

INFLUENCE OF FORAGE CHARACTERISTICS ON GRAZING BEHAVIOR OF WEANED
STEERS

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ABSTRACT

For Virginia, the primary forage base is endophyte-infected tall fescue (*Schedonorus phoenix* (Scop.) Holub). However, the decreased animal performance and disorders caused by the presence of the fungal endophyte *Neotyphodium coenophialum* reduces its suitability for many forage-livestock producers. The endophyte, which is found in the inter-cellular space of tall fescue tissues, forms a mutualistic relationship with the plant and helps the fescue tolerate drought, insect predation, and grazing pressure. The production of toxic ergot alkaloids by the endophyte is associated with decreased performance of animals that graze endophyte infected tall fescue. The objective of the current experiment was to determine how tall fescue type (endophyte free vs novel endophyte) and orchardgrass-legume (alfalfa vs clover) mixture affect grazing behavior of weaned steers. Forty-eight weaned steers (218 ± 18 kg) were blocked by frame score (medium and large), and randomly assigned within block to four treatments with three replications in a 2x2 factorial design. Each treatment consisted of 2 paddocks with the combination of tall fescue and legume-orchardgrass mixture as follow: alfalfa-orchardgrass mixture (A)/E-, clover-orchardgrass mixture (C)/E-, A/E++ and C/E++. Each group of 4 steers had 24 h access to both tall fescue stand and legume-orchardgrass mixture. Botanical composition of paddocks was determined on d 0, and forage mass, sward height and nutritive value were determined on d 0, 17 and 33. Behavior of steers was scanned every 5 min on d 24 and 25 from 0700 to 1900. The proportion of legumes was higher ($P = 0.001$) in A paddocks(37 %) compared to C paddocks (6%), while the percent orchardgrass was lower ($P = 0.040$) in A

(33 %) than C (53 %). The proportion of broadleaf and grassy weeds was higher ($P = 0.013$) in E- paddocks (11 %) as compared with E++ paddocks (3 %). Sward height and herbage mass were similar between treatments during the experiment ($P > 0.05$). Nutritive value (CP, NDF and ADF) did not differ between fescue paddocks ($P > 0.05$). However, within mixed swards, CP was higher and NDF was lower in A compared with C ($P < 0.05$). Acid detergent fiber was ($P < 0.05$) lower in A than C only on d 0 and 17. Average daily gain did not differ between treatments ($P > 0.05$). Steers spent higher ($P = 0.002$) proportion of the daylight time grazing in treatments with C (56 %) as compared with A (50 %), and in treatments ($P = 0.023$) with E++ (55 %) compared with E- (52 %). Time spent ruminating, idling and lying, as well as steps taken per day, did not differ between treatments ($P > 0.05$). Regardless of differences in weeds between E- and E++, these results indicate that fescue type affected grazing behavior of calves. Difference between mixed swards in proportion of ground cover with legumes and orchardgrass, as differences in nutritive value between A and C may have affected grazing behavior of calves between mixed swards, instead of a legume species effect within mixtures.

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CHAPTER I INTRODUCTION

Grasslands cover over 40 % of the earth's surface. Approximately, one third of global stock of carbon is sequestered in grassland (White et al., 2000). The United States is one of the five countries with the largest grassland area (White et al., 2000).

In the last decades, human actions have profoundly changed the extent, condition, and capacity of major ecosystems. Agriculture has expanded at the expense of grasslands and forests, especially in temperate regions. Non-native species have negatively affected ecosystems, increasing competition with native species, which can eventually lead to decreases in biodiversity (White et al., 2000). Grazing systems may increase biodiversity as compared with extensive row-cropping. Increasing diversity in grazing systems would lead to higher productivity, extended grazing period and sustainability of the system.

Appalachian landscape suits grassland-based beef production (Scaglia et al., 2008). Typical pastures in this area have a high proportion of well adapted tall fescue (*Schedonorus phoenix* (Scop.) Holub) ecotypes, among other perennial and annual cool season grasses.

Tall fescue, the primary forage base for Virginia, forms a mutualistic association with the endophyte *Neotyphodium coenophialum* that reduces the suitability of this forage for many forage-livestock producers. The endophyte produces ergot alkaloid and this toxin is responsible for decreased performance of animals grazing endophyte infected tall fescue (E+). In addition, the endophyte helps the plant cope with extremes in environmental conditions such as drought, insect predation and grazing pressure (Thompson et al., 2001).

Since alkaloids are produced by the endophytic fungus, endophyte-free tall fescue (E-) does not contain the toxic alkaloids that are produced in endophyte-infected fescue. Cattle that grazed tall fescue have shown a negative relationship between ADG and infestation level (Thompson et al., 1993). Although the ADG of animal that grazed E- was not affected compared to those grazing E+, E- is less drought, insect and grazing pressure resistant (Elbersen and West, 1996) and less persistent (Hopkins and Alison, 2006) as compared with E+ tall fescue.

Recently, tall fescue cultivars infected with a non-alkaloid producing endophyte have been developed. Theoretically, these cultivars should be similar to E+ in agronomic factors (drought and insect tolerance) with no adverse effect on animal performance. Primary evaluations of novel endophyte tall fescue (E++) in animal behavior and ADG have shown that animals grazing E++ perform similar to those grazing E- and better than those grazing E+ (Boland, 2005; Parish et al., 2003; Stewart, 2006).

Less than the desirable amount of legumes are found in Virginia pastures. Most of the common legumes found in Virginia pastures are cool-season perennials. Among them are alfalfa (*Medicago sativa* L.), white (*Trifolium repens* L.) and red (*Trifolium pratense* L.) clover (Ball et al., 2007). Legumes form a symbiotic association with *Rhizobium* spp and fix N. Grasses grown in mixture with legumes can benefit from the nitrogen fixed by the legumes. Mixed diets of legumes and grasses, have higher nutritive value as compared with grass alone. Also, E+ tall fescue grown in mixture with legumes dilute the harmful effects of E+ tall fescue to the grazing animals, thus making the mixture a more suitable option than grass monoculture.

Cattle and sheep prefer a mixed diet with approximately 70 legumes and 30% grass (Boland, 2009; Parsons et al., 1994; Rutter et al., 2004). Most of these studies have been done using monocultures, specifically comparing preference between white clover and perennial

ryegrass (*Lolium perenne* L.). This information has been extrapolated to legumes and grasses in general (Rutter, 2006). Little work has been done to understand cattle preference among grasses and among legumes.

The hypothesis of the current study was that grazing behavior of steers would not be affected by fescue type (E- and E++) and legume species (alfalfa and white clover) within grass mixtures, and steers should perform similar among legume species within mixed swards or among tall fescue types. The objective of the current experiment was to determine if E- vs E++ tall fescue and alfalfa vs clover in mix with orchardgrass affect grazing behavior of weaned steers.

Grazing behavior and performance of newly weaned steers was evaluated in adjacent paddocks of endophyte free or novel endophyte tall fescue monocultures with mixed swards of orchardgrass with either alfalfa (A) or white clover (C). During 33 days, groups of 4 calves were assigned to 4 treatments, with two paddocks each, combining one tall fescue monoculture with one mixed sward (A/E-, A/E++, C/E- and C/E++). Calves had 24 h access to the 2 paddocks. Grazing behavior was scan sampled in a two days trial during the 12 h of daylight period.

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CHAPTER II

LITERATURE REVIEW

Tall Fescue

General characteristics. Tall fescue (*Schedonorus phoenix* (Scop.) Holub) is an introduced, perennial cool season bunch grass, which is well adapted to the southeastern United States. Among the reasons for its widespread use are: high forage quality, resistance to grazing pressure, high response to fertilization, and tolerance to wide range of soil and environmental conditions. Tall fescue can provide more grazing days/year than most introduced cool-season grasses. Tall fescue is one of the most sought out grass for stockpiling for winter grazing. Although several other forages can be used for stockpiling, tall fescue is the most productive because of its significant growth prior to the winter months and constantly high quality (Ball et al., 2007).

Endophyte infected tall fescue. For Virginia, the primary forage base is endophyte-infected Kentucky 31 tall fescue (E+). Tall fescue sets the standard against which agronomic performance of other grasses is measured. However, the decreased animal performance and disorders caused by the presence of the fungal endophyte *Neotyphodium coenophialum* reduces its suitability for many forage-livestock producers.

Generally, the nutritive value (CP, NDF and ADF) of E+ tall fescue indicates that it should support the nutritional requirements of several classes of livestock. However, decreased ADG, reduction in milk production, and poor conception rates have been observed in animals grazing tall fescue (Paterson et al., 1995). The decline in performance of animals grazing E+ tall fescue is associated with fescue toxicosis, caused by ergot alkaloids produced by the endophyte

Neotyphodium coenophialum found in the intercellular space of the fescue plant (Thompson et al., 2001). The fungus has a mutualistic association with the plant, which attributes to the drought and insect tolerance and grazing resistance of infected tall fescue (E+). When infected tall fescue is grazed by cattle, animals experience elevated body temperature, high respiratory and heart rates, and as a result grazing time and intake are reduced (Thompson et al., 2001).

Endophyte free tall fescue. As alkaloids are produced only in infected plants, endophyte free tall fescue (E-) does not produce deleterious effects on animals as E+ does (Paterson et al., 1995). Thompson et al. (1993) analyzed the results of 12 studies along Southern states comparing performance of beef steers grazing E+ vs E- fescue. Data was collected in spring and summer. Tall fescue treatments included low endophyte (< 5% infected) and high endophyte (100% infected). Steers grazing E+ fescue had lower ADG compared with those grazing E- in both seasons. A strong negative relationship between endophyte infestation level and ADG was found (Thompson et al., 1993). The effects of endophyte on cow/calf performance and cow milk production were studied in 2 120-d experimental periods, during summer and fall (Peters et al., 1992). In summer, cows grazing E+ had lower DMI, but this effect was not observed in the fall. Average daily gain was greater for both cows and calves grazing E- in summer and fall (Peters et al., 1992). Hancock et al. (1988) studied the effect of prior diets containing E+ tall fescue or bluegrass (*Poa pratensis* L.) or orchardgrass (*Dactylis glomerata* L.)-legume mixtures on the performance of steers during 56 days in feedlot. Steers that grazed E+ fescue prior to feedlot had lower BW, body protein and fat. Average daily gain was similar among treatments during the feedlot trial. Steers that previously grazed E+ tall fescue were not able to compensate for the lower ADG observed during the grazing period (Hancock et al., 1988).

Although E- fescue does not affect animal health and performance, E- is not as persistent and high yielding as E+ tall fescue. West et al. (1993) compared drought tolerance of E+ and E- tall fescue. Plots were irrigated by gradient in 2 growing seasons, from July to October. Tiller density of E+ tall fescue was less affected by drought compared with E-. In addition, at the end of the growing season, E- had only 62% of the original tiller density compared with 100% for E+ (West et al., 1993). Drought tolerance of E+ fescue may have been associated with the physiological response of each fescue to reduced water stress (Elbersen and West, 1996). Elbersen and West (1996), evaluated stress response of E+ and E- tall fescue during 3 17-d periods of no irrigation, during June and July. Stomatal conductance was lower and leaf rolling was more severe in E- as compared to E+, resulting in desiccation of growing points in water stress situations. Similarly, leaf elongation, tiller density, and dry weight per tiller were lower in E- as compared with E+ (Elbersen and West, 1996). In addition to lower drought tolerance, the persistence of E- fescue is also affected by the absence of the endophyte in the plant. Hopkins and Alison (2006) observed that the E- fescue stand declined after 3 yr of grazing during spring and fall, in Louisiana, but not in Oklahoma.

