

SPATIAL ALLOCATION OF FORAGES AND ITS IMPACT ON GRAZING BEHAVIOR,
DIET SELECTION AND DRY MATTER INTAKE OF BEEF STEERS

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ABSTRACT

Previous research on grazing behavior has shown that ruminants will select a mixed diet. The use of adjacent monocultures is an essential tool for determining dietary preference of forages. Much of the work to date has been conducted with white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). Partial preference for white clover over ryegrass has been reported consistently and partial preference for legumes is thought to occur regardless of the legume and grass species being evaluated. Two forage species, tall fescue (*Festuca arundinacea* Schreb. or *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and alfalfa (*Medicago sativa* subsp. *sativa* L.), which had not been evaluated together previously as adjacent monocultures were grazed by beef steers in the present set of experiments. Steers exhibited a partial preference for alfalfa of 61 to 65% when given a choice of grazing alfalfa or tall fescue as adjacent monocultures, regardless of the ground area proportion of the two forages offered. Steers grazing tall fescue monocultures spent more time ruminating ($P = 0.02$) and tended to graze less time ($P = 0.06$) than steers in adjacent monoculture treatments. Time spent idling, number of prehensions and mastications, and bite rate were similar ($P > 0.05$) among treatments. Steers grazing tall fescue monocultures spent less time standing, more time lying, were less active and took fewer steps ($P \leq 0.05$) than steers in adjacent monoculture treatments. Grazing behavior was examined when alfalfa had not been in the previous diet of the steers. Cattle without previous experience grazing alfalfa spent 78% of the time grazing alfalfa, whereas after having

experience grazing it they spent a lower ($P = 0.04$) proportion of their time grazing alfalfa (72%). Overall proportion of the day spent grazing both forages was lower ($P = 0.0001$) when alfalfa was novel (40%), compared to when steers were experienced grazing both forages (46%). Proportion of the day spent idling was greater ($P < 0.0001$) when alfalfa was novel (35%), compared to when both forages were familiar to the steers (26%). Previous research has reported that ruminants exhibit a diurnal pattern of preference by decreasing the proportion of white clover consumed from morning to late afternoon while increasing the proportion of perennial ryegrass in the diet. This is thought to be a strategy to increase fiber intake before nightfall or as a response to higher carbohydrate levels in grass in the afternoon. In the present study, proportion of grazing time in alfalfa was higher ($P = 0.02$) in the afternoon (76.8 %) than in the morning (72.1 %). While fiber concentration was higher in the tall fescue, carbohydrate concentrations were similar. Steers were not attempting to increase fiber intake in the afternoon in the present study. Dry matter intake of steers grazing adjacent monocultures of alfalfa and tall fescue was estimated with n-alkanes. Diet composition was estimated using n-alkanes and long chain alcohols (LCOH) in several different combinations. The use of LCOH added additional characterization of the forages, but diet composition estimates were not different ($P \geq 0.22$) than when estimated using four different n-alkanes. Laboratory analysis costs may be reduced if n-alkanes alone can adequately characterize the forages being consumed, depending on the forage species in question. Meteorological conditions impacted DMI with intake being less in hotter conditions. Steers had similar partial preferences for alfalfa over tall fescue ($P = 0.13$, 79% and 70% alfalfa in yr 1 and 2, respectively) even though total DMI differed between

years ($P = 0.002$, 9.4 kg d^{-1} and 4.5 kg d^{-1} in yr 1 and 2, respectively). Lower DMI in yr 2 was attributed to hotter air temperatures. When animals are consuming two different forages as adjacent monocultures such as in the current experiments, it is important to determine the proportion of each forage in the diet before calculating DMI using odd chain n-alkanes of the forage along with a dose even chained n-alkane. Dry matter intake can be overestimated if the proportion of the forages consumed is not estimated and accounted for in the equation. This would apply to other studies utilizing mixed swards or any diet containing multiple components that differ in concentration of the n-alkane being used for DMI estimation. Analysis of n-alkane concentration should be performed on each item in the diet and the proportion of each item in the diet estimated so that the right value can be used in the calculation. Differences in marker concentrations between years also indicate the importance of analyzing those concentrations in the feed or forage at the time of fecal collection and not using values reported from previous research.

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CHAPTER 1

INTRODUCTION

Approximately 20% of the total land area on Earth's surface is comprised of grazing lands (Illius and Hodgson, 1996). With such a large percentage of the planet dedicated to this purpose it is vital to conduct research on grazing behavior to better understand diet selection of grazing herbivores. The most inexpensive source of feed available to producers is usually grazed herbage. Pasture-based production systems offer low cost production while also catering to the consumer desire for a product produced in an environmentally friendly manner that promotes improved animal welfare (Marotti et al., 2002a). Maintaining sheep and beef cattle on pasture has been shown to be productive throughout the world (Caradus et al., 1995; O'Riordan, 2000). The meat of animals finished on grass is healthier (Mulvihill, 2000; Moloney et al., 2007) and pasture-raised beef is in high demand (Lozier et al., 2004). However, voluntary intake of grazing ruminants is often lower than in those animals consuming processed feeds (Gibb and Orr, 1997). Research on methods to increase intake and performance of ruminants on pasture can lead to development of management strategies by which higher level of production may be obtained. An understanding of the preferences of grazing animals and how they selectively graze is needed to effectively utilize pasture lands (Rutter et al., 2004a). Economic and physical productivity of grazing systems is fundamentally linked to the efficiency of each individual animal (Marion et al., 2005).

Legumes have high feeding value relative to grasses (Crampton et al., 1960; Beever et al., 1986). However, grazing of legumes alone can cause frothy bloat in ruminants. There are both animal and agronomic benefits gained by growing legumes in mixtures with grasses. Incidence of bloat can be reduced by the inclusion of at least

60% grass in sward mixtures (Vallentine, 1990). Nitrogen (N) fertilization of grasses can be reduced as additional nitrogen (N) is mineralized from organic soil N from the legumes (Sprent and t'Mannetje, 1996). The additional N can improve yield and overall forage quality of grass legume swards (Sleugh et al., 2000). Unfortunately, mixed swards containing grasses and legumes are inherently unstable due to differing plant physiology and selective grazing by livestock (Parsons et al., 1991). The legumes face competition in mixed swards with grasses (Fales et al., 1996; Hoveland et al., 1999) and may only account for 5 to 20% of the herbage on offer (Clark and Ulyatt, 1985; Nolan et al., 2001).

Ruminants' preference for white clover (*Trifolium repens* L.) over perennial ryegrass (*Lolium perenne* L.) has been well documented (Parsons et al., 1994a; Penning et al., 1997; Marotti et al., 2002b; Rutter et al., 2004a). This preference for clover over grass leads to livestock selectively grazing it within the pasture (Curl et al., 1985; Ridout and Robson, 1991). Further growth of clover is decreased due to the removal of photosynthetic tissue (Parsons et al., 1991). Clover may become depleted in mixed swards due to selective grazing (Bedell, 1968). Livestock may incur a foraging cost in that overall intake may be reduced due to excessive time spent searching for their preferred forage when it is no longer as prevalent in the pasture (Parsons et al., 1994a; Parsons et al., 1994b). The higher nutritive value of the preferred forage may not be enough to compensate for the lower nutrient ingestion rate. A better understanding of why animals select their diet in the way they do should provide insights into alternate methods of presenting forages to livestock (Chapman et al., 2007). Offering grasses

and legumes as separate monocultures may moderate some of the production issues (mentioned above) that arise in mixed swards.

The following literature review presents research that has been conducted on the diet selection of sheep and cattle. Possible explanations for why animals choose mixed diets will be discussed as well as the potential implications that diet selection presents for livestock production, along with methodologies for conducting the research. The majority of the research to date has been conducted with ryegrass and white clover swards. Alfalfa (*Medicago sativa* subsp. *sativa* L.) is the most commonly grown forage legume in the US, covering nearly 10 million ha (Sheaffer and Evers, 2003). Tall fescue (*Festuca arundinacea* Schreb. or *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) is the most commonly grown grass used for pasture in the southeastern US, covering over 14 million ha (Ball et al., 2002). Evaluating two forage species with such widespread use in agriculture provides information that can be used in a broad range of applications in animal production. The set of experiments described here evaluated grazing behavior of cattle on pastures of these two forages grown as adjacent monocultures. The experiments conducted were designed to expand on the current knowledge of diet selection of grasses and legumes by grazing livestock.

CHAPTER 2 LITERATURE REVIEW

Dietary preference and selection

To evaluate and discuss the diets of livestock a clear distinction should be made between preference and selection (Newman et al., 1992; Parsons et al., 1994a).

Preference is defined as what the animal selects to eat when physical constraints are minimized, such as those associated with finding and ingesting their food (Parsons et al., 1994a). In other words, what the animals 'want' to consume. Selection is defined as a function of preference modified by opportunity for selection (Hodgson, 1979). An example of selection would be what occurs when an animal must search through a mixture of species in a sward to find its' preferred forage. The need to search thereby limits the ability of the animal to consume what it wants. In dietary choice experiments researchers want to minimize physical constraints on the ability of animals to consume what they want so that the animals' preference can be measured. Planting the forages in spatially separated but adjacent monocultures can accomplish this requirement. In this spatial arrangement, animals are free to graze between the distinct areas eliminating the constraint imposed when it must seek out a preferred meal within a dense mixture of forages (Rutter, 2006).

The distinction between active selection and indifference (i.e. grazing randomly) should also be addressed. If the forages being evaluated are only offered in equal ground area proportions, for example 50% white clover and 50% ryegrass, and the animals selected 50% white clover and 50% ryegrass in their diet then the distinction could not be made as to whether the animals were grazing indiscriminately and

consumed those proportions because that is what they had on offer or if they actively selected those proportions (Parsons et al., 1994a). Two additional treatments with other proportions of the white clover and ryegrass, for example 25:75 and 75:25, can be added to an experiment to determine if selection is active or random. If within those additional treatments the diets selected differ from the proportions on offer then random grazing can be ruled out (Rutter et al., 2004a; Rutter, 2006).

Mixed diets

Van Dyne and Heady (1965) evaluated the diets of Hereford steers and crossbred wethers (whiteface x Suffolk) that had esophageal fistulas. Animals grazed during the summer on mature California rangeland containing various grasses (57 to 61%), forbs (33 to 44%), legumes (5 to 3 %), shrubs (0 to 5%), and unidentifiable plant (9 to 12 %). They found that grasses averaged 55% of the diet and that cattle consumed more grass than sheep, whereas sheep selected a higher proportion of legumes than cattle. Bedell (1968) evaluated the diets of esophageally fistulated mature ewes (Willamette and Suffolk) in western Oregon. The ewes were grazing subterranean clover in mixture with either ryegrass or tall fescue. He reported that sheep preferred clover to the grasses and that a higher proportion of clover was in their diet than was present in the sward. Behavior was not a focus of his work however he proposed that management strategies could be manipulated to match this observation of preference in the animals' diets. Curll et al. (1985) used esophageally fistulated wethers (Suffolk x Scottish halfbred) to evaluate the diet when grazing ryegrass and white clover mixed swards. They also reported that clover was preferred to ryegrass over a range of clover

content in the sward (2 to 48%) and at different levels of herbage mass (0.5 to 4.8 t DM ha⁻¹). Clark and Harris (1985) conducted a study examining sheep grazing ryegrass and clover in a mixed sward or as monoculture strips. They originally reported that sheep did not show a preference for clover in mixed swards. However, Ridout and Robson (1991) reanalyzed the data and found it to be misinterpreted by Clark and Harris (1985). There was a preference for clover shown by their data after statistical analysis was revised using a selection coefficient:

$$\Theta = (\text{proportion of clover in diet} / \text{proportion of grass in diet}) / (\text{proportion of clover in sward} / \text{proportion of grass in sward}).$$

Briseño de la Hoz and Wilman (1981) studied mixed swards of white clover and grasses after being grazed by either sheep or cattle or being cut and harvested. Although diet selection was not directly measured by observation or intake determination, they reported that sheep “actively sought out the clover” over the grasses, which included perennial ryegrass, Italian ryegrass (*Lolium multiflorum* Lam.) and Pecora timothy (*Phleum pretense* L.). They did not make this observation with cattle and found their grazing to have an effect on the sward similar to frequent cutting and proposed that cattle exerted little selection either for or against white clover. Similarly, Bedell (1973) reported that sheep selected subterranean clover (*Trifolium subterranean*) while cattle selected for tall fescue or perennial ryegrass, however, here again diet selection was not directly measured.

Although these early reports of animal diets are beneficial, much of the data published throughout the 1960's to the 1980's is presented using different terminology to describe their results. A clear distinction between 'preference' and 'selection' was not

always made and makes interpretation and comparison between experiments more difficult. For example, examination of extrusa samples from fistulated animals in comparison to the proportion of forages species present (as in Bedell, 1968 and Curll et al., 1985) may not be a true measure of preference but a measure of apparent selection (Newman et al., 1992). Hodgson (1979) published a collection of terminology for use in grazing experiments that gives researchers a common usage for terms, making comparisons between experiments more straightforward. Measurement of preference and selection as defined by Parsons et al. (1994a) and Hodgson (1979) have been used in the more recent literature in grazing experiments.

Parsons et al. (1994a) conducted a study with mature half-bred ewes (Border Leicester x Cheviot), both dry and lactating, to evaluate diet preference with clover at 20, 50, or 80 % of the total ground area. They found that intake rate for ewes grazing clover was higher than when grazing grass (2.92 vs. 5.84 and 2.38 vs. 3.41 g DM min⁻¹ for lactating and dry ewes, respectively). The ewes chose a mixed diet within each of the different percentages of clover available and were not grazing randomly. The ewes could have chosen a diet of 100 % clover which would have maximized intake rate; however over the study they consistently chose a mixed diet. On d 6 the percentage of clover in the diet was 44, 79, or 72% for 20, 50, or 80% clover treatments, respectively. Parsons et al. (1994a) also reported that physiological state (dry or lactating) did not have an effect on preference. However, the ewes' diet prior to the study did seem to influence diet selection. The ewes initially had a preference for the species opposite of what they had grazed previously. Those with ryegrass as their background diet ate 81% clover the first day compared with 70% clover intake for those that had grazed clover

previously. But by d 6 the ewes from a ryegrass background decreased their clover intake to 55% and the clover background ewes increased clover intake to 75% showing a gradual lapse back to their original diet. A diurnal pattern of preference was observed with ewes grazing more clover in the morning and more grass in the afternoon. Possible explanations for this observation are discussed in a later section.

Harvey et al. (1996) offered non-lactating sheep a choice of grazing adjacent monocultures of ryegrass and clover with either 25 or 75% of ground area in clover. The remaining ground area was ryegrass. Two N fertilization treatments were applied to ryegrass every two weeks; high N (30 kg ha⁻¹) and low N (5 kg ha⁻¹). Sheep grazed either high- or low-N ryegrass prior to the study to also evaluate the effect of background diet on subsequent diet preference. No differences were found in dietary preference of sheep that had low-N ryegrass as a background diet. Those with high-N ryegrass prior to the study did vary in the proportion of time eating clover from the adjacent monocultures if the grass was high-N but not if it was low-N (Table 2-1). This is in agreement with the finding of Newman et al. (1992) who evaluated diet selection of Scottish half-bred ewes (Border Leicester x Cheviot) grazing turves of perennial ryegrass and white clover. They also reported that sheep preferred the opposite forage to what they had consumed as their previous diet (Newman et al., 1992). Intake rate reported by Harvey et al. (1996) was higher for clover (4.28 g DM min⁻¹) than for either low- or high-N grass (2.96 or 3.08 g DM min⁻¹) which corresponds with the data of Parsons et al. (1994a) and others that clover can be consumed at a higher rate. There may have been a nutritional effect on preference related to N in this experiment.

Cosgrove et al. (1996) used Friesian and Friesian-cross dairy heifers (4 mo old) to determine if cattle show a preference for white clover when offered as an adjacent monoculture to ryegrass (50% ground area each). They reported that there was an effect of season with heifers grazing 65% clover in February but only 49 and 45% in December and May, respectively. Total amount of time spent grazing decreased over the grazing period (8.3, 7.2, and 5.2 h, December, February and May respectively). As a result, the lower partial preference for clover in May was a result of less time spent grazing clover, not an increase in time spent grazing ryegrass, and may be explained by reduced palatability of the clover in May. Because there was only one ratio of ground area examined, the proportion of clover and ryegrass in the diet may have been the result of active selection or indifference. However, the results indicate that seasonal patterns in preference may exist and may be related to changes in plant palatability.

Penning et al. (1997) used mature Scottish half-bred ewes (Border Leicester x Cheviot) and British Saanen does to evaluate their preferences grazing adjacent monoculture swards of 20 or 80 % clover by area with the remaining area being perennial ryegrass. There was no effect of percent area indicating that animals were not grazing at random. Goats had a 52% preference for clover while sheep had a 70% preference. Sheep grazed for a longer period of the day than did goats (663 vs. 520 min, respectively). Sheep grazed longer per day in the 20% clover treatment (734 min) than the 80% clover (592 min) as did goats (587 vs. 453 min, 20 and 80% clover respectively). As Hodgson (1979) noted, to determine preference there must be minimal constraints on the animal. The researcher made calculations to try to ensure herbage was not depleted however a decrease in sward surface height (and subsequently

herbage mass) did occur in the study by Penning et al. (1997). They Goats were observed to stop grazing during rainfall while sheep were not affected, indicating an environment constraint on the goats.

Torres-Rodriguez et al. (1997) evaluated diet preference of young heifers when grazing three adjacent monoculture combinations: white clover: ryegrass (W: Rye), birdsfoot trefoil (*Lotus corniculatus* L.): ryegrass (L: Rye), or birdsfoot trefoil: red clover (*Trifolium pratense* L.) (L: Red). They predetermined that white clover and bird's-foot trefoil were the preferred forages of those offered. The sward height of those preferred forages was cut to different heights (4, 6, 8, or 10 cm) while the alternative forage was cut to 10 cm. They found that grazing time decreased as height of preferred forage increased in the L: Rye and W: Rye treatments. In contrast, grazing time increased in the plot with two legumes (L: Red) as height of birdsfoot trefoil increased. Heifers exhibited a partial preference for legume in W: Rye with 68% of the time spent grazing white clover, and in L: Rye with 70% of the time spent grazing birdsfoot trefoil. In the L: Red treatment, heifers spent similar proportions of time grazing each legume (55: 45% for L: Rye).

Cosgrove et al. (1997) also evaluated birdsfoot trefoil in adjacent monocultures with ryegrass or red clover. The dietary preference of Friesian and Friesian-cross dairy heifers was recorded in summer and fall. Heifers preferentially consumed birdsfoot trefoil for a greater proportion of the time spent grazing when adjacent to ryegrass in both seasons (75 and 67% in summer and fall, respectively). The animals decreased the total amount of time spent grazing in the fall and specifically they grazed birdsfoot trefoil less (4.1 h d^{-1}) compared to in the summer (7.1 h d^{-1}) whereas the amount of time

spent grazing ryegrass was similar in both seasons (2.3 and 2.0 h d⁻¹ in summer and fall, respectively). It was not clear why this change occurred and was not thought to be due to changes in sward structure or herbage mass. The animals decreased their total time spent grazing adjacent monocultures of birdsfoot trefoil and red clover in the fall compared to summer as well (9.0 and 5.9 h d⁻¹, respectively). However, there was no difference in the proportion of time spent grazing birdsfoot trefoil compared to red clover. Birdsfoot trefoil was grazed 53 and 54% of the time in summer and fall, respectively. Because these proportions were not different from the proportion being offered it is uncertain if they preferred equal proportions of these two legumes or if they were grazing indifferently. Testing of these legumes at different proportions of ground area would clarify this.

Harvey et al. (2000) evaluated dietary preference over 48 h on adjacent monoculture of ryegrass and white clover at varied sward heights and stocking densities using both yearling and mature Scottish half-bred ewes (Border Leicester x Cheviot). Animals in the high (29.8 ewes ha⁻¹) and low (21.3 ewes ha⁻¹) stocking density treatments spent similar time grazing white clover during d 1 (45 and 55% of time) however on d 2 those in the high stocking group spent more time grazing clover (67%) compared with the low stocking group (57%). Subsequently, the proportion of clover intake (g DM consumed) also increased on d 2 for the high stocking density group (73% of clover in intake) compared with the low stocking density group (63% of clover in intake). There were differences observed between the different sward height treatments from d 1 to d 2. All ewes had been grazing adjacent monocultures of the same sward height (6 cm) prior to the experiment. However, when placed on pastures with differing

sward heights (3 cm clover: 6cm grass or 3 cm clover: 9 cm grass) their diet selection changed during the first 24 h. The ewes with 3 cm clover: 6 cm grass seemed to go against their intake pattern prior to the study (70% clover) and instead favored an intake maximization strategy by spending more time grazing ryegrass on d 1 making up a greater proportion of their intake (54%). This may be partially explained by the observation of Parsons et al. (1994a) that sheep will initially graze the forage opposite to what they previously ate but will over a few days revert back to grazing approximately 70% clover in their diet. After 48 h Harvey et al. (2000) reported that sheep in the low clover treatment (3 cm clover: 9 cm grass) began to revert back to a higher proportion of white clover in their diet (63%). A diurnal pattern was also observed by with ewes grazing more clover in the morning and including more grass in their diet later in the day.

