B1, E5, and F5.
6. Add 200 µL E- control to wells A2, B2, E6, F6, G12, and H12.
7. Add 200 µL E+ control to wells A3, B3, E7, F7, G11, and H11.
8. Run sample tillers through the leaf squeezer, rinsing with 1 mL PBS-Tween. Collect as much of this rinse as possible. Add 200 µL to the appropriate well(s) matching setup 'map'.

9. Cover plate and leave at 4°C overnight.

Day 2:

IV. Antisera 2

1. Repeat step II, being sure to wash out any remaining grass fragments from the wells.

V. Enzyme-conjugate

1. Mix 20 µL of Protein A-alkaline phosphatase (in –80°C freezer) with 20 mL of PBS-Tween as in step I2.
2. Remove plate(s) from incubator. Wash 3X with PBS-Tween.
3. Add 200 µL of conjugate solution to each well.
4. Cover the plate(s) and return to 30°C incubator for 2 hours.

VI. Substrate

1. Measure 20 mL Diethanolamine solution per plate. Cover and leave at room temperature.
2. 20 mg of substrate is required per 20 mL of Diethanolamine. This may be four 5 mg tablets (in freezer) or one 40 mg tablet for 2 plates. Add these to the Diethanolamine and mix until dissolved.
3. Remove plate(s) from incubator. Wash 3X with PBS-Tween. Rinse 2X with distilled H_20 to remove salt and Tween.
4. Add 200 µL of substrate solution per well.
5. When yellow color begins to develop, read plate(s) at 405 nm on spectrophotometer. Generally, data may be saved when the E+ positive controls average above a 1.0 absorbance on the absorbance report.

ELISA Protocol

Reagents needed:

Protein A (e.g. Sigma P3963)

For a 1mg bottle:
Add 1.0 mL PBS (no Tween) = 1 mg mL^{-1}
Divide into 20 µL aliquots
Store at -20°C

Carbonate Coating Buffer

Making 1 liter:

Sodium carbonate	Na_2CO_3	1.59 g
Sodium bicarbonate	$NaHCO_3$	2.93 g
pH 9.6		

Phosphate Buffered Saline (PBS)

Making 1 liter:

Sodium chloride	NaCl	8 g
Potassium phosphate	KH_2PO_4	200 mg
Potassium chloride	KCl	200 mg
Sodium phosphate	Na_2HPO_4	1.15 g

PBS-Tween (0.05% Tween)

Making 1 liter of PBS:
500 µL Tween 20

Protein A-Alkaline Phosphatase (e.g. Sigma P9650)

For a 0.5 mg bottle:
Add 1.25 mL of distilled H_2O = 0.4 mg mL^{-1}
Divide into 20 µL aliquots
Store at -80°C

Diethanolamine solution

Making 1 liter:

Diethanolamine (98%)	97 mL Sigma D2286
Distilled H_2O	800 mL
pH (with HCl) to 9.8	

Sigma 104 phosphatase substrate (Sigma 104-105)

15 mg tablet for each 5 mL of Diethanolamine solution

Appendix E

Prolactin Protocol and Data

Prolactin Radioimmunoassay Protocol

Prolactin First Antibody (Ab) Recipe:

1. x/300 = total volume/150,000 = volume of stock first Ab.
2. Total volume = [# of tubes in assay + 30 (error)] * 200μL (amount/tube).
3. Total volume – volume of first Ab = amont of Ab buffer required.

Prolactin Second Antibody Recipe:

1. [# of tubes in assay + 30 (error)] * 100μL = total volume.
2. Total volume/12 = volume of stock second Ab.
3. Total volume – volume of second Ab = amount of RIA buffer required.