Novel endophyte tall fescue. Novel endophyte tall fescue (E++) has an endophyte that helps give it the positive agronomic characteristics of E+. Nevertheless, the novel endophyte does not cause the production of toxins found in other E+ fescue varieties (Nihsen et al., 2004). Moreover, initial investigations suggest that animal performance is not compromised by the presence of the novel endophyte. Hopkins and Alison (2006) compared stand persistence and animal performance of E+, E- and E++ tall fescue. After 4 years, GA-5 MaxQ E++ stand was similar to GA-5 E+ stand in Louisiana (58% E++ vs 64% E+) and in Oklahoma (99% E++ vs 92% E+). Steers' ADG was greater for cattle grazing E++ compared with those on E+ and was

similar to those on E-. Bouton et al. (2002) inoculated two highly productive tall fescue varieties (Jesup and GA) with non-ergot alkaloid-producing endophyte strains to assess pasture persistence and animal performance. For each tall fescue variety, treatments included E+, E- and E++. Animal toxicosis was evaluated in 3 spring and 2 fall trials. Only E+ produced ergot alkaloids. No animal response (serum prolactin depression and body temperature increment) to the toxin was observed in cattle grazing E++ and E- (Bouton et al., 2002). Persistence of E++, after two years of grazing, was intermediate between E- and E+. Even though the agronomic characteristics of E++ were close to desired levels of E+, cattle grazing E++ improved performance as compared with those on E+ (Bouton et al., 2002). Vibart et al. (2008) evaluated persistence of Jesup tall fescue with three levels of infection (E+, E- and E++). After five years of intensive winter grazing, the persistence of E+ was the greatest (73%), followed by E++ (66%) and the lowest for E- (59%).

Parish et al. (2003) evaluated grazing behavior, performance and physiological response to level of infections in Jesup, GA-5 and Kentucky 31 tall fescue grazed by steers and heifers in a 3-yr trial. Cattle grazed tall fescue pastures during fall and spring. During 4 5-d trials, animals wore grazing recorders to determine grazing behavior. In addition, steers were dosed with chromic oxide to determine intake. Cattle that grazed E++ and E- did not decrease serum prolactin levels, as did those grazing E+. Rectal temperature was higher in cattle on E+ fescue as compared with E- and E++. Average daily gains were greater in cattle grazing E++ (755 g in spring and 815 g in fall) as compared to those grazing E+ (400 g in spring and 485 g in fall), but similar to those grazing E- (840 g in spring and 855 g in fall). Similar trend was observed in daily DMI. Daily DMI of steers on E++ (14 g DM kg BW⁻¹ in spring and 11 g DM kg BW⁻¹ in fall) was greater than those grazing E+ (10 g DM kg BW⁻¹ in spring and 9 g DM kg BW⁻¹ in

fall). Steers spent more time grazing E- as compared with E+ and idling time was greater in E+ than E- and E++ in the spring (Parish et al., 2003). In the fall, steers grazing E++ fescue spent more time grazing than those on E+ (Parish et al., 2003). In a 3 yr study in Georgia, Franzluebbbers and Seman (2009) studied the effect of infection status of tall fescue on ADG of steers. Steers that grazed E++ performed similar to those that grazed E-, and better than those that grazed E+ in fall (1570 g ha⁻¹ E++, 1790 g ha⁻¹ E- and 1120 g ha⁻¹ E+), winter (1340 g ha⁻¹ E++, 1570 g ha⁻¹ E- and 1120 g ha⁻¹ E+) and spring (2350 g ha⁻¹ E++, 2350 g ha⁻¹ E- and 1570 g ha⁻¹ E+). In summer performance of steers was similar (1460 g ha⁻¹ E++, 1230 g ha⁻¹ E- and 1230 g ha⁻¹ E+) between tall fescue types (Franzluebbbers and Seman, 2009). In Virginia, Stewart (2006) compared performance of beef steers that grazed Kentucky 31 E+ and E-, and Q4508 E++ tall fescue, between May and September in two consecutive years. Nutritive value (CP, NDF and ADF) was similar between tall fescue types, but ADG was lower in steers grazing E+ (300 g d⁻¹) as compared with those that grazed E- (540 g d⁻¹) and E++ (450 g d⁻¹). In a parallel study, DMI of steers that grazed these fescue types was estimated each year in May, July and September using *n*-alkanes. With the exception of July of the second year, DMI of steers on E- was the greatest in both years (Stewart, 2006). Matthews et al. (2005) studied the effect of different fescue types on hay DM digestibility and DMI of steers. Endophyte infected fescue had lower DM digestibility and steers consumed less DM as compared with those eating E- and E++ (Matthews et al., 2005).

Watson (2004) studied the response of cow/calf pair to tall fescue type in a 3-yr trial. Treatments included GA-5 E+ fescue and GA-5 AR542 E++. Calves on E++ fescue had greater ADG (1150 g steers and 1030 g heifers) and weaning weight (256 kg steers and 237 kg heifers) as compared with those on E+ (ADG: 970 g steers and 900 g heifers; weaning weight: 227 kg

steers and 217 kg heifers). Also, birth weight of calves was greater for cows grazing E++ (38.6 kg) as compared with E+ (32.7 kg). Fescue type did not affect pregnancy rate (Watson et al., 2004).

Gunter and Beck (2004) reviewed studies that evaluated endophyte effect on plant persistence and animal performance. They concluded that E++ has similar persistence compared with E+, but resulted in 47% greater ADG of cattle grazing E++ compared to those grazing E+. In addition, the cost of replacing E+ with E++ shows that if the discount of E+ calves at sale is included, it would take 3 yr to recover the investment (Gunter and Beck, 2004).

Legumes

Most of the common legumes cultivated in United States are cool-season perennials. Among them are alfalfa (*Medicago sativa* L.), a cool season legume from Iran and Central Asia, that grows particularly well in summer, white and red clover, Mediterranean legumes that grow better at lower temperatures than alfalfa (Ball et al., 2007). Legumes form a symbiotic association with *Rhizobium* ssp fixing N. Grasses also benefit from nitrogen fixed by legumes through mineralization.

In southeastern United States, where the main forage grass is infected tall fescue, legumes are usually incorporated to the pasture for two main reasons: to increase the nutritive value of the diet of grazing animals and/or to dilute toxin effects of infected tall fescue. Legumes can be interseeded in grass pastures or cultivated in adjacent pastures as pure stands.

Hoveland (2002) studied the benefit of including alfalfa in mixture with Jesup E+ and E- tall fescue on ADG of beef steers, during spring and fall. Average daily gain of steers that grazed E- was the greatest in spring (1130 g d⁻¹). However, inclusion of alfalfa in mixture with E+ (560

g d⁻¹) and alfalfa pure stand (970 g d⁻¹) resulted in greater ADG of steers as compared with those that grazed E+ pure stand (430 g d⁻¹). Similar trend was observed when grazing pressure was increased (Hoveland et al., 2002). White clover cultivars (Durana, Patriot and Regal) in mixture with E+ and E- tall fescue were evaluated to assess the response on animal performance during spring and fall (Bouton et al., 2005). Results from spring show that mixtures with clover improved ADG of steers as compared with tall fescue monocultures fertilized with N in E+ (1400 g d⁻¹ on mixture vs. 840 g d⁻¹ on monoculture) as in E- (1480 g d⁻¹ on mixture vs. 1260 g d⁻¹ on monoculture). Differences in ADG of steers grazing E+ (1400 g d⁻¹ in spring and 550 g d⁻¹ in fall) and E- (1480g in spring and 770g in fall) are not such when clover is included as mixed swards. In the fall, data were inconsistent due to low proportion of clover in the pastures. However, in pastures where white clover accounted for 40%, animal performance did improve as compared with tall fescue monocultures (Bouton et al., 2005). Intake estimations, conducted measuring forage disappearance, in steers grazing monocultures of alfalfa and tall fescue, and 1/3 and 2/3 alfalfa-tall fescue adjacent monocultures showed a linear negative relationship between intake and proportion of grass in the pasture (Seman et al., 1999).

Several cultivars of alfalfa and other cool season legumes were investigated to determine persistence among cultivars under severe grazing conditions (Brummer and Moore, 2000). Persistence varied among cultivars, and thus would be important to select the proper cultivar for a given grazing management scheme. However, there are legume species that are well adapted to intensive grazing pressure, such as white clover. Although alfalfa is not known to withstand intensive grazing pressure, some cultivars of alfalfa, like Alfagraze and XGrazer, have performed well under such conditions (Brummer and Moore, 2000).

Hoveland et al. (1999) studied the effect of endophyte infection level of tall fescue on biomass yield and stand density of cool season legumes. Red and white clovers, and alfalfa were evaluated. Infected tall fescue is highly competitive, so, E+ tall fescue in mixtures with legumes may compromise legumes' persistence (Hoveland et al., 1999). Infection level of tall fescue (E+ or E-) did not affect biomass yield and stand density of red and white clover during 3 yr. Infection level did not affect biomass yield of alfalfa when harvested every 3 weeks. However, harvesting every 5 weeks and at high seeding rate of the legume (22 kg ha⁻¹), alfalfa was able to compete better with E-, increasing biomass yield, as compared with E+ (Hoveland et al., 1999). Selection of legume cultivars that are resistant to grazing pressure and competitive with tall fescue is important to ensure persistence of the grass-legume mix. Incorporation of legumes to tall fescue pastures improves animal performance and reduces deleterious effects of tall fescue.

Grazing behavior

The ingestive behavior of animals was described from a mechanistic approach by Hodgson (1982). He expressed intake as the product of rate of biting, intake per bite and daily grazing time. The rate of biting has a narrow range of variation and lower compensation capacity when the other two variables are modified. In daily allocation of paddocks, lactating dairy cows decreased intake rate as herbage mass and height of perennial ryegrass (*Lolium perenne*, L.) decreased, with no variation in bite rate (Barrett et al., 2001). Increasing the period of starvation from 2.5 h to 16.5 h modified grazing behavior of lactating dairy cows, increasing DMI per bite and grazing time, but had no effect on intake rate (Chilibroste et al., 1997). McGilloway (1999) working with dairy cows grazing perennial ryegrass found no relation between bite rate and sward height.

Intake per bite is highly variable due to sward characteristics, and these variations can be compensated partially by increments in daily grazing time (Hodgson, 1982). In particular situations, like when tropical grasses with high proportion of stem content are used, grazing time cannot compensate, thus ultimately bite size limits daily intake of cattle (Stobbs, 1973). Also, in short swards of perennial ryegrass reduction in daily DMI was observed due to lower intake per bite (Gibb et al., 1997). As sward height decreased, grazing jaw movements increased linearly, and grazing time tended to increase, but these changes in grazing behavior were not able to compensate a reduction in intake per bite (Gibb et al., 1997). In this experiment (Gibb et al., 1997), the highest sward height (9 cm) resulted in lower daily DMI as compared with medium sward height (7 cm), possibly due to the linear increase in rumination time as sward height increased. Hence, the variables in the sward that affect intake per bite, will finally determine differences in daily DMI.

Sward structure. Sward structure can be characterized as the combination of both surface height and bulk density, and thus these variables can explain herbage mass. Griffiths et al. (2003) studied the influence of sward characteristics on bite size, different sward heights of perennial ryegrass at vegetative stage were compared. A positive relationship was found between bite depth and sward height. When the sward was divided into leaf and pseudo-stem, pseudo-stem showed a strong negative relationship with bite depth (Griffiths et al., 2003).

Barrett et al. (2003) studied bite dimensions as related with sward structure of vegetative and reproductive swards of perennial and annual ryegrass (*Lolium multiflorum*, L.). Bite depth was the only parameter of the bite significantly affected by sward characteristics, and it was highly correlated with sward height. In addition, bite depth was inversely correlated with bulk density (Barrett et al., 2003). Hodgson (1985) determined the leaf horizon as the limit of the bite

depth. Benvenuti (2006) studied the influence of tensile resistance of stems on bite dimensions in artificial microswards using *Panicum maximum* Jacq. The increment of tensile resistance and density of stems in the microsward reduced mass and depth of bites taken by steers (Benvenuti et al., 2006).