Cosgrove et al. (2001) observed behavior and measured intake of sheep grazing in one of the following treatments: white clover only, ryegrass only, mixed sward of ryegrass and clover (22% clover by mass), or choice of the two forages as adjacent monocultures (50% ground area each). They found that over a two week period the animals in the choice treatment maintained 70% clover in their diet and had 25% greater intake than sheep grazing ryegrass only (1345 vs. 1080 g DM d⁻¹, respectively). Animals grazing clover only did not differ in intake (1300 g DM d⁻¹) from those grazing in the choice treatment. Marotti et al., (2001) used these same forage treatments as Cosgrove et al. (2001) to evaluate the effects on milk yield of Friesian dairy cows. Cows grazed in the forage treatment for 6 d and milk yield was measured from d 4 to 6. Those offered a choice had a diet of 70% clover. Milk yield of those cows grazing

adjacent monocultures was 11% higher ($2.4 \text{ kg cow}^{-1} \text{ d}^{-1}$ more) than cows grazing a traditional mixed sward and 28% higher than cows grazing grass only.

Marotti et al. (2002b) further evaluated the grazing behavior and intake of Romney ewes with the same treatments as used in their 2001 experiment. The mixed sward in this experiment was 16% clover by mass. Background diet of the sheep was the mixed sward. As reported previously in the literature, intake rate of sheep grazing clover ($5.0 \text{ g DM min}^{-1}$) was greater than of those grazing grass ($3.5 \text{ g DM min}^{-1}$). Consequently, sheep needed to graze longer in the grass only treatment (310 min d^{-1}) to attain a daily intake of 1080 g DM d^{-1} compared to sheep in the choice (280 min d^{-1}) or clover only (270 g DM d^{-1}) treatments. Those sheep had a daily intake of 1360 and 1340 g DM d^{-1} , respectively. Ewes in the choice treatment spent 62% of the time grazing clover which amounted to a proportion of 70% clover in the diet.

The majority of the studies that have been conducted evaluating diet selection of adjacent monocultures have been of short duration. Rook et al. (2002) considered the long term dietary preferences of ewes (Border Leicester x Cheviot) when allowed to graze adjacent monocultures of perennial ryegrass and white clover for a period of 12 weeks. Initially the ewes showed a partial preference for white clover with it accounting for 60% of their diet. This led to a decrease in the sward surface height of clover in the first weeks of the study while ryegrass increased somewhat but then also started to decline. The proportion of clover in the diet dropped to 28% after two weeks but rose back to 52% after 4 weeks. By the end of the 12 wks the proportion of clover in the diet had decreased to 23%. Whereas the amount of clover consumed varied, the sheep were still trying to maintain a high percentage of clover in their diet as indicated by the

selection coefficient (Θ) of clover which stayed above 1. The animals made a choice to reduce total intake from 1.8 kg DM d⁻¹ in wk 1 to 1.0 kg DM d⁻¹ by week 12 and maintain a mixed diet even though they had to graze longer due to the depletion of the clover sward. These animals had lower energy requirements because they were not lactating so animals with a higher energy requirement (lactating) may not make such a trade off for total intake.

Rutter et al. (2004b) studied the dietary preference of yearling dairy heifers (Holstein x Friesian) when grazing adjacent monocultures of perennial ryegrass and white clover with treatments consisting of either 25 or 75% white clover by ground area with the remaining percentage in ryegrass. Additionally, half the heifers were dosed with intra-ruminal anti-bloat devices in a 2 x 2 factorial design. Animals were monitored for incidence of bloat while preference measurements were conducted. During the week prior to each measurement period (4 total, 48 h each), the heifers grazed adjacent monocultures of 50% ryegrass, 50% clover to ensure their background diet would not influence preference. Heifers were also fitted with CIDR's to prevent estrus behavior that might interfere with grazing behavior measurements. There was no interaction between anti-bloat treatment and forage treatment. Mild bloat was reported on seven occasions with the majority of those having not received an anti-bloat device and grazing in 75% clover. Overall, heifers in the 25% clover treatment spent 64% of their time grazing clover while heifers with 75% clover spent 77% of their time grazing clover. A diurnal pattern of preference for clover in the morning and grass in the afternoon was observed, which was similar with prior studies involving sheep (Parsons et al., 1994a;

Harvey et al., 2000). The authors concluded that mild bloat may play a role in diet selection, but would not likely be the primary explanation.

Rutter et al. (2004a) again evaluated adjacent monocultures with treatments of either 25 or 75% white clover by ground area with the remaining percentage in ryegrass. In this study the dietary preference of Holstein-Friesian lactating dairy cows was evaluated. The cows grazed an area of 50% ryegrass, 50% clover for 1 wk prior to each measurement period (6 total) which lasted for 48 h. Here again results were similar to previous studies with a partial preference for clover observed regardless of forage treatment and a diurnal preference for clover in the morning changing over to a preference for grass later in the day. Those with 25% clover had 63% clover in their diet while cows with 75% clover had 84.5% clover in their diet. Intake rate was higher when cows were eating clover ($41.3 \text{ g DM min}^{-1}$) than when eating grass ($27.5 \text{ g DM min}^{-1}$). This corresponds with previous reports that sheep had higher intake rate when eating clover (Parsons et al., 1994a; Harvey et al., 1996; Marotti et al., 2002b).

Whereas most of the literature reviewed thus far has been conducted on separate but adjacent monocultures, Rutter et al. (2005) conducted a study evaluating white clover and ryegrass sown in strips compared to a mixed sward. The widths of the strips were varied (108, 36, or 12 cm) to determine the minimum width at which the same benefits of adjacent monocultures that have been shown in previous experiment could be achieved, such as increased daily intake. Paddocks were grazed by beef heifers (Simmental x Holstein) that were dosed with n-alkanes to estimate daily intake and diet composition. In the 108 and 36 cm treatments, the heifers' diets contained 59 and 60% clover, respectively. However, proportion of clover in the diet when grazing 12

cm strips was similar to the mixed sward (36 and 38%, respectively). Daily intake did not differ between any treatments so the increased intake observed in previous studies of adjacent monoculture was not observed here with ryegrass and clover sown in strips.

Poli et al. (2006) also conducted a study looking at diet selection of dairy heifers (Friesian-cross) grazing different forages offered in strips. However, they used three different legumes instead of a legume and a grass. Paddocks were sown in strips of birdsfoot trefoil mixed with white clover (BW) and strips of a red clover monoculture (RC). These strips were then allocated to provide different treatments based on percent ground area (20 BW: 80 RC, 33 BW: 67 RC, 67 BW: 33 RC, and 80 BW: 20 RC). On the first day animals preferentially selected the forage that made up the lowest proportion of the two. For example in the 20 BW: 80 RC treatment, they grazed BW 68% of the time and in the 20 BW: 80 RC treatment they grazed RC 65% of the time. Over three days the animals tended to switch to the opposite forage, most likely due to a decrease in the sward height of the other forage that was preferentially grazed initially.

What leads ruminants to select a mixed diet?

The literature reviewed here shows there are aspects of diet selection that have been measured repeatedly over a number of experiments in various locations and with different animal species. There seem to be similarities that carry over between the diet selection of cattle and sheep. Both select mixed diets that are usually higher in proportion of white clover. Both show a diurnal preference for white clover in the morning and ryegrass in the afternoon. This implies there may be biological explanations for those similarities that can apply to both of these animals. There are a

number of theories that might explain why ruminants select a mixed diet, some of which have been partially disproved and others that need further examination.

Spatial memory. Ruminants do not seem to be selecting a mixed diet simply because they have poor spatial memory of where food is located. Bailey et al. (1989) showed that steers (Angus x Hereford and Barzona x Hereford) could remember location of food resources in a maze. Not only did they find the food but patterned their choices from the greatest to the least food reward. Edwards et al. (1996) studied the spatial memory of non-lactating ewes (Masham) and found that they could remember where a food source was located both without the use of visual cues. However, sheep could locate food more quickly if a visual cue was present. Laca (1998) evaluated yearling cross-bred steers (*Bos taurus* x *Bos indicus*) in an experimental arena with food located in feeders. Spatial distribution of the food was varied to create a series of treatments and animal response was monitored from a tower. He also reported that cattle have good spatial memory and can forage efficiently by avoiding locations where they have recently visited. Dumont and Petit (1998) also showed that sheep (Limousin) were able to remember where preferred food resources were located. It seems that poor spatial memory is not explanatory of why ruminants select a mixed diet.

Intake maximization. Some have proposed that intake rate maximization is a component of diet selection in herbivores (Owen-Smith and Novellie, 1982; Black and Kenney, 1984; Thornley et al., 1994; Distel et al., 1995). However, this theory has been challenged and does not seem to fully explain what drives domestic ruminants to select a mixed diet. Illius and Hodgson (1996) stated that there is insufficient evidence for intake rate maximization in herbivores, and that animals often select a mix of food times

while disregarding the intake rate associated with each. As mentioned in the experiments reviewed above, intake rate of sheep and cattle when grazing clover is higher than when grazing grass (Parsons et al., 1994a; Harvey et al., 1996; Cosgrove et al., 2001; Marotti et al., 2002b). Consequently, if animals were simply maximizing intake rate they would select a diet of 100% clover, not the mixed diet observed.

Carbon and nitrogen. Westoby (1974) suggested that animals are selecting a mixed diet that will optimize a mixture of nutrients while maintaining a certain level of gut fill. Provenza et al. (1996) reported that lambs will eat a variety of foods even when one of the foods on offer can meet their nutritional needs. The ratio of carbon (C) to N in grasses and legumes differs with legumes generally having a higher proportion of N than grasses (Whitehead, 1995), thus by choosing mixed diets of grasses and legumes, animals may be balancing for certain levels of C and N to meet energy and protein requirement. There are energetic costs associated with excess N in the diet because it requires the animal the process that surplus N, meaning the animal must burn more energy, which in turn changes the animal's energy budget (Rutter, 2006). Kyriazakis and Oldham (1993) showed that sheep (Suffolk x Scottish wethers) can select from feeds that vary in protein content to meet their CP requirement. Lambs (Finn x Polypay x Suffolk) have been shown to have a preference for feed high in protein after a meal high in energy and vice versa (Villalba and Provenza, 1999a, b). This seems to correlate to the experiments in which animals were given a choice of grass or legume and preferred the forage opposite what they had eaten previously (Newman et al., 1992; Parsons et al., 1994a).

Adaptive rumen environment. Relating to the balance of C and N in the animals diet is the suggestion that animals select a diet to maintain optimal conditions in the rumen. By maintaining both grass and legume in the diet, ruminants can sustain a population of microflora that is adaptive should the animal be in a situation where only one of those forages was present (Parsons et al., 1994a). Cooper et al. (1995) found that when lambs (Suffolk x Greyface) were given a choice between two feeds that were either high, medium, or low energy density they did not choose the most energy dense exclusively but a mixture. In a second experiment with rumen cannulated sheep (Texel x Scottish Blackface) they gave infusions to change the pH and osmolality of the rumen. They observed a decrease in the amount of the high energy dense feed consumed, presumably because the sheep were trying to avoid further decrease in rumen pH and increase in osmolality due to the rapidly fermentable composition of the feed. Maintaining the rumen in a condition that is favorable for rapid cellulolysis and microbial growth while preventing acidosis or ruminitis may be one of the explanations for selection of mixed diets (Cooper et al., 1995). Merry et al. (2002) evaluated different ratios of red clover and ryegrass silage using an artificial rumen. At a ratio of 70% red clover and 30% ryegrass an optimal level microbial protein synthesis was attained. This consistency with the proportions of legume and grass found in grazing studies seems to suggest that the consumption of mixed diets is related to maintaining the rumen environment.

Metabolites in plants and the rumen. To add a further dimension, the energy and protein content of the diet can impact the ability of ruminants to consume plants high in secondary metabolites such as terpenes (Villalba and Provenza, 2005; Villalba

et al., 2006). Freeland and Janzen (1974) suggested that when an animal eats a variety of plants (containing various secondary metabolites), the overall intake rate can be increased because there are various detoxification pathways utilized for processing each particular. In effect, a negative impact that might be seen in an animal from consuming one secondary plant metabolite may be counteracted by consumption of another. The animal's prior exposure to these metabolites is also plays a role to ensure that rumen microflora are properly adapted and able to manage interactions between secondary metabolites of different forages. Smith (1959) reported that mule deer consuming juniper (*Juniperus osteosperma*) and sagebrush (*Artemisa tridentata*) together had higher intake (1.44 lb cwt BW⁻¹) compared with juniper or sagebrush alone (0.78 or 0.42 lb cwt BW⁻¹, respectively). In contrast, intake of scrub oak (*Quercus gambelii*) and sagebrush together was lower compared to a diet of scrub oak alone (2.20 vs. 2.52 lb cwt BW⁻¹) perhaps indicating some negative interaction of the secondary metabolites of those two. However, combining oak, sagebrush, and juniper resulted in the highest intake (2.73 lb cwt BW⁻¹).

Condensed tannins are another type of secondary metabolite found in birdsfoot trefoil, that decrease degradation of protein in the rumen allowing it to bypass fermentation which means less protein is converted to ammonia in the rumen (Min et al., 2000). Schreurs et al. (2007) evaluated rumen metabolites of sheep (Romney) consuming white clover, perennial ryegrass or birdsfoot trefoil. They found that rumen ammonia concentration was higher and peaked faster for those eating white clover than ryegrass or birdsfoot trefoil (1062, 341, or 294 mmol L⁻¹ per kg CP intake, respectively). The DMI of sheep eating white clover (114 g per meal) was lower than for those eating

perennial ryegrass or birdsfoot trefoil (351 or 278 g per meal, respectively). This could indicate a conditioned aversion being formed due to the high ammonia concentration. The animals dealt with this by reducing DMI because no other food items were available to them, similar to that reported by Marotti (2004) in a comparable study (Chapman et al., 2007). The inclusion of starch or other rapidly degradable carbohydrates (such as water-soluble carbohydrates) in the diet can lessen the effect of ammonia (Visek, 1968) which may contribute to the selection of mixed diets. These interactions need to be further explored but may also need to be considered when evaluating diet selection if plant secondary metabolites are present.

While not a plant secondary metabolite, the endophytic fungi found in ryegrass and tall fescue produce alkaloids in the plant that can affect grazing behavior and performance of livestock (Keogh, 1984; Cheeke, 1995; Parish et al., 2003; Boland et al., 2007). Edwards et al., 1993) reported that sheep can distinguish between endophyte infected (E+) and endophyte free (E-) perennial ryegrass and will choose other grasses before consuming E+. Cosgrove et al. (2002) examined how the presence of ergot alkaloids would affect diet selection in a greater variety of forage treatments. Forage treatments consisted of E+ ryegrass, E- ryegrass, and white clover (C) as monocultures and as adjacent monocultures of E+: E-, E+: C, and E-: C. There was no difference in overall time spent grazing between any treatment (all results shown in Table 2-2). As expected, sheep preferred E- to E+ when offered as adjacent monocultures. The authors had hypothesized that sheep in E+: C would have a greater partial preference for white clover than sheep in E-: C due to the presence of the alkaloids. However, there was no difference in the proportion of time spent grazing white clover between

treatments. It has been suggested that consumption of other forages that do not contain ergot alkaloids act to dilute the effects of the toxin (Mette Dahl Jensen and Roulund, 2004), and could explain why no difference between those treatments was observed by Cosgrove et al. (2002).

Predator avoidance. Anti-predation has been suggested as a possible explanation for the dietary selection of sheep and cattle. Domesticated animals are not under the same threat of predation as wild animals due to protective measures by humans (Price, 1984); however there are still situations where livestock are at a significant risk of predation such as in large open rangelands (Scrivner et al., 1985). Sheep and cattle are crepuscular, having their highest levels of foraging activity at dawn and dusk (Albright and Arave, 1997). This is the time at which they are least likely to be under attack by predators and thus concentrate their feeding time to the periods of the day when they are at the least risk (Lima and Bednekoff, 1999). Such diurnal patterns in foraging are not observed in animals living without predators as in the case of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) (Loe et al., 2007). These animals have not been under the threat of predation for more than 5,000 years. They do not have peaks in activity at dawn and dusk as is observed in other herbivores that have evolved under predation. Svalbard reindeer instead have seasonal variation in feeding and ruminating patterns relating to changes in temperature and precipitation (Loe et al., 2007). Domestic livestock are at the highest risk of predation at night and consequently they usually avoid grazing at night (Gluesing et al., 1980). This may contribute to the diurnal patterns of preference that have been reported when livestock are offered white clover and ryegrass in adjacent monoculture.

Diurnal patterns of intake. Cattle have been observed to reduce the amount of mastication when grazing later in the day (which increases bite rate and intake rate to maintain daily herbage intake) perhaps in anticipation of bedding down after dusk when they would have ample time to ruminate before dawn (Rutter et al., 2002), preventing a need to graze at night to maintain gut fill. Gibb et al., 1998) also showed that bite rate was highest at 1900 h (59.4 bites min⁻¹) and lowest at midday (47.5 bites min⁻¹) with a linear increase in DMI and organic matter intake over the course of the day. These mechanics of foraging likely go hand in hand with other diurnal feeding strategies. Herbivores under predation risk may avoid grazing at night by eating high fiber forages with slow passage rates later in the day. This can explain why animals prefer grasses over legumes in the evening (Parsons et al., 1994a; Harvey et al., 2000) because fiber content is higher in grasses compared to legumes. Dumont et al. (1995) observed this diurnal pattern for fiber intake in ewes (Limousin x Ile de France). The animals grazed orchardgrass (*Dactylis glomerata* L.) pastures with strips mowed to maintain the forage in a vegetative state while non-mowed strips were allowed to mature. Sheep showed a diurnal pattern, preferring reproductive swards (with higher crude fiber) to vegetative swards during late day meals.

Diurnal change in non-structural carbohydrates. Another possible contributor to the preference for grass in the afternoon is due to changes that occur in the plant itself. Non-structural carbohydrates (NSC) increase in grasses over the course of a day (Lechtenburg et al., 1972). The export of photosynthate is not at the same rate as carbon fixation during the photoperiod so NSC's accumulate (Fisher et al., 1999). Variation in NSC levels among different tall fescue cultivars has been reported. It was

concluded that those higher in NSC were more preferred by cattle (Hereford, Hereford x Angus) in preference trials (Shewmaker et al., 1997; Mayland et al., 2000). Sheep, goats, and cattle have been shown to prefer hay that was harvested in the afternoon (higher in NSC) to hay harvested in the morning (Fisher et al., 1999; Fisher et al., 2002). In those studies the average NSC for afternoon cut alfalfa hay was lower than afternoon cut tall fescue hay (5.6 vs. 9.5 % DM, respectively). Higher NSC levels in the diet are also beneficial to ruminants due to an increase in the efficiency of microbial protein synthesis in the rumen, more efficient N use for milk production, and a decrease in N excretion (Miller et al., 2001).

Which theory applies? While some of these explanations may seem more or less plausible than others, it is obvious that no one theory can fully characterize the decision making processes that animals are using. It is more probably that a combination of strategies is being used by the animal to meet its ultimate goal or to balance among several objectives (Rutter, 2006). There may be temporal factors an animal must face that might change which strategy it uses to meet those goals, such as changes in environment (heat, cold, excess precipitation, drought, etc.). It is important to recognize all these factors when evaluating diet selection in domestic ruminants.

Evaluation of animal behavior

Direct visual observation of animal behavior is one of the primary methods used to evaluate animal behavior. The majority of the papers reviewed thus far have utilized this method in some way. Visual scan sampling of all of focal animals within a group is usually conducted from an elevated platform, hidden from the view of the animals

(Harvey et al., 2000; Rook et al., 2002; Rutter et al., 2004a, b). Animal behavior (grazing, idling, etc.) and animal location (grass or clover) are recorded on set time intervals. In some experiments visual observations have been combined with the use of video cameras, data recorders, and global positioning systems (GPS). Parsons et al. (1994a) and Penning et al. (1997) set up video cameras on the dividing line between the grass and clover monocultures to record when an animal passed from one to the other. Video recordings are beneficial because they are a permanent record of behaviors and allow monitoring of several areas simultaneously through the use of security video systems. However analysis of these recordings can be time consuming and depending on the location of paddocks being observed, a considerable length of cables may need to be used to connect the cameras to the unit recording the video (Penning, 2004).