Prolactin Tracer Recipe:

1. [# of tubes in assay + 30 (error)] * 10,000 cpm/tube = total cpm required.
2. Total cpm/stock cpm/μL (take 10μL of stock and count to get actual stock cpm/μL) = volume of stock tracer required.
3. [# of tubes in assay + 30 (error)] * 100μL (amount/tube) = total volume.
4. Total volume – volume of stock tracer = amount of RIA buffer required.
5. After preparing tracer solution, check 100μL in gamma counter to see if it is reading 10,000.

Procedure:

Steps 1-10 on next page. Follow sequence.

11. Incubate tubes at room temperature for 1 hour.
12. Centrifuge tubes at 3000 rpm for 15 minutes.
13. Decant supernatant and discard into 'Radioactive' labeled bottles.
14. Let tubes drain for 10 minutes in box labeled 'Radioactive', lined with foil, paper towels and Kimwipes.
15. Place tubes in gamma tray and count the 'pill' precipitant in tubes.

Sequence*		1	2	3	4	5	6	7	8	9	10
Tubes	Tube #	RIA Buffer	Sample	1^{st} Ab	Tracer	Vortex	Incubate	ARS	2^{nd} Ab	PEG	Vortex
TC†	1,2,3				100µL	1 min.	48 hr				1 min.
NSB†	4,5,6	600µL					at room	100µL	100µL	500µL	
Bo†	7,8,9	400µL					°T				
Stds	10-33	200µL	200µL	200µL							
QC†	34-35	300µL	100µL								
QC	36-37	350µL	50µL								
Sample	38-	**		↓	↓			↓	↓	↓	

***Add appropriate item as sequence indicates (1-10)**

****Sample volume (sample & buffer) must equal 400µL.**

† TC = Tracer Control, B_0 = Total Binding, QC = Quality Control, NSB = Non-specific Binding

Total count is the total amount (counts per minute) of tracer added to each tube.

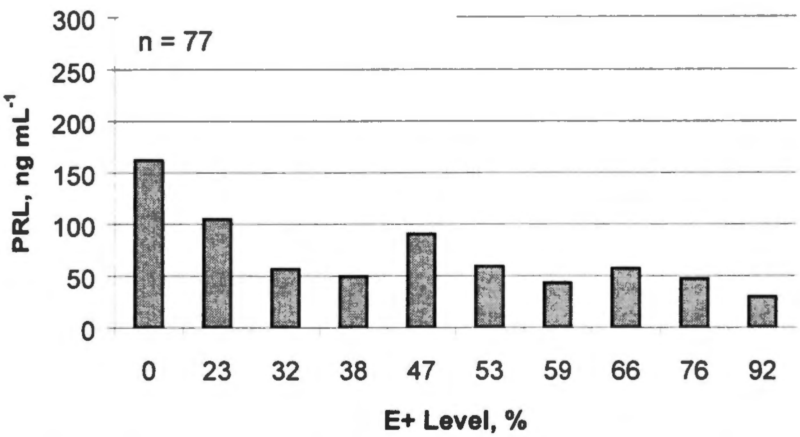
Non-specific binding is the amount of interference of impurities in the assay tubes, buffer, and tracer.

Total binding (%) is the capacity of the working dilution of tracer and antibody to be used as the basis for determining hormone concentrations.

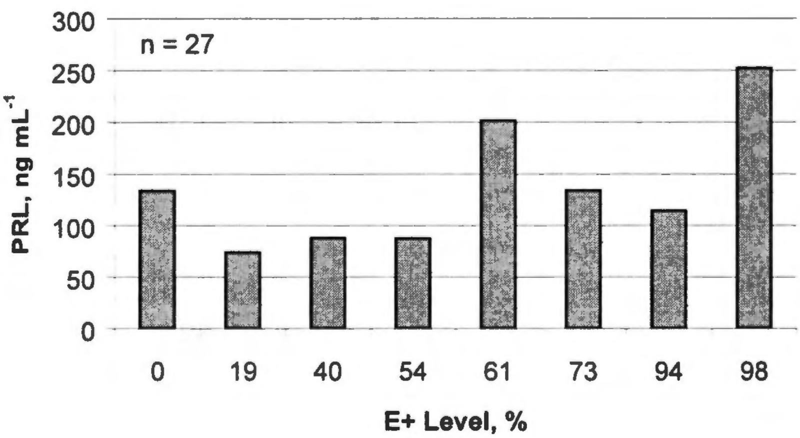
Standards are the known amount of hormone used to construct the standard reference curve.