Daily DMI and intake rate of lactating dairy cows grazing perennial ryegrass were studied in daily allocated paddocks. Sward height, herbage mass over 4 cm, and proportion of leaves decreased, and proportion of pseudo-stem and bulk density increased as the day progressed (Barrett et al., 2001). Intake rate followed a quadratic trend as the day progress, with an increment first and then a decline. To determine if the time of allocation the paddocks had an effect on intake, new paddocks were assigned at different times of the day. As sward characteristics were similar between paddocks, time of allocation the paddocks did not affect bite size and intake rate of cows (Barrett et al., 2001).

Species composition. Mixed swards have greater degree of variation as compared with monocultures. This variation force grazing animals to make decisions based on animal's preference and constraints. Preference was defined by Parsons et al. (1994) as being animal selection when given the minimum physical constraint. Efforts have been made to understand animal preference. In order to minimize constraints, work has been done in monocultures, looking at preference between white clover and perennial ryegrass. Parsons et al. (1994) tested preference of ewes between white clover and perennial ryegrass in short (1 h) and long-term (6 d) periods. Ewes had grazed different pastures previously (clover, ryegrass or 50:50 clover ryegrass). The proportion of clover selected in the diet was compared to 100% clover in the diet and to the proportion of clover in the treatments to test partial preference, different from total preference or indifference. The design included three area based proportions of clover and

ryegrass (clover:ryegrass 50:50, 20:80 and 80:20). Grazing behavior was determined with jaw movement recorders, and location of animals within the two pastures was recorded with video cameras. Dry matter intake of animals was indirectly estimated by changes in live weight and bite rate. Despite that ewes' had greater intake rate of clover as compared to grass, diet preference did not change if expressed as daily DMI or daily grazing time (Parsons et al., 1994). The results showed period and previous experience effects. The 1st h of the experiment, ewes which were previously grazing grass selected more clover in their diet than ewes previously grazing clover. In the 3rd and 6th d, ewes previously grazing grass selected more grass as compared with ewes previously grazing clover. Ewes also showed a daily pattern of preference, with preference for clover declining during the day. The preference of ewes for clover was greater than 20 and 50 % in the 1st, 3rd and 6th d of the trial, and different from 80 % in the 3rd and 6th day of the trial. These results show that ewes prefer a mixed diet with around 70 % of clover (Parsons et al., 1994).

Rutter et al (2004) studied diet preference between white clover and perennial ryegrass of dairy heifers for two days. The two treatments had either 25 % clover and 75 % grass (C25) or 75 % clover and 25 % grass (C75). Preference was measured as proportion of daily DMI, and DMI was calculated as the product of grazing time and intake rate. Intake rate was estimated by changes in live weight between, before, and after 1 h grazing period in each pasture. In each pasture, two different rates of intake AM/PM were calculated. An observer during daylight determined whether heifers were in clover or grass. Intake rate was greater in clover as compared with grass, and in the evening vs. morning. Heifers in treatment C25 showed an active selection for clover (63 % of clover in their diet), which was significantly different from no preference for clover (0 %), total preference (100 %) with no grass in their diet, or indifference (25 %) with the

same proportion of clover in their diet as in the pasture (Rutter et al., 2004). Heifers in treatment C75 selected 85 % of clover in their diet, different from no or total preference, but similar to the proportion in the pastures. On average, heifers selected 74 % of clover in their diet (Rutter et al., 2004), similar to the result reported for sheep by Parsons et al. (1994).

Champion et al. (2004) compared grazing behavior and intake of lactating sheep grazing white clover, perennial ryegrass, adjacent monocultures of white clover and perennial ryegrass (50:50 in area), or a mixed sward of these two species (9% of clover) during an 8 d period. Grazing behavior was recorded using IGER behavior recorders. Also, standing, lying, and walking behavior were recorded. Location of ewes in treatments with adjacent monocultures was recorded every 4 min by an observer. Intake rate was estimated by bite rate and change in live weight before and after a 1 h period of grazing. Composition of the diet of ewes that grazed adjacent monocultures and mixed swards was determined by *n*-alkanes. Sheep in the mixed sward treatment selected a diet with 34% of clover, greater than the 9 % of clover present in the mixture. The treatment with adjacent monocultures had 43% herbage mass of clover and sheep selected 62% of clover in their diet. Intake rate tended to be lower in mixed sward as compared with monocultures. Average daily gain of lambs was significantly lower, and total weight loss of ewes was numerically higher in mixed swards (Champion et al., 2004). The effect of mixtures or adjacent monocultures was also studied in dairy cows by Marotti et al. (2001). Treatments included white clover, ryegrass, adjacent pastures of these two species with equal area or a mixed sward with 22% clover. Cows in adjacent monocultures selected 70% of clover in their diet and produced 11% more milk than cows in mixed sward treatment. Champion et al (2004) suggested that low proportion of clover in the mixed sward is a constraint for animals to express

the preference observed in the adjacent monocultures, and that search for clover increases cost of diet selection.

In monoculture pastures, which allowed animals to select with minimum constraint, the daily intake and performance of sheep (Champion et al., 2004) and cattle (Marotti et al., 2001) was improved as compared to animals grazing mixed swards. Rutter et al (2005) conducted an experiment composed of adjacent strips of clover and grass to evaluate the benefit of mixed swards and adjacent monocultures. Four treatments with different strips' width (108, 36 and 12 cm) and a mixed sward were assigned to groups of 2 Simmental-Holstein beef heifers. Daily DMI and preference (as proportion of intake) were estimated with *n*-alkanes. Daily DMI was similar among treatments. However, heifers grazing the 12 cm strip and mixed sward treatments selected a diet with lower proportion of clover (36-38 %) as compared with the proportion selected by heifers in 36 and 108 cm strip width treatments (59-60 %). Greater proportion of clover selected at higher strip width shows that at these widths, heifers treated the strips as separate monocultures, and they could select a proportion of clover in the diet, similar to the 70% reported before in sheep (Champion et al., 2004; Parsons et al., 1994) and cattle (Rutter et al., 2004).

Harvey et al. (2000) studied the effect of sward height of white clover and perennial ryegrass adjacent monocultures on partial preference for clover by sheep. Three combinations of white clover (c) and perennial ryegrass (g) sward heights (cm) were used (c3g6, c3g9 and c6g6) in a 2 d period. Grazing behavior and position were scan sampled every 5 min, and intake rate was estimated by live weight change before and after 1 h of grazing each pasture-sward height combination. Variations in sward height of grass and clover affected preference. Ewes in treatment c3g6 spent less time grazing clover and more time grazing grass as compared with

those on c6g6 treatment. Daily DMI was calculated with the estimation of intake rate and grazing time recorded. Grazing time and DMI of grass were greater, and of clover lower in ewes grazing on treatment c3g6 as compared with those grazing on c6g6 in the first day of the period. In the second day ewes on treatments c3g6 and c3g9 increased time grazing clover to similar levels than those grazing treatment c6g6, and total grazing time was greater than ewes grazing on treatment c6g6. Results show that constraints affect grazing behavior and DMI by sheep (Harvey et al., 2000).

Torrez-Rodriguez et al. (1997) studied the effect of sward height on preference of heifers grazing adjacent monocultures. This study compared preference between ryegrass and white clover, ryegrass and birdsfoot trefoil (*Lotus corniculatus* L.), and between two legumes, birdsfoot trefoil and red clover. White clover and birdsfoot trefoil were arbitrary defined as preferred species. The sward height of white clover and birdsfoot trefoil varied from 4 to 6, 8 and 10 cm. The sward of the alternative species (ryegrass or red clover) was fixed at 10 cm. The proportion of the grazing time spent by the animals in each pasture was considered as preference (DMI was not measured). When the alternative species was ryegrass, as the sward height of the preferred species increased the total grazing time of heifers tended to decrease (Torrez-Rodriguez et al., 1997). Time spent grazing the preferred species was greater in birdsfoot trefoil and clover-ryegrass treatments, but it was similar between birdsfoot trefoil and red clover. Greater proportion of grazing time spent in white clover or birdsfoot trefoil as compared with ryegrass indicated a partial preference for legumes, and the effect of sward height as a constraint on grazing behavior of cattle. Rutter (2006) concluded that partial preference of sheep and cattle could be extrapolated to a wide range of species of grass and legumes.

Boland (2009) studied preference of beef steers for two species widely used in the US, tall fescue and alfalfa, with a design similar to Parsons et al. (1994) to differentiate partial preference from total preference and indifference. Each steer was attached with a GPS and IGER grazing recorder to determine behavior and position within the 2 pastures. Grazing time was used as indicator of preference. In the 3 treatments with adjacent monocultures, proportion of the grazing time spent in alfalfa was different from 0% or 100% (total preference). In treatments with 25% and 75% of area with alfalfa, proportion of the grazing time that steers spent in alfalfa paddocks was different to the proportion of alfalfa in the treatment, showing partial preference for alfalfa by steers. Steers spent 62% of the grazing time in alfalfa, similar to results reported by Rutter et al (2004).

Partial preference of heifers between legumes in adjacent pastures was studied by Poli et al. (2006). They compared white clover-birdsfoot trefoli mixture vs red clover in 0.2, 0.33, 0.67 and 0.8 proportion of red clover by area. Grazing behavior was only measured during 3 h periods at sunrise and sunset. Averaged over proportions, preference between legumes was similar regardless of legume types (48 % white clover-birdsfoot trefoli, 52 % red clover), and similar to the preference between birdsfoot trefoli and red clover reported by Torrez-Rodriguez et al. (1997).

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CHAPTER III

INFLUENCE OF FORAGE CHARACTERISTICS ON GRAZING BEHAVIOR OF WEANED CALVES

ABSTRACT

Endophyte infected (*Neotyphodium coenophialum*) tall fescue (*Schedonorus phoenix* (Scop.) Holub) is the primary forage base in Virginia. However, the production of toxic ergot alkaloids by the endophyte is associated with decreased gain of animals that graze endophyte infected tall fescue (E+). Endophyte free (E-), novel endophyte (E++) tall fescue and inclusion of legumes in mixed sward have been used to minimize the effects of E+ on cattle. The objective of the current experiment was to determine how tall fescue type (E- vs. E++) and orchardgrass (*Dactylis glomerata* L.)-legume mixture affect grazing behavior of weaned steers. Forty-eight weaned steers were blocked by frame score (medium and large), and randomly assigned within block to four treatments with three replications in a 2x2 factorial design. Each treatment had two paddocks with the combination of one tall fescue stand and one orchardgrass-legume mixture as follows: alfalfa (*Medicago sativa* L.)-orchardgrass mixture (A)/E-, white clover (*Trifolium repens* L.)-orchardgrass mixture (C)/E-, A/E++ and C/E++. Each group of 4 steers had 24 h access to both tall fescue stand and legume-orchardgrass mixture. Botanical composition of the paddocks was determined on d 0, and herbage mass, sward height and nutritive value were determined on d 0, 17 and 33. Behavior of steers was recorded every 5 min on d 24 and 25 from 0700 to 1900. The proportion of legumes was greater ($P = 0.001$) in A paddocks (37 %) compared to C paddocks (6%), while the proportion of orchardgrass was lower ($P = 0.040$) in A (33 %) than C (53 %). The proportion of broadleaf and grassy weeds was greater ($P = 0.013$) in E- paddocks (11 %) as compared with E++ paddocks (3 %). Sward height and herbage mass did

not differ between treatments ($P > 0.05$). Nutritive value (CP, NDF and ADF) did not differ ($P > 0.05$) between tall fescue types. However, within mixed swards CP was greater and NDF was lower in A compared with C ($P < 0.05$). Acid detergent fiber was lower ($P < 0.05$) in A than C only on d 0 and 17. Average daily gain did not differ between treatments ($P > 0.05$). Steers spent greater ($P = 0.002$) proportion of the daylight time grazing in C (56 %) as compared with A (50 %), and more time ($P = 0.023$) in E++ (55 %) as compared to E- (52 %). Time spent ruminating, idling and lying, as well as steps taken per day, did not differ between treatments ($P > 0.05$). Regardless of some differences in species composition between E- and E++ greater proportion of daylight grazing in E++ as compared with E- indicates that fescue type affected grazing behavior of steers. Differences in grazing behavior between mixed swards may be due to species abundance within mixtures and not due to legume species effect.