Behavior data recorders (Rutter et al., 1997b) developed by the Institute of Grassland and Environmental Research (IGER) have become a valuable tool in the analysis of grazing behavior. These data recorders are used to determine the number of jaw movement (prehensions and mastications) and temporal grazing patterns of animals on pasture (Gibb et al., 1998; Cosgrove et al., 2001; Rutter et al., 2004a). The device consists of a computerized data logger and halter mounted jaw movement sensor. The data logger was attached to the halter on the left side of the neck. The jaw sensors are worn as a noseband on the animal and are constructed of rubber tubing filled with graphite powder. Electrical current passes through the sensor and movement of the jaw results in a change in electrical resistance that when analyzed by the software is reported as a prehension, mastication, or other jaw movement dependent on

the size and shape of the resultant energy waveforms when translated graphically. Data are sent from this sensor directly to the data logger where they are recorded onto CompactFlash™ memory cards and later downloaded to a computer for analysis. Analysis is conducted with the Microsoft® Windows™ based software GRAZE (Rutter, 2000).

Monitoring animal movements with the use of GPS has been studied in a variety of settings (Bailey et al., 1989; Rutter et al., 1997a; Ganskopp, 2001; Kawamura et al., 2005; Ungar et al., 2005). In a recent experiment by Hessle et al. (2008), GPS were used along with behavior data recorders. By combining these two technologies they were able to follow animal movement over semi-natural grassland while at the same time recording the animals' grazing behavior. The moisture gradient of the pasture was mapped and by using GIS software ArcMap (ESRI, 2002) the animals' location within those gradients could be followed over the course of the day and combined with the animals' activity being logged by the behavior recorder to determine where animals spent their time grazing.

Estimation of intake

Ideal fecal markers should be completely recoverable in feces, provide an accurate quantitative measurement, be inert and not affect the animal or its diet, and should be similar physically in size and density to digesta contents (Kotb and Luckey, 1972). The n-alkanes of plant cuticular wax have been extensively investigated to be used as markers for intake. The primary technique used for the analysis was described by Mayes et al. (1986) and was recently updated by Dove and Mayes (2006).

The majority of the hydrocarbons present in plants are odd-numbered C chains ranging from C₂₁ to C₃₇ (Mayes and Dove, 2000). The most abundant of these are nonacosane (C₂₉), hentriacontane (C₃₁), and tritriacontane (C₃₃) (Mayes et al., 1986). There are differences between plant species in their concentration of these odd-chain n-alkanes. Because of these differences, it can be estimated in what proportions an animal consumed different species plants or plant parts (Dove, 1992). Fecal recovery of n-alkanes is incomplete due to absorption in the small intestine (Mayes et al., 1988). However if animals are dosed with an external marker of synthetic even-chained alkanes to estimate fecal output then intake can be better estimated (Mayes et al., 1986). Daily herbage intake can be calculated is as follows:

$$I = \frac{\frac{F_j}{F_i} * D_j}{H_i - \frac{F_j}{F_i} * H_j}$$

Where:

I = dry matter intake (kg d⁻¹)

i = odd chain naturally occurring n-alkane

j = dosed even chain external n-alkane marker

D_j = the weight of dosed j (mg d⁻¹)

F_j = the concentration of j in feces (mg kg⁻¹ DM)

F_i = the concentration of i in feces (mg kg⁻¹ DM)

H_i = the concentration of i in forage (mg kg⁻¹ DM)

H_j = the concentration of j in the forage (mg kg⁻¹ DM)

(Dove and Mayes, 1991)

Adjacent odd and even chained n-alkanes, for example C₃₂ which is dosed and C₃₃ from the herbage, should be used in this type of equation and if they have similar fecal recoveries. If recovery of C₃₃ was incomplete then C₃₁ could be substituted in the equation (Penning, 2004).

Controlled Release Devices (CRDs) containing C₃₂ and C₃₆ (Captec, NZ) have been shown to provide accurate intake estimation (Mayes et al., 1995). However, release rate of the CRDs can differ from that stated by the manufacturer and the recovery rate should be verified. This can be accomplished with the use of fecal collection bags attached to some of the animals in the experiment (Penning, 2004).

The modified technique of Dove and Mayes (2006) includes methodology for isolation of separate fractions of very long chain fatty acids (VLCFAs) and long chain alcohols (LCOHs). Bugalho et al. (2004) found that when using LCOH along with n-alkanes it allowed for improved distinction of individual plant species than when using only n-alkanes. Ali et al., (2005) compared n-alkanes, LCOHs, and VLCFAs in the diets of sheep. They suggest that using n-alkanes along with either LCOHs or VLCFAs could provide more accurate estimation of the composition of herbivore diets. The measurement of these additional compounds can be useful when animals are grazing mixed swards or paddocks of adjacent monocultures where animals will be consuming multiple species.

Table 2-1. Time spent eating white clover as a percentage of grazing time during daylight hours when offered ryegrass and white clover in adjacent monocultures. Adapted from Harvey et al. (1996).

Background diet	25% clover by area		75% clover by area		Mean
	LN-grass	HN-grass	LN-grass	HN-grass	
Low-N grass (LN)	74.2	73.9	62.2	71.3	70.4
High-N grass (HN)	72.3	47.0	77.8	89.3	71.6
Mean	66.8		75.1		71.0

Percent clover by area x grass N interaction $P < 0.01$

Percent clover by area x background diet interaction $P < 0.05$

Table 2-2. Time spent grazing (min d⁻¹) by sheep offered white clover (C), endophyte infected ryegrass (E+), and endophyte free ryegrass (E-), alone or in adjacent monocultures. Adapted from Cosgrove et al. (2002).

Period	Treatment	Time spent grazing on			Total
		C	E+	E-	
December	C	440			440
	E+		410		410
	E-			440	440
	E+: E-		150	300	450
	E+: C	240	170		410
	E-: C	280		140	420
	Comparisons		C1: P = 0.6 SEM = 12	C2: P = 0.3 SEM = 9	C3: P = 0.9 SEM = 42
February	C	370			370
	E+		410		410
	E-			390	390
	E+: E-		90	280	370
	E+: C	270	50		320
	E-: C	300		50	350
	Comparisons		C1: P = 0.6 SEM = 10	C2: P = 0.3 SEM = 5	C3: P = 0.9 SEM = 14

SEM and P values apply to comparisons as follows: C1 = E+: C vs. E-: C; C2 = E+: C vs. E-: C; C3 = all treatments

C4 = within treatment E+ vs. E-; December P = 0.6, SEM 122; February C4 = P0.01, SEM = 24

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CHAPTER 3

GRAZING BEHAVIOR AND DIET PREFERENCE OF BEEF STEERS GRAZING ADJACENT MONOCULTURES OF TALL FESCUE AND ALFALFA DIFFERING IN SPATIAL ALLOCATION

ABSTRACT

The use of adjacent monocultures of different forages has become a valuable tool to study the diet preference of grazing animals. Previous research using this technique with white clover and perennial ryegrass has consistently shown that sheep and cattle have a partial preference of around 70% white clover. It has been proposed that sheep and cattle will exhibit a partial preference for a legume over a grass regardless of forage species. Therefore the objective of the study was to determine if beef steers have a partial preference for alfalfa over tall fescue, two forages species that have not been evaluated previously as adjacent monocultures. Behavior data recorders, GPS tracking devices, and pedometers were used to evaluate grazing behavior and diet preference of beef steers grazing tall fescue monocultures or adjacent monocultures of tall fescue and alfalfa at proportions (by ground area) of 50:50, 25:75, and 75:25. Steers exhibited a partial preference for alfalfa of 61 to 65% when given a choice of grazing alfalfa or tall fescue as adjacent monocultures, regardless of the ground area proportion of the two forages offered. A diurnal pattern of preference was not observed. Steers grazing tall fescue monocultures spent more time ruminating ($P = 0.02$) and tended to graze less time ($P = 0.06$) than steers in adjacent monoculture treatments. Time spent idling, number of prehensions and mastications, and bite rate were similar ($P > 0.05$) among treatments. Steers grazing tall fescue monocultures

spent less time standing, more time lying, were less active and took fewer steps ($P \leq 0.05$) than steers in adjacent monoculture treatments. Steers did not exhibit a clear preference for where non-grazing activities were conducted and seemed to be influenced by the proportion of ground area of the forages. The results of this study support the proposal that regardless of forage species, a partial preference for legume will be expressed in beef steers. Further research to determine the factors that drive this preference is needed.

INTRODUCTION

Heterogeneity of grasslands results in animals having to choose what and when to graze among different forages. When animals must search for preferred forage within a mixed sward the animal's ability to eat its desired food becomes constrained. Within this constraint, diet preference of an animal cannot be evaluated. However, this constraint can be minimized when forages are offered as monocultures in adjacent areas (Parsons et al., 1994). This technique has become a valuable tool in research to evaluate dietary choice of livestock. Studies using adjacent monocultures as a grass and a legume have consistently reported that sheep and cattle consume a mixed diet and have a partial preference for legume of $70 \pm 10\%$ (Rutter, 2006).

The number of forage species that have been evaluated as adjacent monocultures in grazing behavior experiments is limited. Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) have been used in most studies as they are the predominant forages used in Western Europe for intensive livestock production systems (Gibb, 2006). An additional legume, Sulla (*Hedysarum coronarium*

L.), has been evaluated and was shown to be partially preferred (74%) over annual ryegrass (*Lolium multiflorum* L.) by ewes (Rutter et al., 2005b). Evaluation of birdfoot trefoil (*Lotus corniculatus* L.) and perennial ryegrass as adjacent monocultures has been conducted and that legume was partially preferred (70%) by dairy heifers (Torres-Rodriguez et al., 1997). It has been suggested that the observed partial preference for a legume over a grass is not unique to only these few forages species and that this preference would be observed regardless of forage species (Rutter, 2006). Grazing behavior and diet preference of beef steers grazing adjacent monocultures of tall fescue (*Festuca arundinacea* Schreb. or *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and alfalfa (*Medicago sativa* subsp. *sativa* L.) has not been reported previously. The present experiment was designed to expand the number of forages species that has been evaluated in this manner and to evaluate forages that are commonly used for grazing livestock in the United States.

Research on diet preference of domestic ruminants grazing forages that have not been evaluated as adjacent monocultures should be conducted in a manner that can differentiate between active selection of animals and indifference (grazing at random). In order to achieve this, multiple different proportions of ground area of the two forages should be evaluated (Parsons et al., 1994). For instance, if only a 50:50 proportion of tall fescue and alfalfa were evaluated and the animals selected a diet of 50% tall fescue and 50% alfalfa then it would not be possible to determine the basis of this result. It could be due to a preference for a diet composed of 50% grass and 50% legume or that the animal encountered that proportion while grazing at random. To eliminate this uncertainty, additional proportions such as 25:75 and 75:25 should be evaluated. If

animals grazing in these additional treatment areas also selected a diet of 50% grass and 50% legume this would indicate active selection. If the proportion of the diet selected by the animal matched the proportion offered to them this would imply they are grazing at random and not being selective.

The objectives of the present experiment were threefold. First, was to evaluate beef steers' pattern of preference for two forages that have not been previously studied as adjacent monocultures. Second, to determine if beef steers offered adjacent monocultures of tall fescue and alfalfa in three different area ratios were grazing randomly or actively selecting what to graze. . Third, to compare grazing behavior of steers grazing tall fescue monocultures to those grazing tall fescue: alfalfa adjacent monocultures.

MATERIALS AND METHODS

Pasture establishment and management

Pastures were established in 2004. All paddocks were sprayed with Roundup[®] (9 L ha⁻¹, glyphosate 41%, Monsanto, St. Louis, MO) in May to eliminate existing pasture species. Two weeks after spraying, foxtail millet (*Setaria italica*, 34 kg ha⁻¹) was planted as a suppression crop. In late July, the millet was harvested for hay and two weeks later the paddocks were sprayed again with Roundup[®] (2 L ha⁻¹) for weed suppression. Tall fescue pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0) and the alfalfa pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0) + 112 kg ha⁻¹ of potash (0-0-60). On September 2 and 5, paddocks were planted with Jesup endophyte-free tall fescue (28 kg ha⁻¹) or with AmeriStand 403T alfalfa (22 kg ha⁻¹).

Nitrogen was applied to the tall fescue paddocks at 34 kg ha⁻¹ in October and then again in March 2005 to promote vigorous growth and tillering. Herbicides were applied in April 2005. All alfalfa pastures received 37 mL ha⁻¹ of Harmony GT[®] (thifensulfuron-methyl 75%, Dupont, Wilmington, DE) and 2 L ha⁻¹ Poast Plus[®] (sethoxydim 13%, BASF, Research Triangle Park, NC) and 1 L ha⁻¹ of surfactant. All tall fescue pastures received 1 L ha⁻¹ of 2-4D (2,4-Dichlorophenoxyacetic acid 46.6%, Helena Holding Co., Wilmington, DE) and 0.6 L ha⁻¹ Banvel[®] (3,6-dichloro-0-anisic acid 48.2%, Micro Flo, Memphis, TN).

Pastures were grazed by stocker steers during the summer grazing seasons of 2005 and 2006. Pastures were fertilized in April, 2006 and 2007 with tall fescue receiving 35-20-45 (224 kg ha⁻¹) and alfalfa receiving 0-46-0 (145 kg ha⁻¹) and Boron (2 kg ha⁻¹). Alfalfa paddocks were sprayed with Harmony GT[®] in April 2007 for control of thistle and with Baythroid[®] (β -cyfluthrin 12.7%, Bayer, Research Triangle Park, NC) for insect control in July 2007 according to manufacturer recommendations.

Treatments and animal management

The procedures used in this experiment were approved by the Virginia Tech Institutional Animal Care and Use Committee. This experiment was conducted at Kentland Farm (37°11' N, 80°35' W), Blacksburg, Virginia, during July and August 2007. A 4 x 4 Latin Square design was used to evaluate behavior of beef steers in treatments that varied by ground area ratio of endophyte-free tall fescue to alfalfa. Treatments were 25:75 (25F75A), 50:50 (50F50A), and 75:25 (75F25A) of tall fescue to alfalfa, respectively, and 100% tall fescue (control). Existing monoculture paddocks of tall

fescue and alfalfa were sub-divided with electrified polytape to create the appropriate treatment ground area ratios. Paddock perimeters were made of electrified 3-strand high tensile smooth wire. Total ground area for each treatment during a period was 0.7 ha. Different paddocks with new growth were sub-divided and used in each subsequent period. Each experimental period was four days with a minimum of four days between periods.

Twelve Angus-crossbred steers (initial BW = 574 ± 14 kg, 28 mo old) were used in the experiment. Steers had previous experience grazing tall fescue and alfalfa as adjacent monocultures. Steers were allotted by initial BW to blocks with three steers per paddock. Each group was then randomly allotted to a treatment for the first period. Steers grazed areas of 50:50 tall fescue and alfalfa for 2 wk prior to the experiment and between experimental periods. Within a treatment area, paddocks of tall fescue and alfalfa were connected by a lane made of electrified polytape through which the steers could move freely from tall fescue to alfalfa. Water troughs and trace mineral salt with poloxalene as an anti-bloat agent (Bloat Guard[®] Pressed Block, Sweetlix Livestock Supplement System, Mankato, MN) were located at the midpoint of the lane. Steers were placed into treatment paddocks by this lane between 1700 and 1800 on d 1 of each period.

Behavioral sampling

Each steer was fitted with a Global Positioning System (GPS) tracking device (Foretrex[®] 101, Garmin International Inc., Olathe, KS) placed inside a transparent waterproof box (Model 2000, Otterbox, Fort Collins, CO) attached to the crown piece of

a nylon halter (Model C14077N, Nasco, Fort Atkinson, WI). The GPS devices recorded steer position every 30 s as a data point of latitude, longitude, and altitude along with the date and time that the position was recorded. Data points from the GPS devices were retrieved using GPS Utility© software (version 4.54, GPS Utility Ltd., UK). Maps of the experimental area were created by designating waypoints around the pasture perimeters with the GPS device. Water and mineral feeders were also identified as additional waypoints. These waypoint data were downloaded from the GPS device to a computer where they were analyzed and each area of tall fescue and alfalfa identified using ArcGIS™ software (version 9, ESRI, Redlands, CA). The spatial join function of the software was used to merge the data points of animal location collected by each GPS device and the paddock maps (Hessle et al., 2008). This created a data file in which each reading of the GPS device is individually identified with the steer's location during the experimental periods. Each GPS data point was assigned a location of "tall fescue", "alfalfa", or "between". The designation "between" indicated time that steers were in the area between the forages where water and mineral blocks were located.

Each steer wore an animal activity monitor (IceTag™, version 2.004, IceRobotics, Midlothian, Scotland, UK) attached to a Velcro® strap on the left rear leg just above the metatarsophalangeal joint. These units measured animal activity 8 times s⁻¹ with an internal accelerometer. Percentage of time spent standing, active, lying, and numbers of steps taken by the steer were recorded. A steer was recorded as being "active" when the steer had leg movement. Data were downloaded from on-board

memory to a personal computer and analyzed by IceTagAnalyser™ software (version 2.009, IceRobotics).

Steers also wore IGER Behavior Data Recorders (Rutter et al., 1997). The device consists of a data logger and jaw sensor. The jaw sensor was a carbon-packed tube serving as an electrical transducer converting movements of the jaw to electronic signals. The data from the sensor were recorded on a Compact Flash memory card in the data logger and analyzed by GRAZE software (version 0.801, Rutter, 2000). Activities reported were time spent grazing, ruminating, and idling (defined as not grazing or ruminating), as well as number of prehensions. Grazing time was defined as time consuming forage with eating bouts joined when the inter-bout interval was less than 420 s. Ruminating time was defined as time performing ruminating mastications with periods joined when the interval was less than 20 s. Idling time was defined as periods when the steer was not identified as grazing or ruminating. All steers were trained to wear these devices prior to the experiment. Training began 2 wk prior to the experiment by fitting a nonfunctional version of the device on each steer for a period of 4 h on first day of training, increasing the length of time by 4 to 8 h each day until steers were acclimated. Training versions were of similar size and weight and fitted to the same halter steers would wear during the trial. Behavior measurements from the first 48 h of each period were analyzed.

Data collected from the devices described above were combined and analyzed by matching the time stamps of the recorded data from the devices using the Excel® (Microsoft®, Redmond, WA) VLOOKUP function. For example, if the GPS data points collected from 08:00:00 to 08:30:59 indicated that a steer was located in the alfalfa

paddock and the behavior data recorder indicated that from 08:00:00 to 08:15:59 the steer was grazing and from 08:16:00 to 08:30:59 the steer was idling. The combined data will report the steer's location and behavior as 15 min grazing in alfalfa and 15 min idling in alfalfa for the respective times of day.

Data were categorized into periods of morning and afternoon to determine the diurnal pattern of grazing during the experiment. Average sunrise occurred at 0632 and sunset at 2016 during the study period. Cattle are crepuscular animals with peak grazing times occurring at dawn and dusk (Phillips, 1993) which is around 30 min before sunrise and 30 min after sunset. To ensure peak grazing times were included, the periods were allocated as follows: AM = 0530 to 1329 and PM = 1330 to 2129. On d 1 the PM period was 1800 to 2129.

Forage sampling and analyses

Hand-plucked forage samples were taken for nutritive value analysis on the day prior to and the day after each experimental period. Samples were collected while walking along a cross-section of the paddock and grabbing a sample every 10 steps from the top 7 to 10 cm of the sward, representing forage being consumed by the steers. Separate samples from each paddock of each forage type were collected between 0730 and 0830 (AM) and between 1700 and 1800 (PM). Forage samples were collected from AM and PM hours to evaluate diurnal changes in carbohydrate concentrations which have been shown to influence diet selection (Ciavarella et al., 2000; Mayland et al., 2000b). Samples were packed on ice directly after collection, placed in a freezer, and later freeze-dried (25L Genesis SQ EL-85, VirTis, Gardiner,

NY). Samples were then ground through a 1-mm screen using a Wiley mill, and analyzed for chemical composition by NIRS (Foss NIRSystems 6500, Foss Tecator, Eden Prairie, MN; AOAC, 2000) at a commercial laboratory (DairyOne, Ithaca, NY). Tall fescue subsamples were further ground to 0.5mm (Cyclotec 1093 Sample Mill, Foss Tecator, Eden Prairie, MN) and analyzed for alkaloid concentration (Agrinostics Ltd. Co., Watkinsville, GA) to ensure they were at low alkaloid levels. Mean alkaloid concentration was 123 ng g⁻¹ (SEM = 23).

Forage mass and sward height measurements were taken on the day prior to and the day after each experimental period. Three areas of 0.25 m² were randomly selected from each paddock of tall fescue and alfalfa and 10 measurements of sward height were taken within those areas at each sampling. The three areas were then clipped to 2.5 cm above ground level, forage placed in cloth bags, and dried in a forced draft oven at 60 °C for 48 h to determine macro DM (%) and forage mass. Forage mass samples were separated into leaf, stem, or dead material and each proportion weighed.