Quality control is a standard unknown plasma sample used in assay to control intra- and inter-assay variation.

Samples are the unknown amount of the hormone that is to be measured.



E-1. Prolactin averages of blood serum of steers grazing tall fescue, July 17, 1996.



E-2. Prolactin averages of blood serum of steers grazing tall fescue, July 30, 1997.

E-3. Prolactin values for steers grazing tall fescue, July 17, 1996

PRL, ng mL^{-1}	Endophyte Level,%	Stocking Density
83.3	0	L
147.0	0	L
487.1	0	L
69.9	0	L
70.2	0	L
94.1	0	L
36.9	32	L
56.3	32	L
76.0	32	L
39.1	54	L
36.9	54	L
115.6	54	L
125.2	90	L
7.3	90	L
27.9	90	L
8.1	92	L
10.4	92	L
22.1	92	L
120.0	22	M
86.8	22	M
57.2	22	M
55.4	22	M
57.3	23	M
130.7	23	M
112.4	23	M
217.0	23	M
142.6	47	M
64.4	47	M
26.1	47	M
127.7	47	M
37.3	53	M
46.0	53	M
62.1	53	M
74.8	53	M
45.0	66	M
12.1	66	M
53.5	66	M

PRL, ng mL^{-1}	Endophyte Level,%	Stocking Density
52.9	66	M
17.3	90	M
9.2	90	M
29.6	90	M
48.0	90	M
190.9	0	H
62.6	0	H
129.3	0	H
122.8	0	H
140.8	0	H
344.1	0	H
35.1	38	H
30.8	38	H
44.3	38	H
40.9	38	H
114.1	38	H
30.0	38	H
23.6	59	H
61.9	59	H
42.8	59	H
15.5	59	H
56.8	59	H
58.0	59	H
97.0	66	H
32.3	66	H
82.4	66	H
31.2	66	H
49.9	66	H
112.4	66	H
66.7	76	H
23.8	76	H
56.3	76	H
42.3	76	H
42.2	76	H
50.5	76	H
36.8	90	H
7.3	90	H
22.6	90	H
42.9	90	H
29.5	90	H

E-4. Prolactin values for steers grazing tall fescue, July 30, 1997.

PRL, ng mL^{-1}	Endophyte Level, %	Stocking Density
144.1	0	L
34.8	0	L
77.3	0	L
278.0	0	L
31.5	40	L
144.2	40	L
65.0	56	L
22.7	56	L
48.1	94	L
72.8	94	L
105.0	98	L
399.3	98	L
38.0	19	M
101.7	19	M
81.6	19	M
114.2	54	M
19.9	54	M
216.4	54	M
232.4	61	M
45.7	61	M
326.3	61	M
113.1	73	M
151.4	73	M
137.7	73	M
161.5	95	M
104.1	95	M
184.8	95	M

Vita

Mark Alan Marsalis was born in Canton, MS on February 7, 1975. He graduated from Madison Central High School, Madison, MS in 1993. He then attended Holmes Community College for two years prior to transferring to Mississippi State University, where he received his Bachelor of Science degree in Biological Sciences in August 1997. In September 1997, he went to work for Wickersham Forestry Services, Inc. as a forester assistant. He returned to academics in 1998 upon arrival at the University of Tennessee, where he began work on a research assistantship under Dr. Henry A. Fribourg. Mark was married to Karen M. Trammell on November 7, 1998. He completed his graduate studies and received his Master of Science degree in Plant and Soil Sciences (Forage Crops Ecology) in the fall of 2000.