INTRODUCTION

The Appalachian area of Virginia is in a temperate region, thus, there are various warm and cool season grasses and legumes that can be used for grass-based beef production systems. A survey conducted by Ball et al. (1987) found that over 90% of the tall fescue (*Schedonorus phoenix* (Scop.) Holub) fields in the US are endophyte-infected (E+). The endophyte (*Neotyphodium coenophialum*) produces ergot alkaloids that are toxic to livestock (Ball et al., 2002; Thompson et al., 2001). A broad range of other alkaloids are also produced by the endophyte, but ergopeptine alkaloids are most closely associated with animal toxicosis (Hill et al., 1991). Cattle grazing E+ tall fescue gain less, produce less milk and are less likely to conceive (Paterson et al., 1995).

Endophyte-free tall fescue (E-) does not contain the toxic alkaloids that are produced in E+ fescue, and therefore, E- does not negatively affect animals consuming it. The ADG of cattle that grazed E- was greater compared to those that grazed E+ (Thompson et al., 1993). In Georgia, Franzluebbbers and Seman (2009) evaluated the performance (ADG) of heifers grazing E- and E+ tall fescue. On average, heifers on E- gained 0.7 kg d^{-1} vs. 0.5 kg d^{-1} for those on E+ tall fescue (Franzluebbbers and Seman, 2009). Cow/calf pair that grazed E- during summer and fall had greater ADG and cows produced 25 % more milk than cows grazing E+ (Peters et al., 1992). On the other hand, the lack of the endophyte in E-, makes the plant less drought and insect resistant and thus often results in decline in stand compared with E+. Stand persistence of E- was significantly lower than E+ after six years of grazing by stocker heifers (Franzluebbbers and Seman, 2009).

Novel endophyte tall fescue (E++) consists of infecting E- tall fescue with non-toxic strains of the fungus. A combination of characteristics between the two fescues (E+ and E-) has been found in novel endophyte fescue. Infection of Jesup tall fescue with AR542 novel endophyte resulted in greater forage yield as compared with E- in Athens, GA, but not in Blairsville, GA (Bouton et al., 2002). After 5 yr of evaluating the effect of endophyte level on persistence of Jesup tall fescue, Vibart et al. (2008) concluded that the stand persistence of E++ was intermediate between E+ and E-. Franzluebbbers and Seman (2009) evaluated the persistence of the tree tall fescue types (E++, E++ and E-) . After 6 yr, they concluded that the stand persistence of E++ tall fescue was greater than E- and similar to E+.

Average daily gain of lambs that grazed E++ was similar to those that grazed E- and greater than lambs on E+ (Bouton et al., 2002). Heifers that continually grazed E++ had similar ADG (0.77 kg d^{-1}) to E- (0.73 kg d^{-1}) and greater than E+ (0.54 kg d^{-1}) when averaged across 6

yr of evaluation (Franzluebbbers and Seman, 2009). Differences in ADG were observed in fall, winter and spring, but not in summer (Franzluebbbers and Seman, 2009). Stewart (2006) found an intermediate performance in steers that grazed E++ (0.45 kg d⁻¹) between May and September as compared with those that grazed E- (0.54 kg d⁻¹) and E+ (0.30 kg d⁻¹) tall fescue. Parish et al.(2003) reported difference in grazing behavior of steers grazing E+ tall fescue vs. E- and E++ across the grazing seasons. Steers on E+ spent lower proportion (36.1 %) of time grazing compared to those on E- (41.9 %), between April and June. Between October and November, however, differences were found between steers that grazed E++ (44.1 %) and E+ (40.5 %), but not for those that grazed E- (Parish et al., 2003). Between June and September, Boland (2005) reported that steers grazing E+ spent more time idling and standing as compared with those grazing E++ and E-. Signs of heat stress were observed in steers grazing E+ during the summer (Boland, 2005).

Incorporation of legumes into E+ the pastures dilutes toxic effects of E+ and increases nutritive value of the diet (Ball et al., 2007). Hoveland et al. (2002) concluded that the ADG of steers grazing alfalfa (*Medicago sativa* L.) in a mixture with E+ was greater compared to ADG of steers grazing E- tall fescue. Average daily gain of steers that grazed white clover (*Trifolium repens* L.)-tall fescue mixtures was greater than those grazing nitrogen-fertilized E+ monocultures when the percentage of clover in the mixture was between 40 % and 60 % (Bouton et al. 2005)

Sheep and cattle prefer a mixed diet, with around 70% of legumes (Parsons et al., 1994; Rutter et al., 2004). Rutter (2006) suggested that partial preference for legumes over grass found mainly between white clover and perennial ryegrass (*Lolium perenne* L.) could be extended to a wide range of species. Torrez-Rodriguez (1997) compared preference of heifers between

birdsfoot trefoil (*Lotus corniculatus* L.) and red clover (*Trifolium pratense* L.) with different sward height of the former species. Regardless of time spent grazing, the preference for birdsfoot trefoil increased as the sward height of this legume increased. However, overall mean preference for birdsfoot trefoil among treatments was 55%. Poli et al. (2006) found similar response to red clover and birdsfoot trefoil-white clover mixture. The overall mean proportion of time spent grazing by heifers was 52 and 48% on red clover and the mixed swards, respectively (Poli et al., 2006).

More information regarding cattle preference among legumes and grasses is needed. The objective of the current experiment was to determine if E- vs E++ tall fescue and alfalfa vs clover in mix with orchardgrass affect grazing behavior of weaned calves.

MATERIALS AND METHODS

Pasture management

The experiment was conducted at the Shenandoah Valley Agriculture Research and Extension Center (SVAREC), Virginia, USA (Latitude: 37° 56' N, Longitude: 79° 13' W; Elevation: 537 m). Climate data for the 33 d of experiment were obtained from the SCAN Site Information for Shenandoah in Virginia of the Natural Resources Conservation Service (<http://www.wcc.nrcs.usda.gov/scan/site.pl? sitenum=2088&state=va>). Soils at the site are primarily Frederick silt loams. Before the experiment began, 5 soil samples were taken to a 15 cm depth and composited together. Mean soil pH was 6.7, and P, K, Ca, and Mg levels averaged 43, 83, 1,267, and 183 ppm, respectively. Soil organic matter averaged 4.9%.

The experiment was conducted on 24, 0.5 ha pastures at SVAREC. In May 2006, pastures were sprayed with 2.3 L ha⁻¹ of Roundup[®] (glyphosate 41 %, Monsanto, St Louis, MO) and planted with pearl millet (*Pennisetum glaucum* L.). Millet was harvested in August 2006 and pastures were sprayed with 2.3 L ha⁻¹ of Gramoxone[®] (Paraquat dichloride 29%; Syngenta, Greensboro, NC). In fall 2006, the 24 pastures were assigned to 4 different forage types: 1) novel endophyte (E++) tall fescue, 2) endophyte free (E-) tall fescue, 3) alfalfa-orchardgrass mixture (A), or 4) white clover orchardgrass mixture (C). On September 12 and 13, 2006, fescue paddocks were seeded with either 28 kg ha⁻¹ of ‘Bronson’ endophyte free, or ‘MaxQ’ novel-endophyte tall fescue. The alfalfa-orchardgrass paddocks were seeded with ‘Ameristand 403T’ alfalfa (11.2 kg ha⁻¹) and ‘Benchmark’ orchardgrass (6.7 kg ha⁻¹). Clover-orchardgrass paddocks were seeded with ‘Benchmark’ orchardgrass at 11.2 kg ha⁻¹, ‘Pinnacle Ladino’ white clover at 2.24 kg ha⁻¹, and ‘Kopu II’ white clover at 4.48 kg ha⁻¹. Between March and April 2008 pastures were fertilized with 55 kg ha⁻¹ of N and 13 kg ha⁻¹ of K according to soil test recommendation. Between May 7 and June 9, pastures were cut for hay and allowed to re-grow until the beginning of the experiment.

Pastures were arranged so each treatment was the combination of one tall fescue stand with one legume-orchardgrass mixture (Figure 3-1). The two paddocks were divided by a fence, but gates remained open to allow steers 24 hour access both paddocks. Treatments were replicated three times.

Animal management

All procedures used in this experiment were approved by the Virginia Tech Animal Care and Use Committee. The Angus steers used in the experiment were part of a larger project entitled “Economic Pasture-based Beef Systems for Appalachia”. Steers were born between

February 22 and May 7, 2008. From late April until weaning, cow/calf pairs were rotationally stocked on tall fescue-bluegrass pastures within two different systems that compared creep grazing methods. A week before the beginning of the present experiment, steers were vaccinated (Pyramid® 5 + Prespense SQ®; Wyeth, Madison, NJ), dewormed with moxidectin (Cydectin®; Wyeth, Madison, NJ) and fence line weaned. On September 17 (d 0 of the experiment), steers were weighed and blocked according to frame score (medium and large). Within blocks, steers were randomly allotted to groups of four and each group assigned to one of the 12 treatment replications (n=48 steers). Stocking rate was 0.25 ha steer⁻¹.

Once in the pastures, steers were provided with free access to water and mineral with anti-bloat agent (Bloat Guard® Pressed Block; Sweetlix Livestock Supplement System, Mankato, MN). Steers were also weighed on d 22 and 33 of the experiment. Two pedometer types were used in the experiment to evaluate activity. On d 22, one pedometer (HJ-105, Omron Healthcare Inc. Bannockburn, Illinois) was attached with Velcro® and Duck® tape to the right rear leg of all steers in two replications (32 steers). In the same two replications, another pedometer (IceTag 2.004, IceRobotics, Midlothian, Scotland, UK) was attached to the left rear leg of one steer, randomly selected, per treatment. Pedometers were removed on d 27. The Omron pedometer is for human use, and data was not correlated to IceTag pedometer. Data from Omron pedometer was not considered in the analysis. Data from IceTag pedometer was downloaded onto a PC with the software IceTag Analyser™ 2.009, and steps per day were registered. Five complete days of data were used in the analysis.

Steer behavior was recorded from two replications per treatment during 3 d, from d 23 to 25. On d 22, the four steers per treatment replication were marked with orange paint in the rear leg, rib or shoulder to be identified from distance. Two, 4 m tripod stands were used for

behavioral observations. Binoculars were used to aid observers. During 3 d, from 0700 to 1900, behavior was scanned at 5 min intervals. An observer in each replication recorded animal location (fescue stand or mixed sward), position (lying down or active) and activity (grazing, ruminating and idling) of each steer (16 steers per observer). Observers rotated within 6 h per location. Grazing was considered when calves were biting or searching forage with head close to the sward (Chilibroste et al., 1997). Activities, positions, and location scan sampled at 5 min intervals were converted to a proportion of the 12 h observation period. Daily grazing time was later converted to proportion spent in each pasture. Weather conditions or animal position sometimes did not allow a full observation, and this was considered as a missing value. During the first day of observation, the proportion of missing data was more than twice the proportion of missing data in the other two days, so the first day was excluded from the analysis. The average of the 4 steers per treatment replication was used in the analysis.

Forage measurements

At the beginning of the experiment, percent ground cover by plant species, dead material and bare ground was visually estimated within 10 randomly located 0.5 m² quadrat per paddock (Tracy and Renne, 2005). The species were classified into target species (tall fescue, orchardgrass, and legumes), bluegrass, and other non-target species. The average of the 10 points per paddock was used in the analysis.