Statistical analyses

Data were analyzed using SAS (version 9.1.3, Cary, NC, USA). The experimental unit for all analyses was the paddock (3 steers) because individuals within a group are not statistically independent (Rook and Penning, 1991). Behavior recorder output and GPS output were combined to obtain the proportion of time spent grazing in alfalfa and tall fescue (time spent grazing alfalfa or tall fescue as a percentage of total time spent grazing). Angular transformation of proportions was conducted to stabilize variance (Parsons et al., 1994). The angular transformed percentage of total grazing

time on alfalfa was used to determine preference for that forage. The TTEST procedure was used to determine if the proportion of time spent grazing alfalfa was significantly different from the proportion of ground area of that forage they were offered. Ruminating and idling time spent on alfalfa were also analyzed to determine if the proportion of forage offered impacted where the steers conducted non-grazing activities. Additional t-tests were conducted to determine if any preference for alfalfa that was observed was absolute or partial by testing for significant difference from 0 or 100% (Parsons et al., 1994; Rutter et al., 2004a, b). Diurnal pattern of preference was analyzed using the MIXED procedure with the model including treatment, time of day (AM or PM), day, and the three-way interaction of these variables.

Behavior data were analyzed using the MIXED procedure and the compound symmetry (cs) covariance structure. The repeated measure was day for all variables. The model for overall behavior recorder output (grazing, ruminating, idling, number of prehensions and mastications, and bite rate) and pedometer recorder data (standing, lying, activity, number of steps) included treatment, day, and treatment x day interaction. A contrast was used to evaluate differences between the control treatment (100% tall fescue) and adjacent monoculture treatments (25F75A, 50F50A, 75F25A).

Forage nutritive value and sward measurement data were analyzed using the MIXED procedure. The model for each forage type included treatment, sampling date (pre or post grazing), and their interaction. Alfalfa and tall fescue nutritive value was compared using forage type, sampling period, and their interaction in the model. The model for nutritive value parameters which have diurnal changes (i.e. carbohydrates) included forage type and sampling time of day (AM or PM) as a main effects and main

effect interactions. The repeated measure was period and the experimental unit was the paddock within period. The paddock selected for use in the period was considered a random effect.

Least squares means are reported for all variables with means separated by Tukey's adjustment. A significance level of $\alpha \leq 0.05$ was set for all analyses with trends defined as $0.10 \geq \alpha > 0.05$.

RESULTS AND DISCUSSION

Forage and sward measurements

There was no effect of treatment ($P \geq 0.58$) or treatment x sampling period interaction ($P \geq 0.31$) for NDF, ADF or lignin concentration of alfalfa. Similarly, for tall fescue no effect of treatment ($P \geq 0.45$) or treatment x sampling period interaction ($P \geq 0.31$) for NDF, or lignin concentration was observed. However, there tended to be a treatment effect ($P = 0.09$, SEM = 0.4) with ADF concentration of tall fescue in the 25F75A treatment (31.3 %) being lower than the 50F50A treatment (32.6 %). The concentration of NDF, ADF, and lignin increased (Table 3-1) from the pre to post grazing sampling period in both alfalfa ($P \leq 0.01$) and tall fescue ($P \leq 0.002$). There was no effect of treatment ($P = 0.49$ and 0.27), sampling period ($P = 0.27$ and 0.17), or treatment x sampling period interaction ($P = 0.69$ and 0.80) for CP concentration of alfalfa and tall fescue, respectively. The lack of differences indicated that regardless of the proportion of tall fescue or alfalfa offered, the nutritive value of each forage was similar among treatments.

Alfalfa usually has superior nutritive value to tall fescue and this was observed when data were analyzed with forage type in the model (Table 3-1). There was an effect of forage and period ($P \leq 0.0001$) for NDF, ADF and lignin concentrations. Neutral detergent fiber tended ($P = 0.08$) to have a forage x period interaction, while this interaction was observed in ADF ($P = 0.006$), but not in lignin ($P = 0.18$). As expected, alfalfa had less NDF and ADF with more lignin than tall fescue. Both forages generally decreased in nutritive value over time however NDF was the only fraction of tall fescue to decrease. Alfalfa was more heavily grazed than fescue as indicated by the greater decrease in herbage mass from the pre to post-grazing measurements (Table 3-2) which is may be why ADF and lignin also decreased. Crude protein differed between forage type ($P < 0.0001$) and only tended ($P = 0.09$) to differ by period.

There was no difference between treatments for overall herbage mass (Table 3-2) of tall fescue ($P = 0.97$) and alfalfa ($P = 0.53$). There were period differences for both forages ($P < 0.0001$, and $P = 0.02$ for alfalfa and tall fescue, respectively) as would be expected with post-grazing mass being lower than pre-grazing. No treatment x period interactions were observed (alfalfa: $P = 0.14$, tall fescue: $P = 0.16$). Other sward characteristics are also reported in Table 3-2.

Diet preference

Steers showed a partial preference for alfalfa in all adjacent monoculture treatments (Table 3-3). Steers grazing in the 25F75A treatment spent 64.8% of their total grazing time on alfalfa. This was different ($P = 0.012$) from the proportion of 75% alfalfa offered to them, was more ($P = 0.001$) than 0% and less ($P = 0.001$) than 100%.

Steers grazing in the 50F50A treatment spent 61.1% of their total grazing time on alfalfa. This was not different ($P = 0.555$) from the proportion of 50% alfalfa offered to them, was more ($P = 0.0002$) than 0%, and less ($P = 0.0002$) than 100%. Steers grazing in the 75F25A treatment spent 60.6% of their total grazing time on alfalfa. This was different ($P = 0.003$) from the proportion of 25% alfalfa offered to them, was more ($P = 0.0004$) than 0%, and was less ($P = 0.001$) than 100%. In all three adjacent monoculture treatments the proportion of grazing time in alfalfa was different than 0 and 100%, indicating that their grazing preference was partial and not absolute. These results support the idea that regardless of the forage species, a partial preference for legume will be exhibited.

A summary of results from adjacent monoculture studies conducted over the years evaluating 50:50 adjacent monocultures is presented in Table 3-4. Partial preference for legumes has been reported for sheep ranging between 60 and 91.8% and for cattle between 60 and 78%. From the animal classes reported, beef heifers are the ones that can be compared most closely to steers used in this study. The results were similar to those reported here with a 60 % preference for legume.

If considering the present results alone as estimates of total DMI of the two forages, it should be noted that proportion of time spent grazing alfalfa and tall fescue may not always be equivalent to an estimate of DMI. It has been previously observed that there may be differences in intake rates (g DM min^{-1}) of grass compared to legume. Cattle have been shown to have higher intake rates on white clover than ryegrass (Marotti, 2004; Rutter et al., 2004a). If the same pattern were true for alfalfa and tall fescue and total time spent grazing in each forage type were used to estimate DMI then

this method would underestimate DMI of alfalfa and overestimate DMI of tall fescue. Whereas some have reported no difference between proportion of time grazing a forage and proportion of intake from a forage even though intake rates differed (Parsons et al., 1994), it is generally suggested that grazing time alone should not be considered an absolute estimate of DMI (Rutter, 2006). Intake rates can be measured by use of short-term change in BW (Penning and Hooper, 1985) or with the use of esophageally fistulated animals (Forbes, 1988). Intake rates should be measured at several times over the course of a day due to potential diurnal variation in intake rate (Gibb et al., 1998). The intake rates calculated from animals grazing monocultures of the forages under evaluation can then be used to calculate DMI using daily grazing time of each forage. Another method is to estimate DMI through the use of markers such as n-alkanes and perform a total fecal collection through the use of a collection harness and bags. A subsequent chapter will evaluate DMI of beef steers grazing adjacent monocultures of tall fescue and alfalfa using this method.

Diurnal pattern of preference

There was no difference between treatments ($P = 0.29$), periods (AM vs. PM, $P = 0.29$), or days ($P = 0.17$) in the proportion of total grazing time spent grazing alfalfa. Mean percentage of grazing time spent grazing on alfalfa in the morning period was 62.3 % compared with 71.9 % in the afternoon (SEM = 3.8). This differs from previous research which has observed a diurnal pattern of preference in both cattle and sheep with a decreasing preference for legume from morning to afternoon (Parsons et al., 1994; Rutter et al., 2004a, b). Diurnal pattern was less pronounced in dry (non-pregnant) heifers (Rutter et al., 2004b) which would be the most similar of previous

studies to the cattle in the present study in terms of stage of growth (although different gender). Steers in the present study were maintained on adjacent monoculture paddocks prior to the start of the study and thus the results are not likely due to previous diet, which has been shown previously to influence diet selection (Parsons et al., 1994). Harvey et al. (2000) observed that the diurnal change in diet selection of sheep was not due to decrease in the proportion of clover grazed (which was relatively constant over the day) but was due to an increase in the proportion of grazing time on ryegrass. In the present study there was no difference between treatments ($P = 0.27$), periods (AM vs. PM, $P = 0.57$), or days ($P = 0.22$) in the proportion of time spent grazing tall fescue. Mean percentage of time grazing fescue in the morning period was 28.2 % compared with 23.3 % in the afternoon (SEM = 3.8).

Previously observed increases in preference for grass in the afternoon have been attributed to a higher level of crude fiber in grasses compared to legumes (Rutter, 2006). Grazing fiber-rich forages with low passage rates in the afternoon helps maintain gut fill and decrease the need to graze at night (Rutter, 2006) when animals would be more at risk to predation (Gluesing et al., 1980). This theory does not explain results shown here because tall fescue was not the preferred forage in the afternoon even though it was higher in NDF and ADF than alfalfa (Table 3-1).

Diurnal patterns of preference in animals have also been attributed to changes in forage carbohydrate concentration over the day. Fisher et al. (1999) and Mayland et al. (2000b) reported that tall fescue hay cut in the afternoon, which was higher in total non-structural carbohydrates (TNC), was preferred over hay cut in the morning. Similarly, afternoon cut alfalfa hay which was higher in TNC was preferred over morning cut hay

(Fisher et al., 2002). Ciavarella et al. (2000) reported that sheep grazing shaded and unshaded areas of *Phalaris aquatica* L. selected 2.6 times more forage in the unshaded area which was higher in both water soluble carbohydrates (WSC) and starch. They could not determine if any specific individual component of WSC was responsible for preference. Water soluble carbohydrates did not differ ($P \geq 0.23$) between forages in either the morning or the afternoon in the present study (Table 3-5) so it would not have been a factor in influencing preference in this case. Non-fiber carbohydrate (NFC) and starch concentrations in the present study were higher ($P < 0.0001$) in alfalfa than tall fescue at both the AM and PM sampling time (Table 3-5). Simple sugars, starch, fructans, soluble fiber, and organic acids comprise NFC, while TNC does not include organic acids or soluble fibers such as pectins (Hall, 2007). Mayland et al. (2000a) reported that organic acid concentration was not related to diet preference of cattle among eight tall fescue cultivars.

A theory of why domestic ruminants choose a mixed diet is that they are maintaining a balance of C and N, which are needed for energy, and microbial protein synthesis (Rutter, 2006). Synthesis of microbial protein from rumen degradable protein (RDP) is critical to ruminants and its concentration in feeds or forages may influence diet selection (Kyriazakis et al., 1999). Dairy cows have been shown to select diets to maintain level of RDP rather than metabolizable protein (Tolkamp et al., 1998). Bacterial efficiency (g of bacterial N kg⁻¹ of DM digested) in continuous cultures increases as TNC: RDP ratio decreases, with maximum fermentation (bacterial efficiency, nutrient digestion, and total VFA production) reached when TNC was $\geq 37\%$ (Stokes et al., 1991). Hoover and Stokes (1991) suggested that RDP may need to be as high as 15%

of DM for optimal microbial growth. Alfalfa in the present study was 18.4% (SEM = 0.5) RDP and tall fescue was 9.5 % (SEM = 0.3). If intake rates did not differ between forages, the steers in this study would have had 15% RDP in a diet of 62% alfalfa.

Ruminant diets high in soluble fiber have also been shown to improve N utilization efficiency when TDN: CP is less than 5 (Mount et al., 2001). Ratio of TDN: CP in the present study was 2.5 (SEM = 0.05) for alfalfa and 4.2 (SEM = 0.12) for tall fescue. Soluble fiber of alfalfa may be three times higher in alfalfa than in tall fescue (Elizalde et al., 1999) and can lead to higher efficiency of utilization of metabolizable energy (Tyrrell et al., 1992). Experiments evaluating the various forms of C and N discussed here have mostly been conducted using continuous culture, or housed animals being fed concentrates or silage. Future research on this topic under grazing conditions needs to occur to help determine what factors are driving diet selection.

In the present study, steers were introduced to their treatment paddocks in the afternoon at which point data collection began, whereas in previous studies the first reported observation were in the morning hours (Parsons et al. (1994) at 1000, Harvey et al. (2000) at 1030, Rutter et al. (2004a) at 0800, and Rutter et al. (2004b) at 0930). The timing of herbage allocation to cattle has been shown to influence grazing behavior primarily by altering time of day at which major grazing events occur (Gregorini et al., 2008). The effect of time of day of new allocations of pasture on diet selection in adjacent monocultures systems has not been reported. Such research would be beneficial to determine if there is an influence on the diurnal patterns of preference in adjacent monocultures if new forage is offered at morning, mid-day, or afternoon.

Grazing, ruminating, and idling behavior

Behavior data collected from the behavior data recorders is reported in Table 3-6. There was no effect of day ($P = 0.31$), treatment ($P = 0.22$), or a treatment x day interaction observed ($P = 0.67$) for the overall daily amount of time spent grazing. However, steers grazing the 100% tall fescue paddocks had a tendency to graze less time ($P = 0.06$, 2 h d^{-1} less) than steers in the adjacent monoculture treatments. This difference is not surprising due to the greater fiber content of tall fescue (Table 3-1). Similarly, Seman et al. (1999) reported that Angus steers grazing tall fescue monocultures grazed 1.4 h d^{-1} less than steers grazing binary mixtures of tall fescue and alfalfa which was lower in NDF. Marotti (2004) evaluated grazing behavior of lactating dairy cattle on white clover or ryegrass monocultures, a binary mixture of the two, or the two forages as 50:50 adjacent monocultures. During daytime observations, cows in the ryegrass monoculture spent the greatest amount of time grazing (496 min d^{-1}) and those in clover monocultures the least (385 min d^{-1}), while grazing time in the mixture (464 min d^{-1}) and adjacent monoculture (420 min d^{-1}) treatments were intermediate. Here again, cattle spent more time grazing in grass alone than in adjacent monocultures of grass and legume.

An effect of day ($P = 0.0003$) was observed for daily time spent ruminating. Steers spent less time ruminating on d 1 (412 min) than d 2 (524 min). This may be the result of being moved from an area they had been grazing for 4 d to a new paddock with regrowth on d 1. Ruminating time has been shown to increase over time in grass paddocks as leaves are removed from the sward and the proportion of less desirable, more fibrous material increases in the sward (Orr et al., 2004). The increase in fiber

from pre to post-grazing for both tall fescue and alfalfa is (Table 3-1) indicating that sward conditions likely promoted the observed increase in ruminating time from d 1 to 2.

An effect of treatment ($P = 0.01$) was observed for daily time spent ruminating. Steers in the 25F75A treatment ruminated less overall than steers grazing 100% tall fescue or the 50F50A treatment. This can be attributed to the 25F75A steers having a lower ($P = 0.02$) ruminating time on d 1 (318 min). Seman et al. (1999) reported that over time, steers grazing binary mixtures of tall fescue and alfalfa had increasing levels of NDF in their diet as quality of the sward declined during the study. In that study the percentage of alfalfa leaf selected by steers decreased from 70 to 50% after 5 d of grazing which led to an increase in ruminating time on subsequent days.

Steers grazing 100% tall fescue spent more time ($P = 0.02$) ruminating than the adjacent monoculture treatments. These results are very similar to previous study of lactating cows which spent 546 min d^{-1} ruminating on a ryegrass monoculture compared to cows on adjacent monocultures of clover and ryegrass that spent 412 min d^{-1} ruminating (Marotti, 2004). Greater ruminating time was attributed to higher NDF and ADF in ryegrass compared with clover, similar to in the present study. There was no interaction of treatment x day observed ($P = 0.22$) for time spent ruminating.

There was no effect of day ($P = 0.48$), treatment ($P = 0.41$), or treatment x day interaction ($P = 0.98$) observed in the number of prehensions per day. There tended to be an effect of day ($P = 0.06$) on the number of mastication with fewer on d 1 (32,842) than d 2 (38,767). This is probably due to the lesser time spent ruminating on d 1 than d 2 discussed above. No effect of treatment ($P = 0.49$), or treatment x day interaction ($P =$

0.40) was observed in the number of mastications per day. Contrast showed no difference between the tall fescue monoculture and the adjacent monoculture treatments in number of prehensions ($P = 0.34$), mastications ($P = 0.59$), or bite rate ($P = 0.88$). Marotti (2004) reported that cows grazing white clover or adjacent monocultures of ryegrass and white clover took around 8,000 fewer mastications per day compared with those grazing ryegrass monocultures or a mixed sward of 20 % white clover. This was due to less mastications needed for diets with a large proportion of clover. Legumes like clover and alfalfa require less mastications than grasses due to their lower NDF and ADF concentrations (John et al., 1988; Malbert and Baumont, 1989). In the present study, the tall sward height and stemmy nature of the alfalfa may have lead to a greater number of mastications needed to consume that forage, and may be an explanation for why no treatment effect on mastication rate was observed. Rumination has also been shown to increase as leaf: stem ratio decreases (Orr et al., 2004).

There tended to be a day effect on bite rate ($P = 0.06$) with a greater rate on d 2 (63 bites min^{-1}) compared with d 1 (56 bites min^{-1}). As sward height and herbage mass decline, an increase in biting rate and time spent grazing usually occurs (Hodgson, 1985; Jamieson and Hodgson, 1979). Steers in the 75F25A treatment had a numerically greater bite rate (11% more), more prehensions (19% more), and averaged 45 min d^{-1} more time grazing compared with the other adjacent monoculture treatments. The large decrease in sward height and herbage mass between pre and post-grazing measurements of alfalfa in the 75F25A treatment paddocks as compared with other tall fescue: alfalfa treatments (Table 3-2) may explain this difference in grazing behavior.

The difference in these sward characteristics is likely due to a more rapid decrease in sward height and herbage mass of alfalfa in the 75F25A treatment paddocks because there was a smaller proportion of that forage available to the steers, whereas it was the forage they preferred to graze. When the amount of leaf mass present declines, cattle have been shown to take more and smaller bites (Chacon and Stobbs, 1976). Steers in the 75F25A treatment took numerically more steps d^{-1} (Table 3-7) as well. Increasing number of steps taken by steers has been correlated with decreases in herbage mass (Boland et al., 2007). Sheep have been reported to do more work (increased grazing time) in order to maintain the preferred proportion of white clover in their diet despite a greater decrease in herbage mass of white clover compared to ryegrass in an adjacent monoculture pasture (Rook et al., 2002). Sheep have also been shown to spend more time walking in search of alfalfa when moved to paddocks that only contain small percentage of that forage in a mixed sward (Gluesing and Balph, 1980).

Time spent idling was not affected by day ($P = 0.23$), treatment ($P = 0.38$) or treatment x day interaction ($P = 0.64$). Contrast revealed no difference ($P = 0.33$) between the tall fescue monoculture and the adjacent monoculture treatments.

Land use of steers for non-grazing activities

Analyses were also conducted to evaluate land use of the steers when they were not grazing. This was done to indicate if the land use (time devoted to an actively in a particular area) differs as the proportion of ground area of the forages offered changes. The proportion of time spent ruminating and idling in the alfalfa swards are presented in Tables 3-8 and 3-9, respectively. Steers grazing in the 50F50A and 75F25A treatment

spent their time ruminating in alfalfa at similar proportions ($P = 0.240$ and 0.196 , respectively) to the proportion of that area offered to them. Steers grazing in the 25F75A treatment ruminated in alfalfa at a proportion of time less than ($P = 0.006$) the proportion of area offered. In all three adjacent monoculture treatments the proportion of ruminating time in alfalfa was different than 0 and 100%, showing that steers did not have an absolute preference of ruminating location. These results seem to indicate that there was no clear preference exhibited for where they spent time ruminating and it was most likely a random occurrence that was somewhat influenced by the proportion of each forage they were offered.

Steers grazing in the 50F50A and 75F25A treatment spent their time idling in alfalfa at similar proportions ($P = 0.507$ and 0.725 , respectively) to the proportion of that area offered to them (Table 3-9). Steers grazing in the 25F75A treatment spent less ($P = 0.049$) time idling in alfalfa compared to the proportion of area offered. The proportion of time spent idling in alfalfa in all three adjacent monoculture treatments was different than 0 and 100%, indicating that there was no absolute preference of idling location. In an experiment evaluating ewes grazing adjacent monocultures containing 20, 50, or 80% white clover (with the remaining proportion in ryegrass), there was an effect of the proportion of clover offered on where the animals spent their time idling (Parsons et al., 1994). In that study the ewes spent less of their time idling on clover when less clover was offered.