On d 0, 17 and 33 of the experiment, five 0.25 m² areas, in each paddock, were randomly chosen. The sward height within each area was measured with a rising plate meter. Herbage mass was then clipped at 2.5 cm stubble height, and samples were dried in a forced draft oven for 48 h at 55 °C, and weighed. Herbage mass (g DM m⁻²) for each paddock was obtained from the average of the 5 samples. Sward height was estimated with a rising plate meter in 30 random

points per paddock, walking in a 'W' pattern, and the average of the 30 points was used in the analysis.

Nutritive value of the forage was determined on d 0, 17 and 33 from samples collected by hand-plucking. Each paddock was walked in 'W' pattern and, every 5 steps, a sample was grabbed from the sward. Approximately 500 g of sample was collected per paddock. Samples were dried in a forced draft oven for 48 h at 55 °C and ground to pass a 1 mm screen in a Willey mill. Laboratory analysis performed included micro DM, ADF, NDF and CP. Percent micro DM was determined placing dried samples in oven overnight at 100 °C (AOAC, 2000). Crude protein was determined using N combustion method (AOAC, 2000) with a Perkin Elmer 2410 Nitrogen Analyzer. Percent of NDF and ADF were analyzed in an Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY) (Goering and Van Soest, 1970; Van Soest and Wine, 1967).

Statistical analysis

Forage and animal data were analyzed with Proc MIXED of SAS (SAS Institute Inc., Cary, NC; 2007). The experiment had a 2x2 factorial design with day as the repeated measure. The model, for variables measured in pastures and animals, included fescue and legume type and the interaction between both forage types. Percent ground cover was measured only on d 0, so no time effect was included in the model. Experimental unit was the two pastures (fescue stand and mixed sward) within treatment. Time spent grazing in each pasture is not only related to species preference by steers but also to sward characteristics (Hodgson, 1985). Hence, to analyze proportion of grazing time in each pasture, herbage mass of both mixed sward and fescue stand, from d 17, were included in the model as a covariate. Day 17 is the most representative day in sward characteristics for d 23 to 25 when observations were made. Orthogonal contrasts were

run to compare treatments with pasture A (A/E- and A/E++) vs C (C/E- and C/E++), and treatments having E⁻ (A/E- and C/E-) vs E⁺⁺ (A/E⁺⁺ and C/E⁺⁺), in each sampling day. Day effect was analyzed with linear and quadratic contrasts. To estimate how well the rising plate meter would predict herbage mass, a regression was conducted for sward height and herbage mass collected from respective quadrats, within each forage type. Results are presented as least square means. A significance level of $\alpha \leq 0.05$ was determined for the analysis. Trends were defined for level of α between 0.15 and 0.05.

RESULTS AND DISCUSSION

Weather conditions

Between September 17th and October 20th 2008 average air temperature was 14 ± 3 °C (Figure 3-2). Minimum and maximum air temperatures for the 33 d were 8 ± 3 °C and 20 ± 4 °C. Total precipitation was 53 mm with a maximum of 20 mm occurring on d 9. On d 24 and 25 of the experiment, when behavior was recorded, average air temperature was 15 °C and 13 °C respectively, and no precipitation was registered.

Forage Evaluation

Percent ground cover of forage species measured at the start of the experiment is presented in Tables 3-1 and 3-2. Within mixed swards, percentage of legume ground cover was greater ($P = 0.001$) in alfalfa-orchardgrass mixtures (A) (37 %) compared to clover-orchardgrass (C) (6 %). Orchardgrass ground cover was greater ($P = 0.04$) in C (53 %) than A (33 %). The C mixtures had more standing dead material (6 %) compared to A mixtures (3 %) ($P = 0.021$).

Bluegrass accounted for a high proportion (38 and 40% in E++ and E-, respectively) of ground cover in tall fescue stands and was similar between them ($P = 0.86$). The proportion of tall fescue was not different ($P = 0.16$) in E++ (34 %) compared to E- (24%). Franzluebbbers et al. (2009) compared persistence of E++, E- and E+ tall fescue over 7 years in Georgia. Percent of ground cover in E++ was greater (73%) compared to E- (67%) tall fescue. Percent ground cover did not differ between E++ and E+ (76%) tall fescue (Franzluebbbers and Seman, 2009). In North Carolina, Burns et al. (2006) reported greater losses in stand of tall fescue in Jesup E- (75 %) after 3 yr of grazing compared with E++ fescue (42 % reduction). In the third year of the pastures, proportion of E++ was greater (47 %) and of E- was lower (18 %) compared to the present study (34 and 24 % E++ and E-, respectively) (Burns et al., 2006). After 4 years of intensive winter grazing, the proportion of tall fescue tended to be greater in E++ (66%) as compared with E- (59%) pastures (Vibart et al., 2008).

Proportion of broadleaf and grassy weeds was greater ($P = 0.013$) in E- as compared with E++. A higher proportion of weeds ($P = 0.05$), as percentage of forage dry matter, in E- tall fescue (11 %) than E++ (3 %) was also reported by Burns et al. (2006)

Herbage mass and sward height did not differ between mixed swards or tall fescue stands ($P > 0.05$) during the experiment (Table 3-3 and 3-4). However, herbage mass decreased linearly during the experiment in A mixtures ($P = 0.013$). In C mixture, as in tall fescue stands, herbage mass remained similar over the course of the experiment ($P > 0.05$).

A regression of herbage mass on sward height using the rising plate meter was done to evaluate how well it might predict yield. Regressions differed among pastures. Adjusted R^2 was greater in legumes-orchardgrass mixtures (Adjusted $R^2 = 0.56$, $P < 0.001$ in A and Adjusted $R^2 = 0.45$, $P = 0.001$ in C) compared with E- (Adjusted $R^2 = 0.25$, $P = 0.020$). Regression was not

significant in E++ treatments ($P = 0.10$). Other studies have reported low R-square values for grass-legume mixtures (Sanderson et al., 2001). Using rising plate meter to estimate herbage mass in grazed pastures has been generally inaccurate. Sward height decreased linearly in A and C during the experiment ($P < 0.001$). Negative linear effect of time on sward height was significant in E++ ($P = 0.012$), and tended to be significant in E- ($P = 0.09$).

Most studies in diet preference are short-term and do not consider constraints in forage availability (Boland, 2009; Parsons et al., 1994; Rutter et al., 2004). However, Rook et al. (2002) studied diet preference of sheep between perennial ryegrass and white clover in a long-term trial. They observed that sward height of clover decreased sharply in the first month and stabilized between 1.2-1.6 cm. Sward height of ryegrass decreased gradually during the 12 weeks of experiment. Different rates of decrement of sward height between clover and ryegrass indicate that the sheep attempted to maintain a high proportion of clover in their diet even though it had declined in abundance. We observed a similar trend in sward height of mixed swards and tall fescue stands. Mixed swards and E++ had a linear decrease in sward height during the experiment. However, the reduction between d 0 and d 33 in sward height was 71 % higher in mixed swards as compared with tall fescue stands. The data on sward height indicates there may have been a preference for legume-grass mixtures by steers.

The proportion of legume cover was different between A and C treatments, and this was reflected in nutritive value (Table 3-5). On d 0, A mixtures had greater CP, and lower NDF and ADF compared with C mixtures ($P < 0.001$). For CP and NDF, these differences continued until the end of the experiment ($P < 0.05$). Acid detergent fiber was similar between mixed swards on d 33 ($P = 0.96$). The linear effect of time in CP was significant in A ($P < 0.001$) and C ($P = 0.001$). Neutral detergent fiber and ADF increased linearly in A ($P < 0.001$) during the

experiment, but time had no effect on NDF ($P = 0.87$) and ADF ($P = 0.89$) in C. The reduction in nutritive value during the experiment was more dramatic in A compared with C mixtures. The legume component of A (37%) was initially greater than C (6%), so the reduction in nutritive value may indicate that steers preferentially selected legumes in mixtures and reduced their abundance.

Within tall fescue stands, changes in nutritive value (Table 3-6) during the experiment were similar. There was no treatment effect in CP, NDF or ADF. Crude protein decreased linearly during the experiment ($P < 0.001$). Neutral detergent fiber and ADF showed a quadratic effect with time ($P < 0.001$).

Steers performance and behavior

Average daily gain of steers did not differ when tall fescue types were compared in the first ($P = 0.89$) and second period ($P = 0.35$) (Figure 3-4). Similarly, no differences ($P = 0.96$) were found in ADG of steers when mixed swards (A and C) were compared in the first period (Figure 3-3). Average daily gain tended to be greater in steers grazing on A compared with C from d 22 to 33 ($P = 0.124$). Between tall fescue stands, forage yield and nutritive value were similar during the experiment. Despite differences in nutritive value between mixed swards, content of CP was high in both A and C. Stewart (2006) compared performance of steers grazing E+, E- and E++ tall fescue during 2 consecutive years. Performance of steers grazing E+ was smaller (300 g d⁻¹), but, as in the present study there was no difference between the AVG of steers grazing E- (540 g d⁻¹) and E++ (450 g d⁻¹) tall fescue. Daily gains reported by Stewart (2006) were smaller compared with the present study, but performance of yearling steers was measured between May and September, hence including the summer, when lower ADG should be expected.

In the present study, calves had free access to both tall fescue and mixed swards, and this may have helped to equalize ADG among treatments. Bouton et al. (2005) evaluated animal performance on differing levels of endophyte infection in tall fescue monocultures and in mixed swards with white clover. Steers' ADG were greater on E- (990 g d⁻¹) compared with those on E+ (430 g d⁻¹). Differences in performance of steers grazing E- or E+ tall fescue disappeared when white clover was included to form mixed swards.

Activity, position and pasture location data from observations are shown in Figure 3-5 and 3-6. Proportion of daylight recorded as missing data due to poor weather conditions and other factors did not differ between treatments ($P > 0.05$). Among activities idling time tended ($P = 0.076$) to be greater in steers on A (24 %) compared with those on C (20 %). Idling time was similar ($P = 0.50$) between steers on tall fescue stands (E- 23 % vs E++ 21 %), and ruminating time did not differ between steers grazing tall fescue stands (E- 20 % vs E++ 20 %; $P = 0.26$) or mixed swards (A 22% vs C 18%; $P = 0.83$). Grazing time, as proportion of 12 h daylight period, was lower ($P = 0.002$) in steers grazing on A (50 %) than those grazing on C (56 %), and in steers grazing on E- (52 %) compared with those on E++ (55 %) ($P = 0.023$). The proportion of total grazing time spent in each pasture (fescue stand or mixed sward) was not different between steers on tall fescue types ($P = 0.21$) and mixed swards ($P = 0.29$) (Figure 3-7 and 3-8). Steers tended ($P = 0.14$) to be more active (standing or walking) and spend less ($P = 0.14$) time lying down on C (active 69 %; lying 28%) as compared with A (active 65 %; lying 32%) (Figure 3-9). Within tall fescue stands, lying ($P = 0.24$) and active ($P = 0.26$) time did not differ between steers on E- and E++ (Figure 3-10). Number of steps per day were similar ($P = 0.33$) between steers on mixed swards (Figure 3-11), and tend ($P = 0.15$) to be higher in steers on E- compared with those on E++ tall fescue stands (Figure 3-12).

Few studies have evaluated the effect of legumes or mixed swards on grazing behavior. Poli et al. (2006) measured the time cattle spent grazing red clover and birdsfoot trefoil-white clover mixed sward with different proportions of each pasture. Heifers had free access to both pastures but only 3 h in the morning and 3 h in the evening during 2 d. Treatments had 80, 67, 33 or 20 % area of red clover and the rest of mixed sward. Overall proportion of grazing time spent in red clover was 52 % (Poli et al., 2006), similar to results found by Torrez-Rodriguez et al. (1997) in birdsfoot trefoil and red clover pastures with similar preference between red clover (45%) and birdsfoot trefoil (55%). If cattle show indifference among legume species, it would be expected that grazing behavior in alfalfa and clover mix pastures would have been similar in our study. However, grazing time was higher in steers on C than on A.

Torrez-Rodriguez et al. (1997) studied preference between red clover and birdsfoot trefoil as affected by sward height of birdsfoot trefoil. Heifers had free access to both paddocks of the respective species during 3 d. Grazing time spent in birdsfoot trefoil and total grazing time increased as the sward height of birdsfoot trefoil increased. If it is assumed that sward height is correlated with abundance, then, these results would differ from results found in the present study where daily grazing time was greater and proportion of legumes was lower in steers grazing on C as compared with those grazing on A. However, in the present study legumes were in mixed swards with adjacent pasture of grass. Boland (2009) and Rutter (2004) demonstrated that steers have greater preference for legumes compared to grasses. Rook (2002) working with sheep in adjacent monocultures of ryegrass and clover (50:50 by area) observed that as the sward height of clover was reduced the preference for clover in the diet was also reduced. However, proportion of time spent in clover was always more than 50%, and sheep increased daily grazing time (Rook et al., 2002). Similarly, in the present study calves may have grazed more time in C

compared to A due to lower abundance of legumes in the C mixture. Their other forage option was a tall fescue pasture which should be less preferred.

Poli et al. (2006) observed that within each 3 h period (morning or evening), the proportion of time grazing red clover increased as the proportion of red clover by area increased. In summary, results of grazing behavior in legumes show that grazing time is similar between species, but it can be constrained by relative abundance of legume species or sward height. Poli et al. (2006) did not report nutritive value of pastures. In the present study, the proportion of legumes (A 37 % and C 6 %) and the nutritive value in the mixed swards differed between treatments. As in previous studies (Poli et al., 2006; Torrez-Rodriguez et al., 1997) these difference between mixed swards may have influenced grazing behavior of cattle.

Additionally, grazing time was affected by the fact that calves had free access to tall fescue stands next to the mixed sward. Rutter et al. (2004) reported a partial preference between white clover (74%) and ryegrass (26%) by dairy heifers when grazing adjacent paddocks with 25 or 75% of clover and the rest area of ryegrass. They also reported that grazing time between treatments did not differ (Rutter et al., 2004). Boland (2009) working with steers in adjacent monocultures of E- tall fescue and alfalfa at proportions of 25, 50 and 75% of each species reported an overall mean of 60% of the grazing time spent in the legume, with similar daily grazing time among treatments. These results indicate that daily grazing time would not be affected by proportion of legumes in the treatment, in a range between 25 and 75 % legumes, when offered as adjacent monocultures with grass. Champion et al. (2004), working with sheep, found that daily grazing time tended ($P = 0.062$) to be greater in ryegrass-clover mixture (9 % of clover) as compared with ryegrass and clover monocultures, or combined as adjacent pastures 50 % in area. If in the present study proportion of legumes in the mixed swards would have been

more than 50%, a similar daily grazing time would have been expected between treatments, with around 70% of the grazing time spent on the legume. Instead of more than 50% legumes in the mixture, our study consisted of mixed swards with 37% of legumes in A and 6% in C mixture. At this low proportion of legumes, we might expect differences in daily grazing time like those found in mixtures by Champion et al. (2004). Overall, the differences and trends that we found in daily grazing time (A 50 % and C 56 %; $P = 0.002$), active time (A 65 % and C 69 %; $P = 0.14$), and idling time (A 24 % and C 20 %; $P = 0.076$) may have been due to the low proportion of legumes in C compared with A, and not due to different preference between alfalfa and clover.

Similarly, to have a mixed legume swards next to grass swards should influenced the decision of how much time to graze per day when steers were grazing E- and E++ tall fescue. Species composition and nutritive value were similar between tall fescue stands. Sward characteristics, which could affect intake, did not differ. Intake per bite is the main variable determining daily intake, and grazing time has low compensation capacity (Hodgson, 1982). Bite size is highly correlated with sward height of grass in vegetative state (Griffiths et al., 2003), and negatively correlated with bulk density (Barrett et al., 2003). Forage availability is the combination of sward height and density along the sward. In our experiment, tall fescue pastures had been cut for hay in summer. In the fall, pastures were in vegetative state and had similar sward height and mass. Hence, it would not be expected that forage characteristics would have affected intake rate, or grazing time in the tall fescue swards.

Previous studies of grazing behavior on E- and E++ tall fescue have reported indifference in diet preference by cattle between these two tall fescue types. Boland (2005) studied the behavior of steers grazing E- and E++ tall fescue, among other grasses, between May and September. The time (min d^{-1}) spent grazing (E- 388, E++ 395), ruminating (E- 102, E++ 113),

idling (E- 120, E++ 112), or lying (E- 263, E++ 288) were similar between steers grazing these two fescue types (Boland, 2005). These results differ with the results found in the present study, where daily grazing time was higher in steers grazing E++ as compared with E-. However, the stands in Boland's study (2005) were more than 90 % of tall fescue in all fescue types. In the present study, proportion of ground cover with tall fescue was 34 % in E++ and 24 % in E-, and there were differences in proportion of ground cover by non-target species. If some of the weedy non-target species were unpalatable, this might have affected grazing time in pastures where they were more abundant as in the E- pastures (Table 3-2).

Parish et al. (2003) evaluated level of endophyte infection on grazing behavior of cattle in central Georgia from March to November. As Boland (2005) found, overall proportion of time grazing was similar between E- (39%) and E++ (39%) tall fescue (Parish et al., 2003). Parish et al. (2003) did not report proportion of ground cover with tall fescue in each treatment. As in the present study, nutritive value among fescue types was similar.

Our results between grazing behavior of steers on E- and E++ differ from previous studies (Boland, 2005; Parish et al., 2003). Lower proportion of tall fescue in the pastures, as adjacent mixtures with legumes might have influenced daily grazing time of the steers.

TABLES AND FIGURES

Table 3-1. Least square means of percent ground cover in alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).

Pasture	Legumes	Orchard- grass	Non-target species		Dead material	Bare ground
			Bluegrass	Other sp.		
A	37	33	12	1	3	13
C	6	53	14	3	6	18
SE	4.55	5.54	4.77	0.88	0.66	2.47
<i>P</i> -value	0.001	0.040	0.75	0.27	0.021	0.18

Table 3-2. Least square means of percent ground cover in endophyte free (E-) and novel endophyte (E++) tall fescue.

Pasture	Tall fescue	Non-target species		Dead material	Bare ground
		Bluegrass	Other sp.		
E-	24	40	11	11	14
E++	34	38	3	10	13
SE	4.59	5.95	1.79	1.81	2.96
<i>P</i> -value	0.16	0.86	0.013	0.90	0.81

Table 3-3. Least square means of herbage mass (g DM m⁻²) and sward height (cm) in alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).

<u>Herbage mass (g DM m⁻²)</u>				
<u>Day</u>	<u>A</u>	<u>C</u>	<u>SE</u>	<u>P value</u>
0	169	147	15	0.31
17	124	129	15	0.82
33	112	124	15	0.59

<u>Sward height (cm)</u>				
<u>Day</u>	<u>A</u>	<u>C</u>	<u>SE</u>	<u>P value</u>
0	10.4	10.1	0.6	0.69
17	9.5	8.9	0.6	0.55
33	6.7	6.5	0.6	0.82

Table 3-4. Least square means of herbage mass (g DM m⁻²) and sward height (cm) in endophyte free (E-) and novel endophyte (E++) tall fescue.

<u>Herbage mass (g DM m⁻²)</u>				
Day	E-	E++	SE	<i>P</i> value
0	173	158	27.5	0.71
17	161	170	27.5	0.82
33	170	171	27.5	0.99

<u>Sward height (cm)</u>				
Day	E-	E++	SE	<i>P</i> value
0	9.9	9.6	0.6	0.78
17	10.0	9.9	0.6	0.94
33	7.7	7.5	0.6	0.92

Table 3-5. Least square means of crude protein (CP), NDF and ADF content in alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).

<u>CP (g kg⁻¹)</u>				
Day	A	C	SE	<i>P</i> value
0	303	207	86	< 0.001
17	203	165	86	0.005
33	189	160	86	0.028

<u>NDF (g kg⁻¹)</u>				
Day	A	C	SE	<i>P</i> value
0	466	637	161	< 0.001
17	550	626	161	0.003
33	586	633	161	0.048

<u>ADF (g kg⁻¹)</u>				
Day	A	C	SE	<i>P</i> value
0	246	325	66	< 0.001
17	280	321	66	< 0.001
33	327	326	66	0.96

Table 3-6. Least square means of crude protein (CP), NDF and ADF content in endophyte free (E-) and novel endophyte (E++) tall fescue.

<u>CP (g kg⁻¹)</u>				
Day	E-	E++	SE	<i>P</i> value
0	184	191	63	0.48
17	150	150	63	0.98
33	138	133	63	0.56

<u>NDF (g kg⁻¹)</u>				
Day	E-	E++	SE	<i>P</i> value
0	641	640	77	0.93
17	610	598	77	0.27
33	645	640	77	0.65

<u>ADF (g kg⁻¹)</u>				
Day	E-	E++	SE	<i>P</i> value
0	321	310	49	0.14
17	293	281	49	0.10
33	316	311	49	0.47

A	A	C	C
E-	E++	E-	E++

Figure 3-1 Treatment arrangement in the experiment. Alfalfa-orchardgrass mixture (A), clover-orchardgrass mixture, endophyte free (E-) and novel endophyte (E++) tall fescue. Groups of four calves grazed each 2 paddocks and had 24 h access to both.

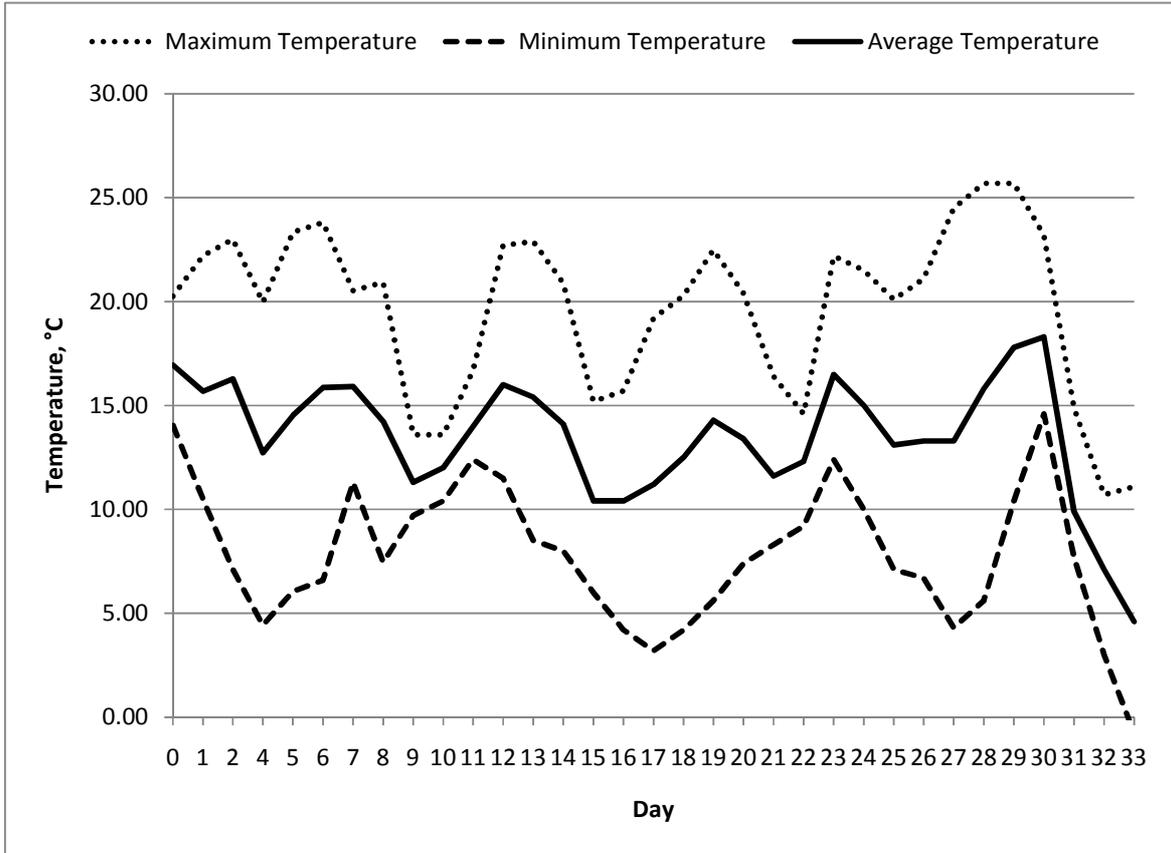


Figure 3-2. Average, minimum and maximum daily temperatures over a 33-d period (September 17 to October 20 2008).