Pedometer data

There was an effect of day ($P < 0.0001$) on the proportion of the day spent standing, lying, or active. On d 1 steers were more active (5.6 vs. 4.4%, SEM = 0.2), spent more time standing (53.0 vs. 48.0%, SEM = 1.6) and less time lying (40.7 vs. 46.8%, SEM = 1.7) than on d 2, respectively. However, the number of total steps d^{-1} was not affected by day (d 1 = 2549, d 2 = 2601, SEM = 114; $P = 0.61$). These pedometers recorded activity through the use of an internal accelerometer which is triggered by the force of movement. If total number of steps was not greater on d 1 then the greater activity measure may indicate that there was more force behind each step, perhaps indicating a greater intensity in the steers movement while grazing that would likely be stimulated by the introduction to a new paddock with a re-growth of forage. Stuth et al. (1987) reported that grazing is most intense on the first and second days after being rotated into a new paddock compared with the following days.

Treatment means for behavior data collected from the IceTag pedometers are reported in Table 3-7. The proportion of the day spent standing ($P = 0.21$) or lying ($P = 0.15$) was not different among the forage treatments and no treatment x day interactions were observed for either activity ($P = 0.33$ and 0.49 , standing and lying, respectively). However, contrast of treatments reveal that steers in the tall fescue monoculture spent less time standing ($P = 0.05$) and more time lying ($P = 0.03$) than steers in the adjacent monoculture treatments. Ruminants commonly synchronize time spent lying with rumination (Peterson and Woolfolk, 1955; Rushen et al., 2007). Champion et al. (2004) reported that ewes grazing ryegrass only had more lying bouts due to the need to ruminate more frequently than ewe grazing adjacent monocultures of ryegrass and

white clover. Similarly, in the present study steers grazing tall fescue spent more time lying and ruminating.

There was an effect of treatment ($P = 0.05$) on the activity level of the steers. Steers grazing in the 100% fescue treatment were less ($P = 0.05$) active than steers in the 75F25A treatment and tended ($P = 0.10$) to be less active than those in the 50F50A treatment. The contrast of treatments also show that steers in 100% tall fescue spent less time active ($P = 0.01$) than those in adjacent monoculture treatments. This difference in time spent active was likely a result of 10 % more time spent lying by steers in the tall fescue only treatment. No treatment x day interaction for activity level was observed ($P = 0.89$). There was no effect of treatment ($P = 0.13$) for the number of steps d^{-1} . Contrast of treatments indicated steers in 100% tall fescue took fewer steps ($P = 0.03$) than those in adjacent monoculture treatments. This is probably due to the lesser proportion of the day those steers spent active. Champion et al. (2004) reported that sheep grazing adjacent monocultures of ryegrass and white clover walked further and faster than sheep grazing mixed swards, grass monoculture, or white clover monoculture. Having the forages as separate monocultures should have decreased time needed to search for preferred forage. However, the net benefit was reduced because the extra time spent walking between the monocultures led to an increase in energy costs. This may be overcome by planting the grass and white clover in strips of a width sufficient enough to allow for easy selection without the need to walk a further distance (Champion et al., 2004). Rutter et al. (2005a) examined diet preference of yearling beef heifers grazing three strip width treatments (108, 36, and 12 cm) and a mixed sward of ryegrass and white clover. The critical scale that allowed heifers to still

be able to select their desired proportion of white clover was between 36 and 12 cm (Rutter et al., 2005a). Additional research with alfalfa and tall fescue evaluating different strip widths would be beneficial to determine possible planting regimes for producers. Planting in strips of adequate width would allow animals to select their preferred diet without constraint while the proximity of alfalfa to tall fescue would provide additional nitrogen to the grass.

CONCLUSIONS

The results from this study indicate that beef steers do not graze at random and have a partial preference for alfalfa of 62.2% when given a choice of grazing alfalfa or tall fescue as adjacent monocultures. Forage nutritive value and sward characteristics influenced grazing behavior and led to differences between tall fescue monoculture and adjacent monoculture treatments. The research presented here expands our knowledge of diet selection of a pair of forages that have not been evaluated previously as adjacent monocultures and seems to add credence the theory that a partial preference for legume over grass will occur regardless of the forage species. Future research with these forages to address the underlying reasons for cattle to exhibit a partial preference for legumes is needed, specifically addressing if animals are selecting a diet to maintain a particular balance of C to N, and if the reason(s) for this partial preference changes depending on forage species being grazed. Additional research on the optimal spatial or temporal allocation of alfalfa and tall fescue would be beneficial for producers wishing to introduce high quality legumes into grazing systems with tall fescue.

Table 3-1. Nutritive value of forages being offered to beef steers as adjacent monocultures in varying proportions

Forage	Period ¹	NDF	ADF	Lignin	CP
		-----% DM-----			
Alfalfa	Pre	29.5 ^d	22.3 ^c	7.3 ^b	26.6 ^a
	Post	35.5 ^c	26.6 ^b	8.5 ^a	25.3 ^a
Tall fescue	Pre	55.9 ^b	31.3 ^a	5.6 ^c	15.2 ^b
	Post	58.9 ^a	32.6 ^a	6.1 ^c	14.4 ^b
Pooled SEM		0.9	0.5	0.2	0.7

¹Pre = pre grazing measurement before d 1, Post = post grazing measurement after d 4

^{abcd} Different superscripts within column indicate difference $P < 0.05$

Table 3-2. Sward characteristics of paddocks being grazed by beef steers in treatments that varied by the proportion of ground area of tall fescue and alfalfa being offered.

Measurement	Period ²	Treatment ¹							
		100F		25F75A		50F50A		75F25A	
		Fescue	Alfalfa	Fescue	Alfalfa	Fescue	Alfalfa	Fescue	
DM (%)	Pre	79	67 ^b	74	71	76	70 ^b	79	4
	Post	75	75 ^a	71	76	72	82 ^a	78	
Sward height (cm)	Pre	19 ^a	36 ^a	19 ^a	34 ^a	21 ^a	28 ^a	20 ^a	2
	Post	16 ^b	24 ^{bx}	17 ^b	22 ^{bx}	18 ^b	16 ^{by}	15 ^b	
Herbage mass (kg ha ⁻¹)	Pre	2,426	2,027 ^a	2,447	1,925	2,386	1,769 ^a	2,603	297
	Post	2,437	1,560 ^b	2,393	1,475	2,009	922 ^b	1,973	
Live material (%)	Pre	44	73	48	69	45	80	44	9
	Post	44	68	49	61	51	74	45	
Dead material (%)	Pre	56	27	52	31	55	20	56	9
	Post	56	32	51	39	49	26	55	
Live mass (kg ha ⁻¹)	Pre	961	1,406	1,107	1,269	1,002	1,325 ^a	988	155
	Post	1,120	1,094	1,183	959	1,024	651 ^b	933	
Dead mass (kg ha ⁻¹)	Pre	1,344	621 ^a	1,340	719 ^a	1,415	372 ^a	1,615 ^a	200
	Post	1,271	466 ^b	1,210	577 ^b	1,017	198 ^b	1,040 ^b	
Leaf mass (kg ha ⁻¹)	Pre	2,426	635 ^a	2,447	543	2,386	584 ^a	2,603	261
	Post	2,437	367 ^b	2,393	469	2,009	283 ^b	1,973	
Stem mass (kg ha ⁻¹)	Pre	-	1,392	-	1,411 ^a	-	1,155 ^a	-	163
	Post	-	1,193	-	1,035 ^b	-	609 ^b	-	

¹100F = tall fescue monoculture, 25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

²Pre = pre grazing measurement before d 1, Post = post grazing measurement after d 4

^{ab} Different superscripts within column within forage measurement indicate difference $P \leq 0.05$

^{xy} Different superscripts within row within forage type and forage measurement indicate difference $P \leq 0.05$

Table 3-3. Preference for alfalfa by steers grazing alfalfa and tall fescue as adjacent monocultures of varying proportions

Treatment ¹	Percent time grazing alfalfa ²	Difference from (P)			SEM ³
		Proportion offered	0%	100%	
25F75A	64.8	0.012	0.001	0.001	3.9
50F50A	61.1	0.555	0.0002	0.0002	2.3
75F25A	60.6	0.003	0.0004	0.001	3.0

¹25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

²Non-transformed percentage units

³SEM=standard error of angular transformed mean

Table 3-4. Diet preference of animals offered adjacent monocultures of two forages at 50:50 ground area ratio¹

Animal species	Physiological state	Herbage choice ²	% Legume	Reference
Sheep	Lactating	PG/WC	79.7	Parsons et al. (1994)
Sheep	Lactating	PG/WC	71.6	Penning et al. (1995)
Dairy sheep	Lactating	AG/Sulla	74.0	Rutter et al. (2005b)
Dairy cows	Lactating	PG/WC	70.0	Rutter et al. (1999)
Dairy cows	Lactating	PG/WC	78.0	Rutter et al. (2001)
Dairy cows	Lactating	PG/WC	73.8	Rutter et al. (2004a)
Sheep	Dry	PG/WC	65.8	Parsons et al. (1994)
Sheep	Dry	PG/WC	71.0	Harvey et al. (1996)
Sheep	Dry	PG/WC	91.8	Newman et al. (1994)
Sheep	Dry	PG/WC	88.4	Harvey et al. (1997)
Sheep	Dry	PG/WC	66.8	Harvey et al. (2000)
Sheep	Dry	PG/WC	70.0	Cosgrove et al. (2001)
Sheep	Dry	PG/WC	60.0	Rook et al. (2002)
Dairy heifers	Dry	PG/WC	65.0	Cosgrove et al. (1996)
Dairy heifers	Dry	PG/WC	68.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/WC	70.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/Lotus	63.9	Rutter et al. (2004b)
Beef heifers	Dry	PG/WC	60.0	Rutter et al. (2005a)

¹Adapted from Rutter (2006).

²PG = perennial ryegrass (*Lolium perenne* L.), WC = white clover (*Trifolium repens* L.), AG = annual ryegrass (*Lolium multiflorum* L.), Sulla = *Hedysarum coronarium* L., Lotus = *Lotus corniculatus* L.

Table 3-5. Carbohydrate fractions¹ of forages being grazed by beef steers in treatments that varied by the proportion of ground area of tall fescue and alfalfa being offered.

Forage	Period	NFC	WSC	ESC	Starch
-----% DM-----					
Alfalfa	AM	32.2 ^b	10.7 ^{ab}	7.7 ^{ab}	3.6 ^a
	PM	36.0 ^a	11.4 ^a	8.2 ^{ab}	4.8 ^a
Tall fescue	AM	17.2 ^d	9.7 ^b	7.0 ^b	1.2 ^b
	PM	20.0 ^c	11.3 ^a	8.5 ^a	1.1 ^b
Pooled SEM		0.6	0.4	0.4	0.3

¹NFC= non-fiber carbohydrates (simple sugars, fructans, soluble fiber, organic acids), WSC=water soluble carbohydrates (simple sugars, fructans), ESC=ethanol soluble carbohydrates (simple sugars)

^{abcd} Different superscripts within column indicate difference $P \leq 0.05$

Table 3-6. Behavior recorder data collected from steers grazing tall fescue or adjacent monocultures of tall fescue and alfalfa in varying proportions.

<u>Treatment</u> ¹	<u>Grazing</u> ------(min d ⁻¹)-----	<u>Ruminating</u> ------(min d ⁻¹)-----	<u>Idling</u> ------(min d ⁻¹)-----	<u>Prehensions</u> ---(bites d ⁻¹)---	<u>Bite rate</u> --(bites min ⁻¹)--	<u>Mastications</u> --(mastications d ⁻¹)--
100F	514	518 ^a	409	32,698	59	34,545
25F75A	636	402 ^b	403	36,704	58	33,421
50F50A	620	494 ^a	326	35,578	57	39,238
75F25A	672	458 ^{ab}	309	44,569	64	36,014
SEM	45	17	47	4,459	4	2,647

¹100F = tall fescue monoculture, 25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

^{ab} Different superscripts within column indicate difference $P \leq 0.05$

Table 3-7. Pedometer data collected from steers grazing adjacent monocultures of Tall fescue and alfalfa in varying proportions.

Treatment ¹	Standing	Lying	Active	Steps
	-----(% d ⁻¹)-----			--(steps d ⁻¹)--
100F	44.2	51.3	4.1 ^b	2,151
25F75A	53.0	40.6	4.9 ^{ab}	2,539
50F50A	52.5	41.6	5.4 ^{ab}	2,761
75F25A	52.1	41.4	5.6 ^a	2,852
SEM	3.2	3.2	0.3	205

¹100F = tall fescue monoculture, 25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

^{ab}Different superscripts within column indicate difference $P < 0.05$

Table 3-8. Proportion of time total time ruminating spent in alfalfa by steers grazing alfalfa and tall fescue as adjacent monocultures of varying proportions

Treatment ¹	Percent time ruminating in alfalfa ²	Difference from (<i>P</i>)			SEM ³
		<u>Proportion offered</u>	<u>0%</u>	<u>100%</u>	
25F75A	28.1	0.006	0.014	0.002	12.1
50F50A	54.3	0.240	0.0001	<0.0001	1.7
75F25A	38.3	0.196	0.016	0.004	7.5

¹25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

²Non-transformed percentage units

³SEM=standard error of angular transformed mean

Table 3-9. Proportion of time total time idling spent in alfalfa by steers grazing alfalfa and tall fescue as adjacent monocultures of varying proportions

Treatment ¹	Percent time idling in alfalfa ²	Difference from (<i>P</i>)			SEM ³
		<u>Proportion offered</u>	<u>0%</u>	<u>100%</u>	
25F75A	43.8	0.049	0.028	0.011	10.4
50F50A	68.1	0.507	0.009	0.020	9.5
75F25A	25.2	0.725	0.040	0.003	8.1

¹25F75A = 25% tall fescue and 75% alfalfa, 50F50A = 50% tall fescue and 50% alfalfa, 75F25A = 75% tall fescue and 25% alfalfa

²Non-transformed percentage units

³SEM=standard error of angular transformed mean

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CHAPTER 4

THE ROLE OF FORAGE NOVELTY IN THE GRAZING BEHAVIOR AND DIET PREFERENCE OF BEEF STEERS GRAZING ADJACENT MONOCULTURES OF TALL FESCUE AND ALFALFA

ABSTRACT

A number of studies have tested the behavior of sheep and cattle grazing adjacent monocultures of perennial ryegrass and white clover. In these studies animals almost always choose a diet of around 70% white clover. Several theories have been proposed to explain this pattern of preference and why domestic ruminants select mixed diets of grass and legume, but only a few species have been evaluated in this manner are limited so two different forage species (tall fescue and alfalfa) were chosen for examination as adjacent monocultures. The theory that ruminants select mixed diets in part based on a plant's 'novelty' was evaluated in this experiment along with the theory that regardless of species evaluated, cattle will have a higher preference for legume than grass. Cattle without previous experience grazing alfalfa spent 78% of the time grazing alfalfa, whereas after having experience grazing it they spent a lower ($P = 0.04$) proportion of their time grazing alfalfa (72%). Overall proportion of the day spent grazing both forages was lower ($P = 0.0001$) when alfalfa was novel (40%), compared to when steers were experienced grazing both forages (46%). Proportion of the day spent idling was greater ($P < 0.0001$) when alfalfa was novel (35%), compared to when both forages were familiar to the steers (26%). The proportion of grazing time in alfalfa was higher ($P = 0.02$) in the afternoon (76.8 %) than in the morning (72.1 %). This differs from previous studies reporting a decline in preference for legume over the day with and

increased preference for grass in the afternoon. Weather and sward conditions may have impacted grazing behavior in this study. Future application of adjacent monocultures in production systems could benefit from additional exploration of long-term performance of cattle grazing in this type of paddock arrangement.

INTRODUCTION

Cattle have been shown to exhibit a partial preference for white clover (*Trifolium repens* L.) over perennial ryegrass (*Lolium perenne* L.) of around 70% when given a choice between the two forages as adjacent monocultures (Rutter et al., 1999; Rutter et al., 2004a, b; Rutter et al., 2005). The previous chapter in the current work examined two forages that had not been previously evaluated as adjacent monocultures: tall fescue (*Festuca arundinacea* Schreb. or *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and alfalfa (*Medicago sativa* subsp. *sativa* L.). Cattle exhibited a partial preference for alfalfa (62%) which supports the proposal that legumes, regardless of species, will be partially preferred over grasses (Rutter, 2006).

There are a number of theories to explain why domestic ruminants consume a mixed diet in these proportions. One such theory suggests that the ‘novelty’ of a forage plays a role in diet selection in short term experiments (Rutter, 2006). Effect of previous diet has been evaluated in sheep grazing different allotments of perennial ryegrass and white clover. Sheep were observed to have a greater preference for the forage that was lacking in their most recent diet (Parsons et al., 1994). Sheep in that experiment had prior experience grazing both these forages, so while the forage was “novel” in the sense that it had been absent from the diet in the previous 2 to 3 wks, neither of the

forages studied was unfamiliar to them. Villalba (2007) stated that animals will prefer familiar over unfamiliar foods which they will regard with caution. Illius and Gordon (1990) suggested that grazing animals may need to 'sample' unfamiliar plants or in unfamiliar areas in order to learn about them and determine whether they are a viable food source. Whereas others reported that animals will readily consume a novel food because the food is new or rare (Ganskopp and Cruz, 1999; Baumont et al., 2000).

Tall fescue and alfalfa are new forages in the body of adjacent monoculture research and therefore the effect that novelty might play in diet preference was considered of interest. The objectives of this experiment were to evaluate 1) diet preference, 2) diurnal pattern of preference, and 3) overall grazing behavior of beef steers grazing adjacent monocultures of tall fescue and alfalfa when alfalfa was a novel forage compared to behavior when steers have previously grazed both forage species.

MATERIALS AND METHODS

Pasture establishment and management

Pastures were established in 2004. All paddocks were sprayed with Roundup® (9 L ha⁻¹, glyphosate 41%, Monsanto, St. Louis, MO) in May to eliminate existing pasture species. Two weeks after spraying, foxtail millet (*Setaria italica*, 34 kg ha⁻¹) was planted as a suppression crop. In late July, the millet was harvested for hay and two weeks later the paddocks were sprayed again with Roundup® (2 L ha⁻¹) for weed suppression. Tall fescue pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0) and the alfalfa pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0) + 112 kg ha⁻¹ of potash (0-0-60). On September 2 and 5, paddocks were planted with Jesup

endophyte-free tall fescue (28 kg ha⁻¹) or with AmeriStand 403T alfalfa (22 kg ha⁻¹). Nitrogen was applied to the tall fescue paddocks at 34 kg ha⁻¹ in October and then again in March, 2005 to promote growth and tillering. Herbicides were applied in April, 2005. All alfalfa pastures received 37 mL ha⁻¹ of Harmony GT[®] (thifensulfuron-methyl 75%, Dupont, Wilmington, DE) and 2 L ha⁻¹ Poast Plus[®] (sethoxydim 13%, BASF, Research Triangle Park, NC) and 1 L ha⁻¹ of surfactant. All tall fescue pastures received 1 L ha⁻¹ of 2-4D (2,4-Dichlorophenoxyacetic acid 46.6%, Helena Holding Co., Wilmington, DE) and 0.6 L ha⁻¹ Banvel[®] (3,6-dichloro-0-anisic acid 48.2%, Micro Flo, Memphis, TN).

Pastures were grazed by stocker steers during the summer grazing seasons of 2005 and 2006. Pastures were fertilized in April, 2006 and 2007 with tall fescue receiving 35-20-45 (224 kg ha⁻¹) and alfalfa receiving 0-46-0 (145 kg ha⁻¹) and Boron (2 kg ha⁻¹). Alfalfa paddocks were sprayed with Harmony GT[®] in April of Yr 1 and Yr 2 for control of thistle and with Baythroid[®] (β -cyfluthrin 12.7%, Bayer, Research Triangle Park, NC) for insect control in July of Yr 2 according to manufacturer recommendations.

Treatments and animal management

The procedures used in this experiment were approved by the Virginia Tech Institutional Animal Care and Use Committee. This experiment was conducted at Kentland Farm (37°11' N, 80°35' W), Blacksburg, Virginia, during August of 2006 (Yr 1) and 2007 (Yr 2). The first experimental treatment (Novelty) was designed to evaluate animal response to grazing endophyte-free tall fescue and alfalfa in equal proportions of ground area when steers had only prior experience grazing tall fescue. The second

experimental treatment (Experience) was designed to evaluate animal response to grazing these two forages again when steers had prior experience both tall fescue and alfalfa. The same steers were used in both treatments within each year with the Novelty period conducted from August 11 to 15 in Yr 1 and August 3 to 5 in Yr 2. The Experience period was conducted from August 26 to 30 in Yr 1 and August 16 to 20 in Yr 2.

Eighteen Angus-crossbred steers (9 steers yr⁻¹, 16 mo old in both yr, Yr 1 initial BW = 392 ± 8 kg, Yr 2 initial BW = 323 ± 9 kg) were used in the experiment. These steers had no previous experience grazing alfalfa and had grazed pastures of predominantly endophyte infected tall fescue and then grazed endophyte-free tall fescue for several months prior to the study. Steers were allotted to groups by initial BW. Each group was then randomly allotted to paddocks with three steers in each paddock, and three paddocks total. Paddocks were 0.22 ha of tall fescue monoculture adjacent to 0.22 ha of alfalfa monoculture. The paddock was subdivided into 3 sub-paddocks with electric poly-tape. One sub-paddock was used for the Novelty period and another sub-paddock was grazed during the Experience period. Within each paddock the steers could move freely between each forage type. Water troughs and trace mineral salt with poloxalene as an anti-bloat agent (Bloat Guard[®] Pressed Block, Sweetlix Livestock Supplement System, Mankato, MN) were provided ad libitum and located at the midpoint of the paddock where the two forage types met. This ensured that the steer's need to drink and consume mineral did not influence into which forage area the steer would travel.