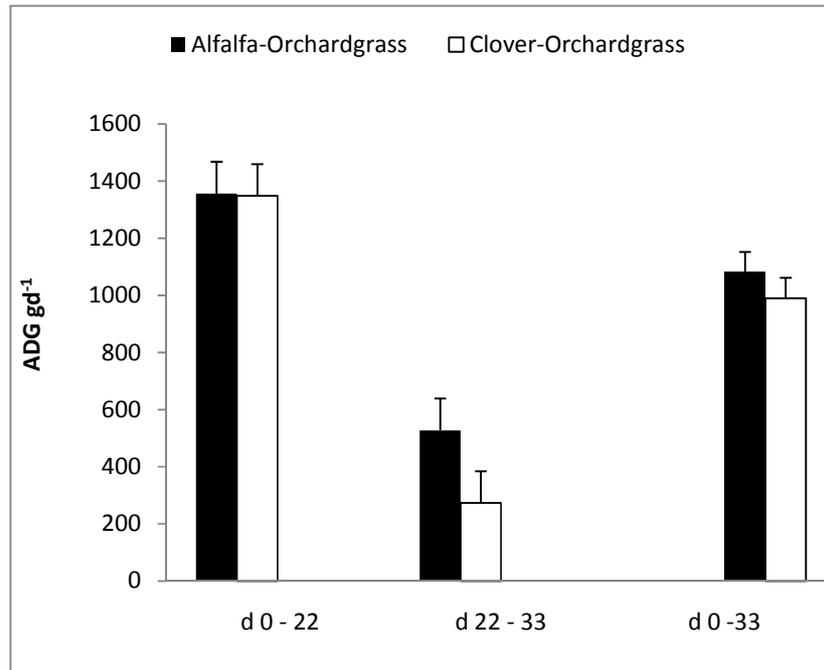


Figure 3-3. Least square means of ADG (gd^{-1}) for 0-22 d, 22-33 d and 0-33 d period in treatments with alfalfa-orchardgrass mixture and clover-orchardgrass mixture. On period between d 22 and d 33 ADG tended to be higher in alfalfa-orchardgrass mixture than in clover-orchardgrass mixture ($P = 0.124$).

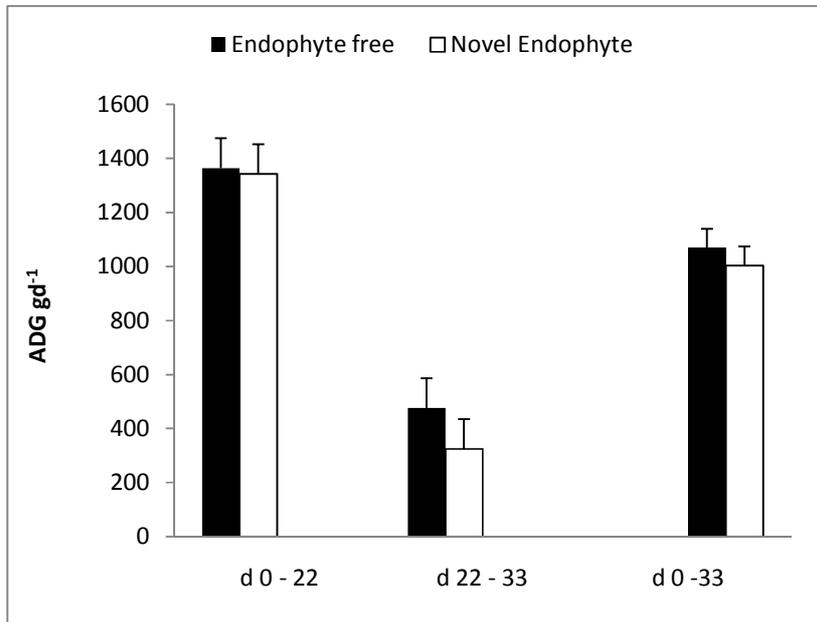
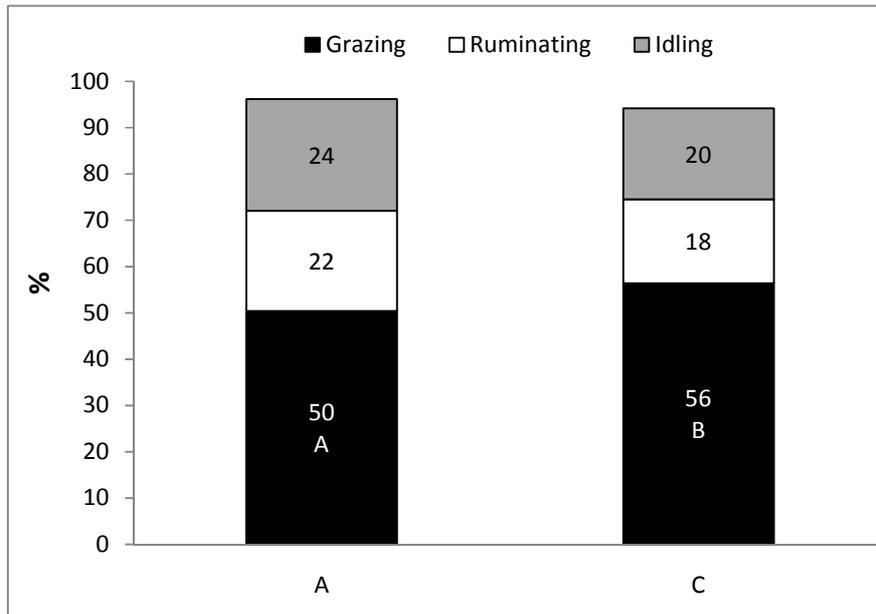
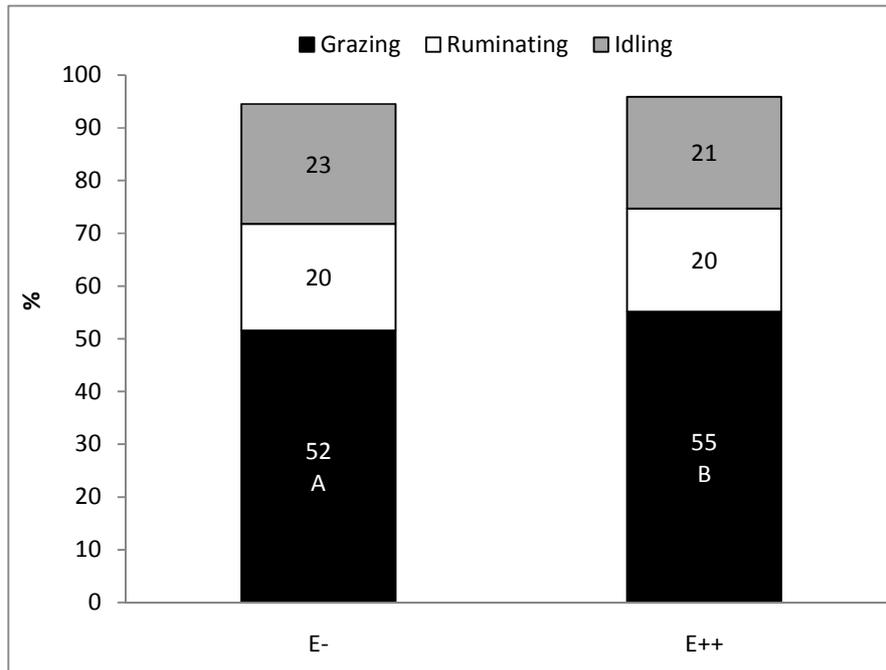


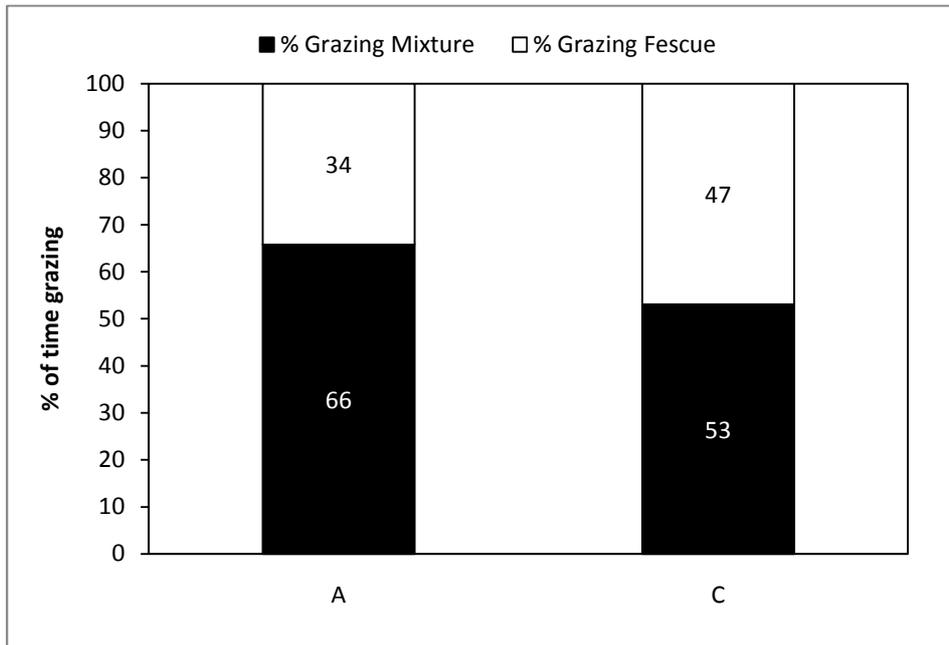
Figure 3-4. Least square means of ADG (gd^{-1}) for 0-22 d, 22-33 d and 0-33 d period in treatments with endophyte free and novel endophyte tall fescue.



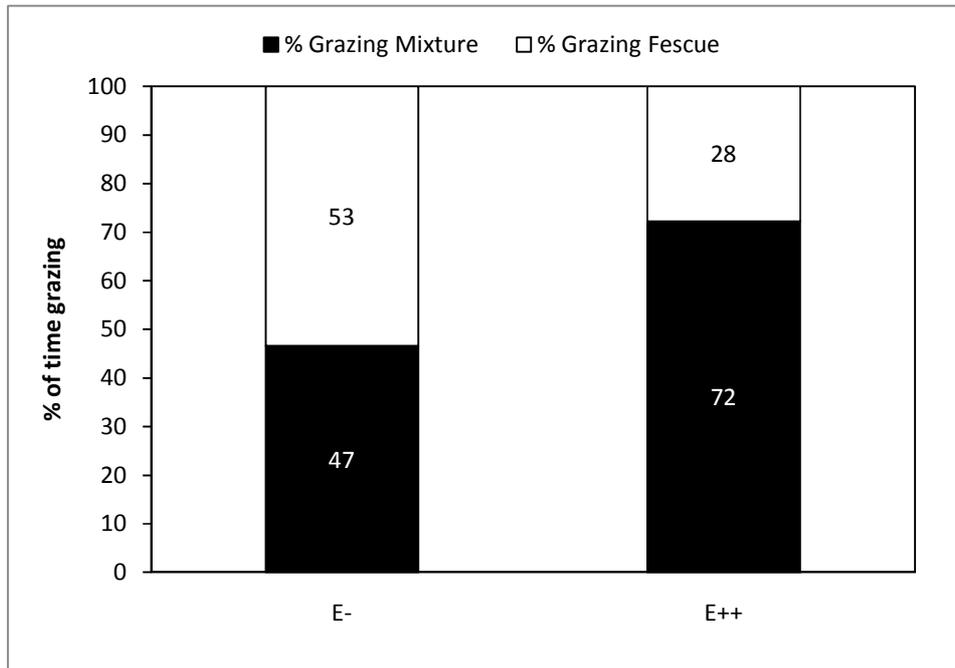
3-5. Least square means of the percentage of 12 h period (7 am to 7 pm) that calves spent grazing, ruminating and idling in treatments with alfalfa-orchardgrass mixture (A), clover-orchardgrass mixture (C).



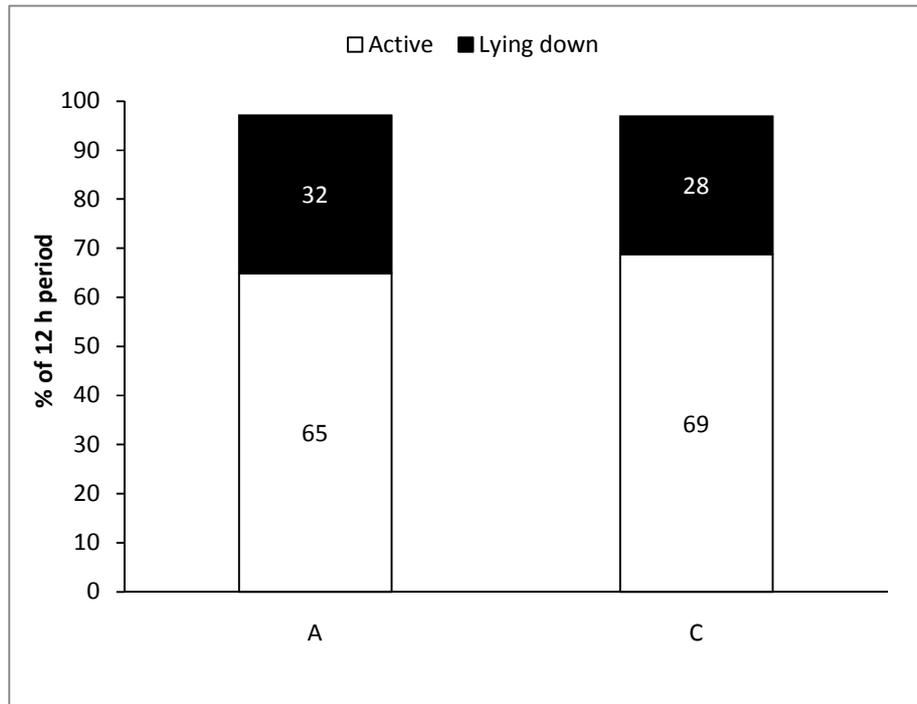
3-6. Least square means of the percentage of 12 h period (7 am to 7 pm) that calves spent grazing, ruminating and idling in treatments with endophyte free (E-) and novel endophyte (E++) tall fescue. Different letters indicate treatment effect on that activity.



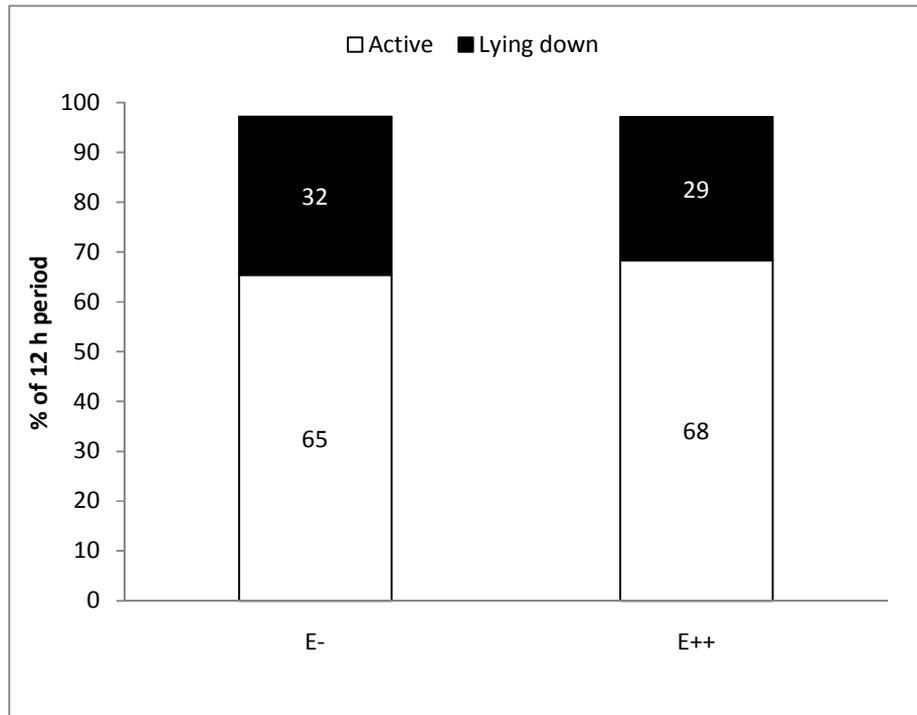
3-7. Least squares means of percent time grazing that animals spent in mixtures and monocultures, in treatments with alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).



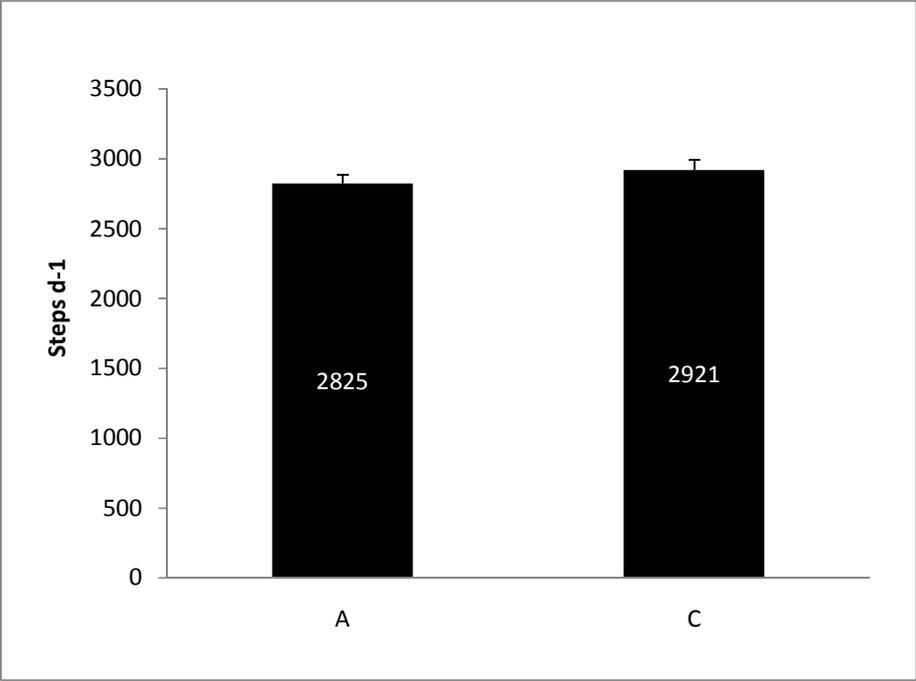
3-8. Least squares means of percent time grazing that animals spent in mixtures and monocultures, in treatments with endophyte free (E-) and novel endophyte (E++) tall fescue.



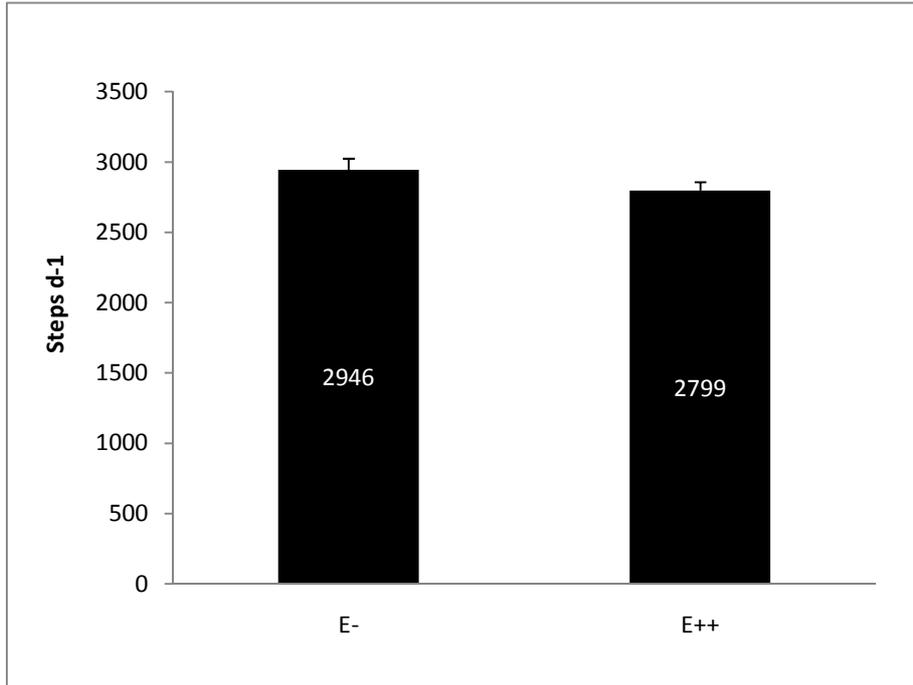
3-9. Least square means of the percentage of 12 h period (7 am to 7 pm) that calves are active (standing or walking) or lying down in treatments with alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).



3-10. Least square means of the percentage of 12 h period (7 am to 7 pm) that calves are active (standing or walking) or lying down in treatments with endophyte free (E-) and novel endophyte (E++) tall fescue.



3-11. Least square means of steps taken per day by calves in treatments with alfalfa-orchardgrass mixture (A) and clover-orchardgrass mixture (C).



3-12. Least square means of steps taken per day by calves in treatments with endophyte free (E-) and novel endophyte (E++) tall fescue.

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CHAPTER IV

CONCLUSIONS

Within tall fescue stands, differences were found only in species composition. Novel endophyte had numerically higher proportion of tall fescue and significantly lower proportion of broadleaf and grassy weeds, as compared with E-. There were no differences in sward characteristics or in nutritive value between these two fescue types during the experiment. Steers spent more time grazing in treatments with E++ than E-. Regardless of some differences in species composition, we concluded that fescue type affected grazing behavior of steers, with steers spending more time grazing in treatments with E++ as compared with E-. Low proportion of tall fescue in the pastures, as adjacent mixed swards with legumes in the treatment, might explain differences in results with previous studies, where no differences in behavior of cattle grazing E- and E++ were found. More research is needed to determine if E++, as part of a mixed diet with legumes, is preferred in similar proportion to E- and other cool season grasses.

Mixed swards presented differences in forage characteristics (nutritive value and species composition) that may confound results in grazing behavior of steers. Proportion of legumes was higher and proportion of orchardgrass was lower in A as compared with C. Nutritive value of C was lower than A during all the experiment. These differences between mixed swards could affected daily grazing time. Higher daily grazing time in treatments with C as compared with A might be due to legume species abundance in mixed swards, instead of a legume type effect. Future research should address preference between legume species of cattle grazing mixed swards with grass.