Behavioral sampling

Behavior Data Recorders (Rutter et al., 1997) were used to monitor grazing behavior. The device consists of a halter mounted data logger and jaw sensor. The jaw sensor was a carbon-packed tube serving as an electrical transducer converting movements of the jaw to electronic signals. The data from the sensor were recorded on a Compact Flash memory card in the data logger and analyzed by GRAZE software (version 0.801, Rutter, 2000). Activities reported were time spent grazing, ruminating, and idling (defined as not grazing or ruminating), as well as number of bites, and ruminating mastications. Bites were recorded as the severing of plant material from the sward during grazing. Mastications were recorded as the chewing activity during rumination. All steers were trained to wear these devices prior to the experiment. Training began 2 wk prior to the experiment by fitting a nonfunctional version of the recorders on each steer for a period of 4 h on first day of training, increasing the length of time by 4 to 8 h each day until steers were acclimated and could wear the device for 4 d. Training versions were of similar size and weight and fitted to the same halter steers would wear during the trial. Steers were brought to the working facility between 0700 and 0800 on d 1 of the novelty and experience periods and were fitted with the behavior data recorders. Steers were put in their respective paddocks between 0800 and 0900, remained there for 48 h, and were then brought back to the working facility where batteries and memory cards were exchanged in the data recorders. Steers were then returned to the paddocks for an additional 48 h period.

Video surveillance cameras in weatherproof enclosures (DCS-5300 in DCS-70, D-Link, Fountain Valley, CA) were mounted 6 m above the ground on poles in each

paddock to monitor steers. Color video containing date and time stamps were recorded digitally during day-light hours onto a computer hard drive and were later reviewed using a digital media player (Windows Media Player, Microsoft Corp., Redmond, WA). Steers within a paddock were identified by a unique color neckband and fluorescent livestock marking paint (Mark-Her, H and W Products). Instantaneous scan sampling (Altmann, 1974) was performed on the recordings. Using this method, the behaviors of each steer was documented “instantaneously” during video review which was conducted on 10 min intervals. Steers were considered to be exhibiting that behavior for the entire 10 min period. At each observation, three variables were recorded: body position (standing or lying), jaw activity (grazing, ruminating, idling), and field location (alfalfa, tall fescue, or on the boundary line between the monocultures). Standing was recorded if the steer was in an upright position while lying was recorded if the steer was in a recumbent position. Grazing was recorded if the steer was observed consuming or searching for forage. Ruminating was recorded if the steer was observed masticating (not grazing and could be standing or lying). Idling was recorded if the steer was not grazing or ruminating (could be standing or lying). Field location was easily identified visually by colored posts located along the dividing line between the two forage areas. Water troughs and mineral feeders were also clearly visible.

Data collected from the Behavior Data Recorders and video recordings were combined and analyzed by matching the time stamp of the recorded data from one device to the data collected on the other devices using the Excel[®] (Microsoft[®], Redmond, WA) VLOOKUP function. Proportion of time for behaviors spent on the boundary line between the two forage monocultures was divided and half the value

added to the proportion of time that behavior was recorded in each forage type. Bite rate while grazing in alfalfa or tall fescue was calculated on a focal steer in each replicate paddock at three times in the morning and three times in the afternoon on d 1 and 3. This was performed on the combined data from the behavior recorder and video surveillance analyses.

Forage sampling and analyses

Hand-plucked forage samples were taken for nutritive value analysis on the day prior to and the day after each experimental period. Samples were collected while walking along a cross-section of the paddock and grabbing a sample every 10 steps from the top 7 to 10 cm of the sward, representing forage being consumed by the steers. Separate samples from each paddock of each forage type were collected between 0730 and 0830 and between 1700 and 1800. Forage samples from AM and PM hours were collected to evaluate diurnal changes in carbohydrate levels which have been shown to influence diet selection (Ciavarella et al., 2000; Mayland et al., 2000). Samples were packed on ice directly after collection, freeze-dried (25L Genesis SQ EL-85, VirTis, Gardiner, NY) and were then ground through a 1-mm screen using a Wiley mill. Samples were then analyzed for chemical composition by NIRS (Foss NIRSystems 6500, Foss Tecator, Eden Prairie, MN; AOAC, 2000) at a commercial laboratory (DairyOne, Ithaca, NY). Tall fescue subsamples were further ground to 0.5mm (Cyclotec 1093 Sample Mill, Foss Tecator, Eden Prairie, MN) and analyzed for alkaloid concentration (Agrinostics Ltd. Co., Watkinsville, GA) to ensure they were at low alkaloid levels. Mean alkaloid level in Yr 1 was 111 ng g⁻¹ (SEM = 18) and in Yr 2 was 51 ng g⁻¹ (SEM = 5).

Forage mass and sward height measurements were taken on the day prior to and the day after each experimental period. Three areas of 0.25 m² were randomly selected from each paddock of tall fescue and alfalfa and 10 measurements of sward height were taken with a ruler within those areas at each sampling. The three areas were then clipped to 2.5 cm above ground level, forage placed in cloth bags, dried in a forced draft oven at 60°C for 48 h to determine macro DM (%) and forage mass.

Statistical analyses

Data were analyzed using SAS (version 9.1.3, Cary, NC, USA). The experimental unit for all analyses was the paddock (3 steers) because individuals within a group are not considered statistically independent (Rook and Penning, 1991). Behavior recorder output and surveillance video data were combined to obtain the proportion of time spent in alfalfa and tall fescue during daylight observations. Angular transformation of proportions was conducted to stabilize variance (Parsons et al., 1994). The angular transformed percentage of the total time of a behavior observed on each forage type was used to determine preference of that behavior occurring on that forage. Diurnal pattern of preference and bite rate was analyzed using the MIXED procedure with the model including treatment, time of day (AM or PM), and their interaction. The model for bite rate also included forage type. Repeated measure was day within yr. Behavior data were analyzed using the MIXED procedure and the compound symmetry (cs) covariance structure. The repeated measure was day for all variables. The model for behavior data (grazing, ruminating, idling, standing, and lying, number of prehensions and mastications) included yr, treatment, day, and their interaction. Repeated measure was day within yr. Forage nutritive value and sward measurement

data were analyzed using the MIXED procedure. The model included yr, forage type (alfalfa or tall fescue), treatment (Novelty or Experience), sampling date (pre or post grazing), and interactions. The model for nutritive value parameters which have diurnal changes (i.e. carbohydrates) included yr, forage type, and sampling time of day (AM or PM), and interactions. The experimental unit was the paddock within period. The paddock selected for use within yr was considered a random effect. Least squares means are reported for all variables with means separated by Tukey's adjustment. A significance level of $\alpha \leq 0.05$ was set for all analyses with trends defined as $0.10 > \alpha > 0.05$.

RESULTS AND DISCUSSION

Forage and sward measurements

Alfalfa NDF, ADF, and CP concentration did not differ by yr or treatment ($P \geq 0.10$). Lignin of alfalfa tended to be higher in the Novelty treatment period than during the Experience period (7.1 and 6.4 %DM, respectively; SEM = 0.2). Year effects ($P \leq 0.04$) were observed for both CP and lignin in tall fescue with lower CP (15.0 and 17.8 %DM; SEM = 0.6) and higher lignin (5.3 and 4.8 %DM; SEM = 0.2) in Yr 2 compared with Yr 1, respectively. Tall fescue NDF and ADF did not differ between years ($P \geq 0.13$), however.

There was an effect of forage type ($P \leq 0.0007$) on nutritive value with alfalfa having lower NDF and ADF, and higher lignin and CP (Table 4-1). Period effects ($P \leq 0.009$) were observed in NDF, ADF, and CP, as forage nutritive value declined after having been grazed for 4 d. There was no effect of yr ($P \geq 0.18$) on overall CP, NDF, or

lignin of the pastures, while ADF tended ($P = 0.07$) to be higher in Yr 1 than 2 (28.4 and 26.6 %, respectively, SEM = 0.5).

There was an effect of yr ($P = 0.02$) on forage mass with it being higher in Yr 1 than Yr 2 (2,717 and 2,250 kg ha⁻¹, respectively; SEM = 127). Forage mass did not differ by treatment ($P = 0.91$) or forage type ($P = 0.21$), and tended ($P = 0.07$) to vary by sampling period with pre-grazing forage mass being greater than post-grazing (2,574 and 2,393 kg ha⁻¹, respectively; SEM = 103). There was no interaction of treatment x forage x period observed (Table 4-2, $P = 0.27$).

All main effects for sward height were significant as well as treatment x forage x period interaction ($P \leq 0.01$, Table 4-3). Sward height was taller in Yr 2 than Yr 1 (25.8 and 19.3 cm, respectively; SEM = 1.5). Given that forage mass was lower in Yr 2, these data help to characterize the general structure of the sward, particularly the alfalfa was taller in Yr 2 than Yr 1 (28.2 and 21.0 cm, respectively; SEM = 1.9) but not as leafy as in Yr 1. This was likely due to an infestation of leaf hoppers before the trial. Alfalfa was taller than tall fescue (24.6 and 20.5 cm, respectively; SEM = 1.3) and pre-grazing height was greater than post-grazing (25.0 and 20.1 cm, respectively; SEM = 1.1).

Diet preference

Steers in the Novelty period spent more ($P = 0.04$, SEM = 2.2; Table 4-4) of their time grazing alfalfa (77.5 %) than in the Experience period (72.1 %). Consequently, steers in the Novelty treatment period spent less ($P = 0.04$, SEM = 2.2; Table 4-4) of their time grazing tall fescue (22.5 %), the forage they were familiar with, than in the Experience treatment period (27.9 %). Past research results of animal response to

novel vs. familiar foods varies. Phillips and Youssef (2003a) conditioned ewes and lambs to one of four different novel forages. Sheep were then offered pastures containing the forage they were experienced with along with the remaining three forages. The sheep spent more time grazing the forage they had experience with regardless of what species it was. While some have reported that animals will avoid or only eat small quantities of novel foods (Provenza and Balph, 1987; Burritt and Provenza, 1989; Provenza et al., 1995; Villalba, 2007), there are those that show animals will prefer foods that are novel, rare, or were lacking in their diet previously (Tuttle et al., 1990; Newman et al., 1992; Parsons et al., 1994). The preference for novelty may be a hedonic behavior and a novel forage item is interpreted as a reward by the animal (Baumont et al., 2000). The preference for rarity however could be somewhat discounted by the work in the previous chapter offering steers different proportions (25:75, 50:50, and 75:25) of tall fescue and alfalfa. If steers were choosing the 'rare' forage then they might have shown a higher preference for tall fescue when it was only 25% of the forage being offered, but instead they grazed on alfalfa 65% of the time. It is not known at what scale an item would be considered 'rare' by these animals. Is it temporal or spatial rarity that matters most? Within temporal terms, is rarity on a scale of hours or days and within spatial terms will several square meters of the sward or a single plant classify it as being rare to the animal? These are questions that have yet to be clearly answered.

Proportion of time spent grazing a forage can be equivalent to the amount of that forage consumed in the diet (Parsons et al., 1994). However, since intake was not measured at the same time these behaviors were recorded, it cannot be assumed that

DMI of alfalfa was equivalent to the proportion of time observed grazing. Cattle in unfamiliar environments with unfamiliar plants may spend more time foraging but actually consume less than with equivalent grazing time in a familiar environment with familiar forages (Hodgson, 1971; Hodgson and Jamieson, 1981; Lyons and Machen, 2000) which may be related to time needed to learn about the new area or learning how to efficiently harvest unfamiliar food items (Flores et al., 1989b). If a novel food differs in its form from familiar foods, it may impact the animals' initial success in efficiently harvesting that novel food (Flores et al., 1989a). Because alfalfa and tall fescue do differ in physical form, this may also have influenced the observed increased time in alfalfa during the Novelty experience period. Ganskopp and Cruz (1999) reported that steers grazing novel forages grazed for numerically longer periods than steers experienced with the forages offered. However, the naïve steers were 21% less efficient in terms of number of bites taken during that time and assuming bite mass did not differ between naïve and experienced steers that could lead to lower DMI.

The preference for alfalfa over endophyte-free tall fescue is not likely to be attributed to any negative association with tall fescue related to endophyte-infected tall fescue (containing ergot alkaloids) that the steers consumed earlier in life. These steers had experience grazing both types of tall fescue and ruminants have been shown to be able to differentiate endophyte-free from endophyte-infected forages (Jones et al., 2000; Cosgrove et al., 2002).

Pattern of preference was also effected by day ($P = 0.0003$) with preference for alfalfa decreasing and preference for tall fescue increasing from d 1 to 4 (Figure 4-1). This may be due to depletion of the most preferred leaves of the alfalfa sward over the

course of the trial. Forage mass of alfalfa was only numerically greater from pre to post grazing (Table 4-3), however growth rate of the forage was not calculated and might account for the lack of difference in pre and post-grazing forage mass. There was an interaction of treatment x day ($P < 0.0001$) observed in pattern of grazing preference (Figure 4-2). From d 1 to 2 the steers' preference for alfalfa increased in the Novelty treatment period and by d 4 had returned to a similar proportion as that observed on d 1. Because these paddocks were unfamiliar to the steers, as was the alfalfa, the steers may have been sampling the new forage and exploring their new surroundings during the first 24 h. Introducing animals to novel foods in unfamiliar environments may cause animals to reject the novel food in favor of a familiar food (Burritt and Provenza, 1997). However, if animals are introduced to new pastures with companion animals or if the area is similar to familiar areas (as they were here), they are more likely to explore their new paddocks as opposed to exhibiting neophobia (Launchbaugh and Howery, 2005). Ganskopp and Cruz (1999) reported that cattle introduced to novel forages will show dietary preferences similar to that of experienced cattle within minutes of being offered the new forages. On d 2 and 3 the steers increased their grazing time on alfalfa and perhaps were still determining what their preferred or optimal legume: grass ratio was going to be, then returning to a lower proportion of time grazing alfalfa on d 4. During the Experience treatment period, steers grazed more alfalfa on d 1 than any other day (Figure 4-2). Parsons et al. (1994) showed a similar pattern in ewes grazing 50:50 adjacent monocultures of perennial ryegrass and white clover with preference for clover being as follows: d 1 = 81.2%, d 3 = 68.0% and d 6 = 70.9%. Even though steers in the present study were grazing adjacent monocultures of tall fescue and alfalfa prior to the

start of the Experience treatment period, the stimulus of being given a new allotment of fresh forage, along with their previously observed partial preference for alfalfa, likely led to this greater grazing time on d 1 in alfalfa. Sheep have been shown to consume a greater proportion of ryegrass if their previous diet was white clover, and more white clover was consumed if their previous diet was perennial ryegrass (Parsons et al., 1994). While alfalfa was not absent from the area the steers grazed before they entered the Experience treatment paddocks, the most desirable leaves of the alfalfa had probably been removed from the sward by the time they were moved to the new paddocks and may have influenced their grazing pattern on d 1.

There was an effect of yr ($P = 0.02$; SEM = 3.0) on time spent grazing in each forage with grazing time in alfalfa being higher in Yr 2 (86.1 %) than Yr 1 (62.8%) and conversely grazing time in tall fescue was higher in Yr 1 (37.2%) than Yr 2 (13.9 %). This may be due to differences in climate and sward conditions between years. Air temperatures in Yr 2 were hotter (Table 4-5) and year-to-date rainfall was lower (479 vs. 443 mm) than in Yr 1. Forage mass of tall fescue did not differ from Yr 1 to Yr 2 (2,448 and 2,286 kg ha⁻¹, respectively; SEM = 179). However, the tall fescue in Yr 2 was observed to contain more dry and dead material, which may have made it less palatable than in Yr 1. Consequently, tall fescue may have been less palatable, leading to more time spent grazing alfalfa which is more tolerant of drought conditions (Sheaffer and Evers, 2003). Crude protein in Yr 2 (15%) was lower than in Yr 1 (18 %) and may have impacted diet selection, particularly if animals were trying to maintain a particular ratio of C: N, which has been suggested as a factor influencing the selection of mixed diets in domestic ruminants (Rutter, 2006). Animals are likely able to balance their diets based

on feedback from nutrients after eating particular foods (Provenza, 1995; Villalba and Provenza, 1999). Because of differences in the tall fescue between years, the steers in Yr 2 may have been receiving more positive post-ingestive feedback when consuming a higher proportion of alfalfa.

Diurnal pattern of behavior

The proportion of grazing time in alfalfa was higher ($P = 0.02$, SEM = 4.6) in the afternoon (76.8 %) than in the morning (72.1 %) and conversely proportion of grazing time in tall fescue was higher in the morning (27.9 %) than the afternoon (23.2 %). There was no interaction of treatment x time of day ($P = 0.60$) for the proportion of time spent grazing within each forage type with AM grazing times being similar between Novelty and Experience treatments and PM grazing times also being similar (Table 4-6). Within treatments, proportions of time spent grazing in AM and PM periods of the day also did not differ (Table 4-6). Previous research of sheep and cattle grazing perennial ryegrass and white clover adjacent monocultures reported a diurnal pattern of preference, as preference for white clover was higher in the morning while preference for perennial ryegrass was higher in the afternoon (Parsons et al., 1994; Rutter et al., 2004a, b). The experiments reported in the previous chapter examining different spatial allocations of tall fescue and alfalfa had similar results to the present experiment, with no decrease in preference for alfalfa over the course of the day and no increase in preference for tall fescue in the afternoon, both of which are contrary to past studies. Parsons et al. (1994) reported ewes differing in physiology (dry or lactating) and differing in background diet (ryegrass/white clover adjacent monoculture, ryegrass only, or white clover only) showed a decrease in preference for white clover from morning to

afternoon. In that study, the high proportion of clover in the diet would return again the following morning. Observations of greater rumination time after eating ryegrass compared with white clover in previous research (Penning et al., 1991) leads to an explanation of why ruminants have been observed to graze more ryegrass in the afternoon (Parsons et al. 1994). If rumination time is less after eating white clover this means the animal will start grazing again sooner than it would after eating and ruminating ryegrass. The animal would prefer to have longer periods of rumination at night when it does not interfere with major periods of intake during the day (Parsons et al., 1994). Being less active at night also puts the animal at less risk of predation (Gluesing et al., 1980). The slower passage and greater time needed to ruminate after eating grass due to its high fiber concentration (Rutter, 2006). It does not appear that steers in the present study selected this strategy since fiber was greater in tall fescue than alfalfa (Table 4-1).

Carbohydrate levels increase over the course of a day due to net photosynthesis, respiration, and translocation in the plant (Mayland et al., 2005). Concentration of total non-structural carbohydrates (TNC) has also been shown to influence diet selection of cattle and sheep. Most notable has been the greater preference for tall fescue and alfalfa hays cut in the afternoon (containing greater TNC) compared to morning cut hays (Fisher et al., 1999; Mayland et al., 2000; Fisher et al., 2002). Greater levels of water soluble carbohydrates (WSC) have been linked to greater preference in grazed forages as well (Ciavarella et al., 2000). This phenomenon does not seem to explain the greater preference of alfalfa in the present study because WSC did not differ in either forage at either time of day (Table 4-7) and thus would not influence a greater preference for one

forage over the other. Non-fiber carbohydrates (NFC) were calculated in this study (TNC + organic acids and soluble fibers) and were higher in alfalfa at both times of day, so they may be influencing the higher preference of alfalfa in both AM and PM periods.

Drought conditions during the study may have led to tall fescue being somewhat more palatable in the morning rather than the afternoon due to the presence of dew, and might explain why they spent more time grazing tall fescue in the AM than PM. Forage with lower moisture content has been shown to be less palatable (Aderibigbe et al., 1982). Dry matter was not measured in the morning and afternoon; however the tall fescue appeared drier and tougher than alfalfa, when hand-plucked forage samples were being collected, particularly in the afternoon. This would indicate a decreased moisture concentration in tall fescue in the afternoon compared with the alfalfa due to greater desiccation in tall fescue over the course of the day. Since hand-plucking of the forage was found to be harder in the afternoon than in the morning, this might imply that it was also harder for the steers to prehend the tall fescue in the afternoon. Intake rate may be reduced when the physical characteristics of a forage make it more difficult to prehend (Inoué et al., 1994). A forage may even be rejected by an animal if harvesting and masticating are more difficult (Phillips and Youssef, 2003a).

Bite rate was affected by forage ($P = 0.0001$) and treatment ($P < 0.0001$). Greater bite rate occurred while grazing alfalfa (65 bites min^{-1}) compared with tall fescue (63 bites min^{-1}). Bite rate during the time when alfalfa was a novel forage was lower (62 bites min^{-1}) than during the Experience treatment period (66 bites min^{-1} , SEM = 1.5). Increase in bite rate with experience has also been previously reported. Lambs that were introduced to a new grass species in subsequent periods along with a forage they

had previous experience with increased bite rate each time a new forage was offered (Phillips and Youssef, 2003b). Bite rate was not affected by time of day ($P = 0.48$) and no interactions were significant ($P \geq 0.30$).

There was no effect of yr ($P = 0.53$) on the amount of time spent standing in either forage type. There was an effect of treatment ($P = 0.001$) with steers standing in the alfalfa more during Novelty treatment period than the Experience period, but was neither proportion was different ($P \geq 0.12$) than 50% (the proportion of forage offered) so it is not clear what is their preferred forage in which to stand (Table 4-4). There was also an effect of day ($P = 0.002$) with the percentage of time standing in alfalfa decreasing from 51% on d 1 to 45% on d 2 (SEM = 1.7). Steers spent a disproportionate amount of time lying in the tall fescue in both the Novelty and Experience treatment periods (Table 4-4) which was greater in Yr 1 than Yr 2 (95 and 88 %, respectively; SEM = 0.9; $P = 0.002$). The proportion of time was greater ($P < 0.0001$) than the 50% of tall fescue area available so this may indicate that steers do have a partial preference to lay on tall fescue rather than alfalfa. This may be because the alfalfa was tall and stemmy and perhaps was not as comfortable to lie on as the tall fescue. Cattle generally prefer to lie on softer areas (Drissler et al., 2005).

Overall grazing behavior

The proportion of the day spent grazing (overall on both forages) was lower ($P = 0.0001$, Figure 4-3) during the Novelty treatment period (39.7%) than in the Experience (46.1%). This difference also created a difference in time spent idling, with greater ($P < 0.0001$) idling time in the Novelty treatment period (35.3%) than in the Experience

(26.2%). Movement to unfamiliar pastures may cause short term (1 to 2 d) changes in behavior, such as increased walking along the fence line or idling, however cattle have been shown to adapt quickly to new surroundings (McIlvain and Shoop, 1971). Lambs with previous experience grazing perennial ryegrass were shown to graze more than lambs that were inexperienced (496 vs. 447 min d⁻¹, respectively; Phillips and Youssef, 2003b). Ganskopp and Cruz (1999) on the other hand reported that steers offered novel forages had longer grazing sessions than steers experienced with all forages being evaluated, however the difference was not significant (39 and 35 min, respectively; $P = 0.17$). Animals may initially be somewhat reluctant to consume an unfamiliar food (Provenza et al., 2003), and this may have decreased overall proportion of total time spent grazing since alfalfa was an unfamiliar forage during the Novelty period. However, within the first 24h of the Novelty treatment period steers were observed grazing predominantly in the alfalfa indicating they adapted to this new food item very quickly.

A yr effect was observed for time spent grazing ($P = 0.02$) and idling ($P = 0.03$). Grazing time overall in Yr 2 was less than Yr 1 (40.1 and 45.7 %, respectively, SEM = 1.0) while idling time in Yr 2 was more than Yr 1 (34.6 and 26.8 %, respectively, SEM = 1.8). This may be related to the difference in climate conditions between years with the afternoon high temperatures reaching as high as 31 °C and 36 °C in the Novelty and Experience periods of Yr 2, respectively (Table 4-5). *Bos taurus* cattle begin to decrease feed intake as air temperature approaches 28 °C, and cease intake completely at 41 °C (Findlay, 1958).

There was no effect of yr ($P = 0.29$) day ($P = 0.12$) on number of prehensions d⁻¹; however they did differ by treatment ($P < 0.0001$). Daily number of prehensions was

higher during the Experience treatment period (39,482) than during the Novelty treatment period (30,026). This difference is probably due to the greater proportion of time spent grazing by Experience treatment steers (Figure 4-3). There was no effect of yr ($P = 0.52$) on time spent ruminating, with trends for day ($P = 0.07$) and treatment ($P = 0.09$; Figure 4-3). Similarly, there was no effect of yr ($P = 0.75$), day ($P = 0.39$), or treatment ($P = 0.37$) on number of mastications by steers. Daily number of mastications during the Novelty treatment period was 32,050, with 34,215 during the Experience treatment period (SEM = 2,894).

CONCLUSIONS

In this experiment novelty did play a role in the amount of time steers allocated to grazing. Steers spent more time grazing the novel forage alfalfa than grazing tall fescue; however more research needs to be conducted to determine if DMI was impacted as well. The present study confirms results presented in the previous chapter that beef steers have a partial preference for alfalfa over tall fescue. Even with the higher preference for alfalfa in Yr 2, the partial preference values along with the previous chapter (62%) are both in range of values reported for preference of legumes offered as 50:50 adjacent monocultures with ryegrass (Table 4-8). Differences in weather and sward conditions can lead to partial preferences becoming stronger or weaker from one year to another. Additional studies on grazing management of tall fescue and alfalfa as adjacent monocultures over a longer period would also be of interest to observe if partial preference varies over time and changing sward conditions. This study along with that in the previous chapter seems to indicate that one of the stronger theories to explain selection of mixed diets is that animals are doing so in order to maintain a

balance of C: N. However, since these experiments were not designed to test that theory, future research to test this in tall fescue and alfalfa adjacent monocultures and perhaps other forage combinations would help further narrow down the strategies being used by the animal that drive diet selection.

Table 4-1. Nutritive value of forages being offered to beef steers as adjacent monocultures.

Forage	Period ¹	% DM			
		NDF	ADF	Lignin	CP
Alfalfa	Pre	31.5 ^c	23.5 ^c	6.4 ^b	25.7 ^a
	Post	35.0 ^b	26.0 ^b	7.1 ^a	24.0 ^a
Tall fescue	Pre	50.4 ^a	29.9 ^a	5.3 ^c	17.1 ^b
	Post	52.0 ^a	30.6 ^a	4.9 ^c	16.0 ^b
SEM		1.5	0.7	0.2	1.1

¹Pre = pre grazing measurement before d 1, Post = post grazing measurement after d 4
^{abcd} Different superscripts within column indicate difference $P \leq 0.05$

Table 4-2. Forage mass (kg ha^{-1}) of alfalfa and tall fescue when paddocks of were offered as adjacent monocultures when alfalfa was a novel forage (Novelty) and when steers had experience grazing both forages (Experience)

Forage	Treatment			
	Novelty		Experience	
	<u>Pre</u> ¹	<u>Post</u>	<u>Pre</u>	<u>Post</u>
Alfalfa	2,633	2,573	2,873	2,322
Tall fescue	2,309	2,380	2,482	2,296

¹Pre = pre grazing measurement before d 1, Post = post grazing measurement after d 4
SEM = 206

Table 4-3. Forage height (cm) of alfalfa and tall fescue when paddocks of were offered as adjacent monocultures when alfalfa was a novel forage (Novelty) and when steers had experience grazing both forages (Experience)

Forage	Treatment			
	Novelty		Experience	
	<u>Pre</u> ¹	<u>Post</u>	<u>Pre</u>	<u>Post</u>
Alfalfa	31.2 ^{ax}	21.6 ^c	25.5 ^{bx}	20.0 ^c
Tall fescue	23.0 ^{ay}	22.1 ^{ab}	20.3 ^{by}	16.7 ^c

¹Pre = pre grazing measurement before d 1, Post = post grazing measurement after d 4

^{abc}Different superscripts within row indicate difference $P < 0.05$

^{xy}Different superscripts within column indicate difference $P \leq 0.05$

SEM = 1.4

Table 4-4. Proportion of behaviors spent in paddocks of alfalfa and tall fescue as adjacent monocultures when alfalfa was a novel forage (Novelty) and when steers had experience grazing both forages (Experience).

Forage	Treatment					
	Novelty			Experience		
	<u>Grazing</u>	<u>Ruminating</u>	<u>Idling</u>	<u>Grazing</u>	<u>Ruminating</u>	<u>Idling</u>
Alfalfa	77.5 ^a	47.2 ^a	16.6 ^a	72.1 ^b	25.6 ^b	14.6 ^b
Tall fescue	22.5 ^b	52.8 ^b	83.4 ^b	27.9 ^a	74.4 ^a	85.4 ^a
	<u>Standing</u>		<u>Lying</u>	<u>Standing</u>		<u>Lying</u>
Alfalfa	50.1 ^a		8.6	45.7 ^b		8.0
Tall fescue	49.9 ^b		91.4	54.3 ^a		92.0
	SEM					
	<u>Grazing</u>	<u>Ruminating</u>	<u>Idling</u>	<u>Standing</u>	<u>Lying</u>	
	2.2	3.5	1.1	1.6	0.9	

^{ab}Differences between treatment within behavior, and within forage type are different $P < 0.05$

Table 4-5. Meteorological conditions during the experimental periods.

Treatment	Day	Precipitation (mm)	Air temperature (°C)			
			AM		PM	
			<u>High</u>	<u>Low</u>	<u>High</u>	<u>Low</u>
<u>Novelty</u>						
Year 1	1	8	21	20	21	18
	2	9	18	17	21	15
	3	0	21	14	27	17
	4	0	26	15	28	19
Year 2	1	0	27	16	31	20
	2	0	28	16	31	21
	3	0	26	18	29	22
	4	0	29	19	30	22
<u>Experience</u>						
Year 1	1	0	26	15	31	18
	2	0	25	14	30	20
	3	0	28	17	32	21
	4	0	28	20	29	20
Year 2	1	0	29	15	36	23
	2	1	28	19	31	18
	3	0	25	16	29	15
	4	0	26	11	34	19

Table 4-6. Proportion of time in the morning (AM) and afternoon (PM) spent grazing in alfalfa and tall fescue when forages were offered as adjacent monocultures.

Forage	Treatment			
	Novelty		Experience	
	<u>AM</u>	<u>PM</u>	<u>AM</u>	<u>PM</u>
Alfalfa	75.3 ^{ab}	78.6 ^a	68.9 ^b	74.9 ^{ab}
Tall fescue	24.7 ^{ab}	21.4 ^b	31.1 ^a	25.1 ^{ab}

^{ab}Differences within row indicate difference $P < 0.05$
SEM = 4.8

Table 4-7. Carbohydrate fractions¹ of forages being grazed by beef steers as adjacent monocultures.

Forage	Period	NFC	WSC	ESC	Starch
		-----% DM-----			
Alfalfa	AM	31.8 ^b	8.3 ^b	6.7 ^b	1.6
	PM	36.0 ^a	10.5 ^a	9.0 ^a	2.1
Tall fescue	AM	22.1 ^d	8.5 ^b	6.1 ^b	1.2
	PM	25.8 ^c	11.4 ^a	9.3 ^a	1.8
SEM		0.8	0.5	0.3	0.3

¹NFC= non-fiber carbohydrates (simple sugars, fructans, soluble fiber, organic acids), WSC=water soluble carbohydrates (simple sugars, fructans), ESC=ethanol soluble carbohydrates (simple sugars)

^{abcd} Different superscripts within column indicate difference $P < 0.05$

Table 4-8. Diet preference of animals offered adjacent monocultures of two forages at a 50:50 ground area ratio¹

Animal species	Physiological state	Herbage choice ²	% Legume	Reference
Sheep	Lactating	PG/WC	79.7	Parsons et al. (1994)
Sheep	Lactating	PG/WC	71.6	Penning et al. (1995)
Dairy sheep	Lactating	AG/Sulla	74.0	Rutter et al. (2005b)
Dairy cows	Lactating	PG/WC	70.0	Rutter et al. (1999)
Dairy cows	Lactating	PG/WC	78.0	Rutter et al. (2001)
Dairy cows	Lactating	PG/WC	73.8	Rutter et al. (2004a)
Sheep	Dry	PG/WC	65.8	Parsons et al. (1994)
Sheep	Dry	PG/WC	71.0	Harvey et al. (1996)
Sheep	Dry	PG/WC	91.8	Newman et al. (1994)
Sheep	Dry	PG/WC	88.4	Harvey et al. (1997)
Sheep	Dry	PG/WC	66.8	Harvey et al. (2000)
Sheep	Dry	PG/WC	70.0	Cosgrove et al. (2001)
Sheep	Dry	PG/WC	60.0	Rook et al. (2002)
Dairy heifers	Dry	PG/WC	65.0	Cosgrove et al. (1996)
Dairy heifers	Dry	PG/WC	68.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/WC	70.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/Lotus	63.9	Rutter et al. (2004b)
Beef heifers	Dry	PG/WC	60.0	Rutter et al. (2005a)

¹Adapted from Rutter (2006).

²PG = perennial ryegrass (*Lolium perenne* L.), WC = white clover (*Trifolium repens* L.), AG = annual ryegrass (*Lolium multiflorum* L.), Sulla = *Hedysarum coronarium* L., Lotus = *Lotus corniculatus* L.

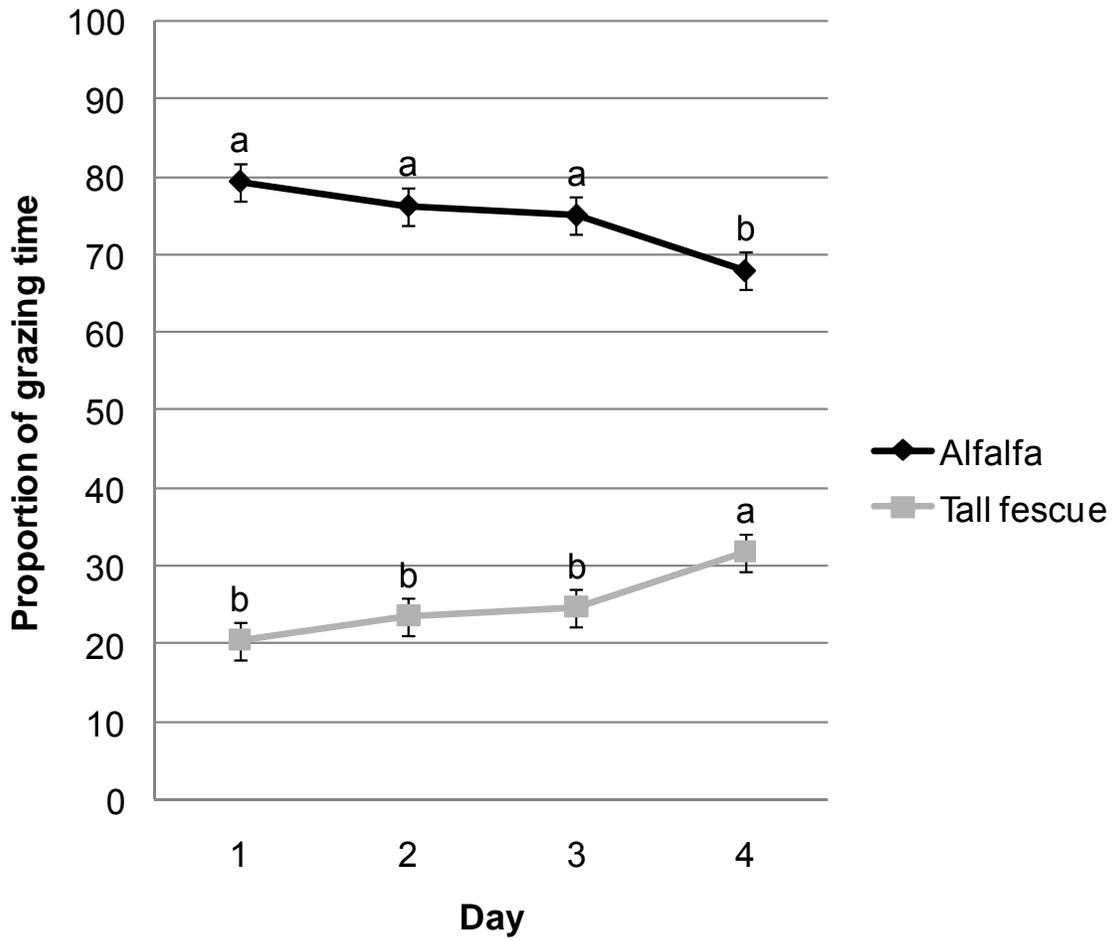


Figure 4-1. Proportion of time spent grazing forages in paddocks of alfalfa and tall fescue as adjacent monocultures.^{ab} Differences within forage indicate difference $P < 0.05$

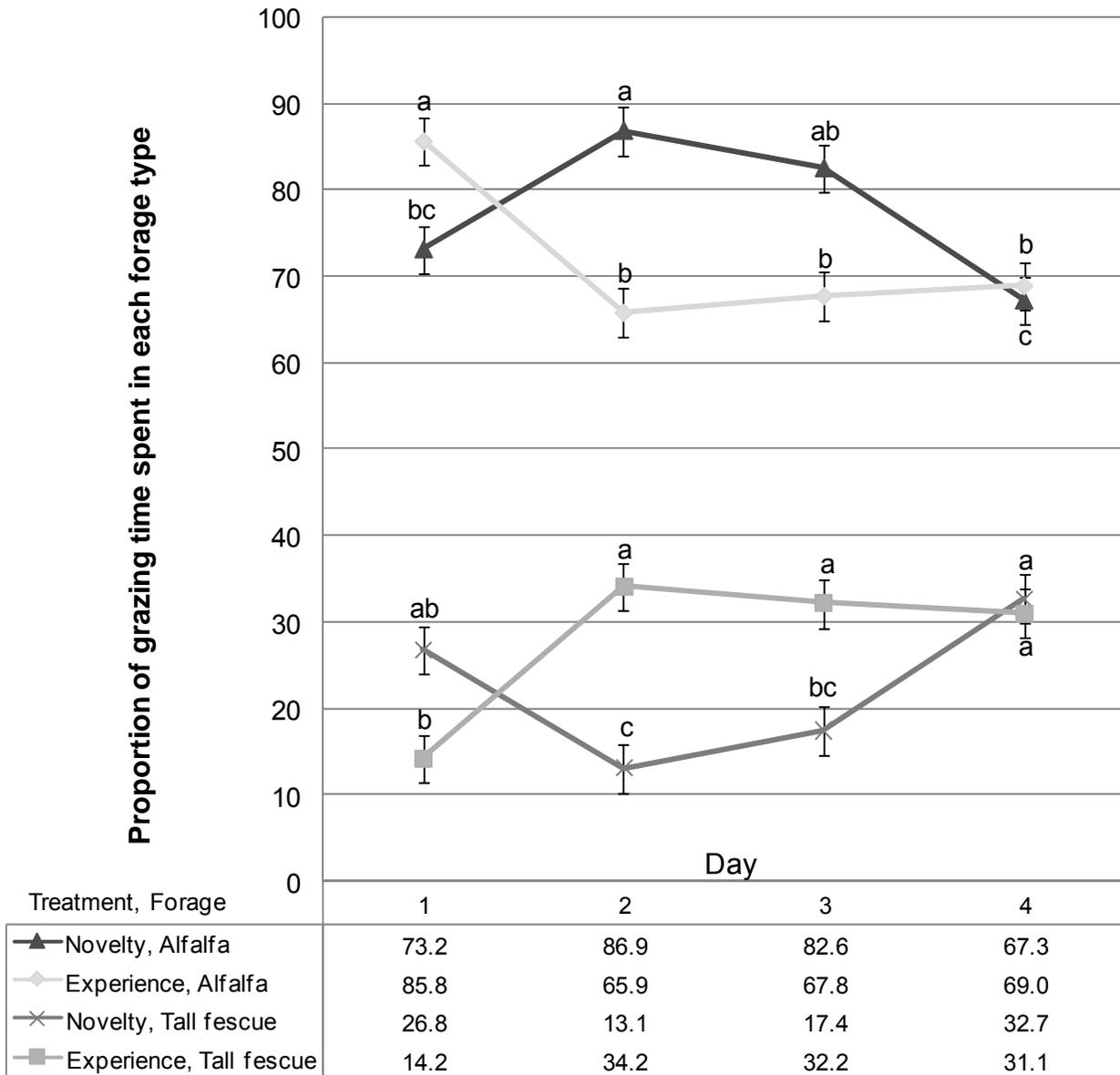


Figure 4-2. Proportion of time spent grazing forages in paddocks of alfalfa and tall fescue as adjacent monocultures when alfalfa was a novel forage (Novelty) and when steers had experience grazing both forages (Experience). ^{abc} Differences within treatment and forage indicate difference $P < 0.05$

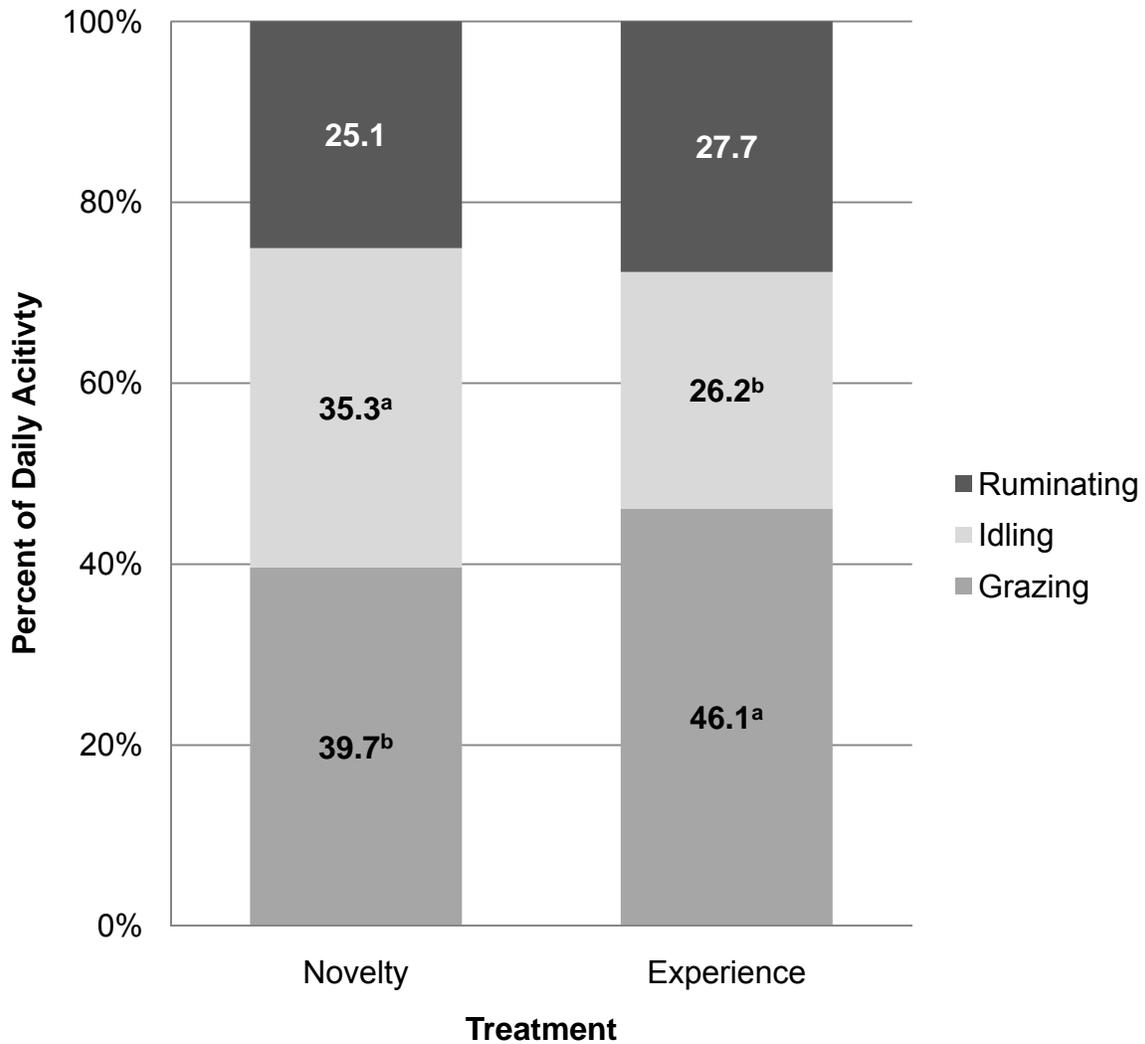


Figure 4-3. Proportion of day steers spent grazing, ruminating, and idling when grazing paddocks of alfalfa and tall fescue as adjacent monocultures when alfalfa was a novel forage (Novelty) and when steers had experience grazing both forages (Experience). SEM: Grazing = 1.0, Ruminating = 1.9, Idling = 1.5. ^{ab} Differences within behavior indicate difference $P < 0.05$

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CHAPTER 5

DRY MATTER INTAKE AND DIET COMPOSITION OF STEERS GRAZING ADJACENT MONOCULTURES OF TALL FESCUE AND ALFALFA

ABSTRACT

The use of n-alkanes has been shown to be a non-invasive and accurate method to estimate dry matter intake (DMI) of grazing herbivores. In recent years the use of other components of the cuticular wax of plants, such as long chain alcohols (LCOH) has been explored for use in estimation of diet composition of animals eating mixed diets. The present study examined different methods of estimating DMI and diet composition of beef steers grazing adjacent monocultures of tall fescue and alfalfa utilizing different chain lengths of n-alkanes and LCOH. The use of LCOH added additional characterization of the forages, but diet composition estimates were not different ($P \geq 0.22$) than when estimated using four different n-alkanes. Diet composition estimation indicated that steers consumed similar ($P = 0.13$) diets of 79% and 70% alfalfa in Yr 1 and Yr 2, respectively. This was 7% more than the observed proportion of time spent grazing that forage in both years during the weeks surrounding this study. Dry matter intake differed ($P = 0.002$) between years values being lower (4.5 kg d^{-1}) in Yr 2 when air temperatures were hotter than in Yr 1 (9.4 kg d^{-1}). This study suggests that if n-alkane profiles of the forages being grazed are distinct, the additional analysis needed to determine LCOH concentrations may not be necessary. Analyzing preliminary forage and fecal samples for n-alkanes and estimating diet composition could reduce costs by eliminating extraneous laboratory analyses.

INTRODUCTION

Estimation of dry matter intake (DMI) and diet composition of free-ranging herbivores is a valuable area of study due to its impact on the health, nutritional status, and productivity of animals as well as by adding to our knowledge of how herbivore foraging behavior can impact biodiversity and the dynamics of the plant community (Ali et al., 2004; Kelman et al., 2003). A considerable amount of research has been conducted using long chain saturated hydrocarbons (n-alkanes) as markers to estimate DMI (Dove and Mayes, 2006, 1991; Mayes et al., 1995; Mayes et al., 1986). Alkanes occur naturally in the cuticular waxes of plants, with the highest concentrations occurring in the odd C chain lengths. These odd-chain length n-alkanes can be used to estimate DMI of animals dosed with even C chain length (artificial) n-alkanes. Dove (1992) reported that between species differences in n-alkane profiles could also be used to determine the proportion of each plant species in the diet. The use of n-alkanes as markers of diet composition relies upon a difference in the n-alkane profiles of these in each plant or food item consumed by the animal being distinct from one another (Bugalho et al., 2002). Another important consideration is the number of different plant species consumed by the animal. As the number of different species increases, the ability of n-alkanes to distinguish one from the next decreases (Mayes and Dove, 2000). The analysis of additional compounds can be used to improve the discrimination between items in the diet (Ali et al., 2004; Bugalho et al., 2002). The long chain fatty alcohols (LCOH), which occur in plants primarily in even C chain lengths, can be used to provide better distinction among plants that are consumed by the animal. Experiments using individually housed animals, in which actual intake and diet

composition could be measured and then compared to the predicted diet composition, have shown that n-alkanes (Lewis et al., 2003) and a combination of n-alkanes and LCOH (Fraser et al., 2006) can accurately predict diet composition. Use of n-alkanes and LCOH has been shown to be a successful method of determining diet composition in grazing trials as well (Kelman et al., 2003). Estimations of diet composition utilizing LCOHs in combination with n-alkanes was shown to provide additional characterization of the diet compared with estimations based on n-alkanes alone (Kelman et al., 2003).

Grazing behavior data alone (grazing time and bite rate), as discussed in the previous chapters, cannot be used to determine DMI or diet composition of grazing animals. Therefore an intake trial was conducted to evaluate the diet of steers grazing forages that had not been previously evaluated as adjacent monocultures. The objectives of this study were to 1) determine the DMI of beef steers grazing adjacent monocultures of tall fescue (*Festuca arundinacea* Schreb. or *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) and alfalfa (*Medicago sativa* subsp. *sativa* L.) across two years using naturally occurring and dosed n-alkanes and 2) to determine diet composition based using n-alkanes and LCOH.

MATERIALS AND METHODS

The procedures used in this experiment were approved by the Virginia Tech Institutional Animal Care and Use Committee. This experiment was conducted at Kentland Farm (37°11' N, 80°35' W), Blacksburg, Virginia, from August 9 to 22, 2006 (Yr 1) and August 2 to 15, 2007 (Yr 2). The experiment evaluated steer DMI when grazing endophyte-free tall fescue and alfalfa in equal proportions of ground area. To determine

proportion of each forage type in the diet two techniques were evaluated: n-alkanes and LCOH.

Pasture establishment and management

Pastures were established in 2004. All paddocks were sprayed with Roundup® (9 L ha⁻¹, glyphosate 41%, Monsanto, St. Louis, MO) in May to eliminate existing pasture species. Two weeks after spraying, foxtail millet (*Setaria italica*, 34 kg ha⁻¹) was planted as a suppression crop. In late July, the millet was harvested for hay and two weeks later the paddocks were sprayed again with Roundup® (2 L ha⁻¹) for weed suppression. Tall fescue pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0); the alfalfa pastures received 168 kg ha⁻¹ of diammonium phosphate (18-46-0) + 112 kg ha⁻¹ of potash (0-0-60). On September 2 and 5, paddocks were planted with Jesup endophyte-free tall fescue (28 kg ha⁻¹) or with AmeriStand 403T alfalfa (22 kg ha⁻¹). Nitrogen was applied to the tall fescue paddocks at 34 kg ha⁻¹ in October and then again in March, 2005 to promote vigorous growth and tillering. Herbicides were applied in April, 2005. All alfalfa pastures received 37 mL ha⁻¹ of Harmony GT® (thifensulfuron-methyl 75%, Dupont, Wilmington, DE) and 2 L ha⁻¹ Poast Plus® (sethoxydim 13%, BASF, Research Triangle Park, NC) and 1 L ha⁻¹ of surfactant. All fescue pastures received 1 L ha⁻¹ of 2-4D (2,4-Dichlorophenoxyacetic acid 46.6%, Helena Holding Co., Wilmington, DE) and 0.6 L ha⁻¹ Banvel® (3,6-dichloro-0-anisic acid 48.2%, Micro Flo, Memphis, TN).

Pastures were grazed by stocker steers during the summer grazing seasons of 2005 and 2006. Pastures were fertilized in April, 2006 and 2007 with tall fescue receiving 35-20-45 (224 kg ha⁻¹) and alfalfa receiving 0-46-0 (145 kg ha⁻¹) and Boron (2

kg ha⁻¹). Alfalfa paddocks were sprayed with Harmony GT[®] in April of Yr 1 and Yr 2 for control of thistle and with Baythroid[®] (β-cyfluthrin 12.7%, Bayer, Research Triangle Park, NC) for insect control in July of Yr 2 according to manufacturer recommendations.

Animals and fecal sampling

Twenty-four Angus-crossbred steers (12 steers yr⁻¹, Yr 1 initial BW = 392 ± 8 kg, Yr 2 initial BW = 323 ± 9 kg, 16 mo old) were used. Steers grazed in groups of three allotted to group by initial BW. Each group was then randomly allotted to paddocks with four paddocks total. Paddocks were 0.22 ha of tall fescue monoculture adjacent to 0.22 ha of alfalfa monoculture. Within each treatment paddock, areas of tall fescue and alfalfa were contiguous so that steers could move freely between each forage type. Water troughs and trace mineral salt with poloxalene as an anti-bloat agent (Bloat Guard[®] Pressed Block, Sweetlix Livestock Supplement System, Mankato, MN) were provided ad libitum and located at the midpoint of the paddock where the two forage types met so that the steer's need to drink and consume mineral did not influence into which forage area the steer would travel.

Steers were dosed on d -7 (0730) with an intra-ruminal n-alkane controlled release fecal marker capsule (CRC) for 300 to 650 kg cattle (Captec Ltd., Argenta, Auckland, NZ). Each capsule contained 8 g of n-dotriacontane (C₃₂) and 8 g of n-hexatriacontane (C₃₆) with a release rate of 400 mg d⁻¹, according to the manufacturer. A period of 7 d was allowed for alkane concentration in the feces to stabilize. Feces were collected at 0730 and 1630 from d 0 to 6. Fecal samples from each steer were collected either by rectal grab sampling or after the steer defecated on the ground.

Samples from the ground were carefully collected avoiding contamination by foreign matter such as soil or plant material. Samples were stored in plastic bags and packed on ice for transport and subsequently frozen for storage prior to analyses in the laboratory at -20 °C.

Total fecal collection was performed with three steers from Yr 1. This allowed for release rate to be determined specifically for the conditions in the present experiment. Steers were halter broken 3 mo prior to the experiment. Two wk before steers were dosed with the CRC the three steers were acclimated to wearing fecal collection bags. This process began by putting the bag on the steers for a period of 4 h on first day of training, increasing the length of time by 4 to 8 h each day until steers were acclimated. The harness and bags used have been described by Tolleson and Erlinger (1989). Fecal collection bags were weighed empty and placed on the steers at 1630 on d⁻¹. At 0730 and 1630 on d 0 to 6, steers were placed into a chute where the bag was removed, the contents weighed, and a new empty bag fitted to each steer. Feces were mixed thoroughly and a subsample collected. A separate grab sample was also collected from the steer while in the chute. Samples were stored in plastic bags and packed on ice for transport and subsequently frozen for storage prior to analyses in the laboratory at -20 °C. Steers were moved back to their respective paddock after each sample collection.

In preparation for analyses, fecal samples were partially thawed in a walk-in refrigerator at 5 °C then brought to room temperature (22 °C). A composite sample for each steer from each day was prepared by weighing an aliquot of approximately 10 g of the AM and 10 g of the PM sample of the given day and combining them in a single

beaker. The beaker was then covered with cheesecloth and the composite sample freeze-dried (25L Genesis SQ EL-85, VirTis). After drying, samples were weighed and then ground to 0.5mm (Cyclotec 1093 Sample Mill, Foss Tecator).

Forage sampling and analyses

Forage mass and sward height measurements were taken on the day prior to the experiment in each year. Three areas of 0.25 m² were randomly selected from each paddock of tall fescue and alfalfa and 10 measurements of sward height were taken within those areas at each sampling. The three areas were then clipped to 2.5 cm above ground level, forage placed in cloth bags, dried in a forced draft oven at 60°C for 48 h to determine forage mass.

Hand-plucked samples of each forage type were taken for nutritive value analysis on d -1, 3, and 6. Samples were collected while walking along a cross-section of the paddock and grabbing a sample every 10 steps from the top 7 to 10 cm of the sward, representing forage being consumed by the steers. Samples were packed on ice after collection and subsequently frozen in the laboratory at -20 °C. Samples were freeze-dried (25L Genesis SQ EL-85, VirTis, Gardiner, NY) and were then ground through a 1-mm screen using a Wiley mill. Samples were then analyzed for chemical composition by NIRS (Foss NIRSystems 6500, Foss Tecator, Eden Prairie, MN, AOAC, 2000) at a commercial laboratory (Dairy One, Ithaca, NY). Tall fescue subsamples were further ground to 0.5mm (Cyclotec 1093 Sample Mill, Foss Tecator, Eden Prairie, MN) and analyzed for alkaloid concentration (Agrinostics Ltd. Co., Watkinsville, GA) to ensure

they were at low endophyte levels. Mean alkaloid level in Yr 1 was 111 ng g⁻¹ (SEM = 18) and in Yr 2 was 51 ng g⁻¹ (SEM = 5).

Hand-plucked samples of each forage type for analysis of n-alkanes and LCOHs were taken from d -2 to d 4. Assuming an approximate 48-h retention time of consumed forage in the gastrointestinal tract (Burns et al., 1991), forage sampling began 2-d before fecal sampling. Samples were obtained while walking along a cross-section of the paddock and grabbing a sample every 10 steps from the top 7 to 10 cm of the sward, representing forage being consumed by the steers. Samples were packed on ice after collection and subsequently frozen in the laboratory at -20 °C. Samples were later freeze-dried (25L Genesis SQ EL-85, VirTis, Gardiner, NY) and then ground through a 1-mm screen using a Wiley mill. Subsamples were further ground to 0.5mm (Cyclotec 1093 Sample Mill, Foss Tecator, Eden Prairie, MN) in preparation for marker extraction.

n-Alkane and LCOH analyses

The protocol of Dove and Mayes (2006) was used for all sample analyses. Samples from both years were prepared and analyzed at the same time. Each freeze-dried and ground sample was analyzed in duplicate, with 0.1 g of feces weighed into 15 x 45 mm glass GC vials and 0.2 g of forage weighed into 16 x 100 mm glass culture tubes. An alkane internal standard solution of 0.3 mg g⁻¹ of n-docosane (C₂₂) and n-tetratriacontane (C₃₄) in n-decane was prepared and 0.11 g was added to each sample. An LCOH internal standard solution of 1-heptacosanol (C₂₇) at 1.3 mg g⁻¹ was prepared in a 50:50 solution (by volume) of ethanol: n-heptane and 0.15 g added to each sample. Ethanoic KOH (1 M) was added to each sample, and then tubes were sealed with

PTFE-lined caps and heated in a dry block heater (DB3, Techne Inc., Burlington, NJ) at 90 °C for 16 h. After heating, tubes were cooled to 50 to 60 °C and n-heptane was added. Tubes were shaken gently then water was added and tubes vortexed for 5 to 10 s. The non-aqueous layer was removed and added to another vial and a second n-heptane extraction performed. Non-aqueous extract was evaporated to a volume of 0.3 ml and gently applied to a column with a 1 ml silica gel bed volume and PTFE frits. Extract was washed into the silica gel bed with n-heptane and hydrocarbons eluted into a vial. Eluate was then evaporated on the dry block heater using a sample concentrator (FSC400D, Techne Inc., Burlington, NJ). The dried hydrocarbon fraction was stored until time for GC analysis.

The crude LCOH fraction was obtained by adding an 80:20 solution (by vol) of heptanes: ethyl acetate to the same silica gel column. The eluate was collected and evaporated on the dry block heater. Sterols and pigments were removed from the dried crude LCOH fraction by re-dissolving with n-heptane and applying solution to an aminopropyl SPE column (Supelclean LC-NH₂, Supelco, Bellefonte, PA) in a positive pressure manifold (Cerex SPE processor, Varian Inc., Palo Alto, CA). Additional n-heptane was applied to the column and run to waste with compressed air applied at a rate sufficient to elute 1 ml of solvent in 30 s. A solution of n-heptane: ethyl acetate at 95:5 (by vol) followed by a 90:10 solution was then added to the column, air pressure applied, eluate collected, and eluate then evaporated to dryness. Acetate derivatives were formed from this purified eluate by adding a solution of acetic anhydride/pyridine (20:80 by vol). Vials were capped, mixed, and heated overnight at 50 °C. After heating, water was added and the solution extracted with n-heptane twice. The top non-aqueous

fraction was collected and evaporated to dryness. Dodecane was added to the alcohol acetates and stored until time for GC analysis.

Analyses were conducted on a gas chromatograph with a flame ionization detector, autosampler, and integrator (GC6890, 7683, Agilent Technologies, Santa Clara, CA). A bonded phase, non-polar capillary column was used (30m x 0.53mm i.d., 1.5 μm film thickness, Rtx⁻¹, Restek Inc., Bellefonte, PA). Splitless sample injection (1.0 μl) was used with helium as the carrier gas (4 ml min⁻¹), hydrogen as fuel gas (35 ml min⁻¹), air as oxidant gas (350 ml min⁻¹) and nitrogen as make-up gas (50 ml min⁻¹). Injector was maintained at 280 °C with the detector at 330 °C. The oven was held at 170 °C for 4 min, ramped to 215 °C (30 °C min⁻¹) and held for 1 min, then ramped to 300°C (6 °C min⁻¹) and held for 16 min. Mixed reference standards were prepared and analyzed at the beginning of every run and after every ten injections along with a blank injection. The n-alkanes standard included the following chain lengths: C₂₁ to C₃₆. The LCOH standard was prepared with the same procedure as samples to obtain the proper derivatives. This standard included LCOH of the following chain lengths: C₁₈ to C₂₈ and C₃₀.

Data analyses

Dry matter intake was estimated using the ratio of C₃₁ (naturally occurring alkane) to C₃₂ (dosed alkane). When the measured concentrations of an n-alkane are low (minimum of 50 mg kg⁻¹ DM) they are not as suitable to use for intake estimation as n-alkanes appearing in greater concentration (Laredo et al., 1991). Because C₃₃ was near or below 50 mg kg⁻¹ DM (Table 5-1), C₃₁ was used for the calculation of DMI.

Recovery rate of the dosed alkane was determined to be 95% based on total fecal collection. Daily herbage intake was calculated is as follows:

$$I = \frac{\frac{F_j}{F_i} * D_j}{H_i - \frac{F_j}{F_i} * H_j}$$

Where:

I = dry matter intake (kg d⁻¹)

i = C₃₁, naturally occurring alkane

j = C₃₂, external alkane marker

D_j = the weight of dosed j (mg d⁻¹)

F_j = the concentration of j in feces (mg kg⁻¹ DM)

F_i = the concentration of i in feces (mg kg⁻¹ DM)

H_i = the concentration of i in forage (mg kg⁻¹ DM)

H_j = the concentration of j in the forage (mg kg⁻¹DM)

(Dove and Mayes, 1991)

The method in which DMI is calculated in this equation required the concentration of the odd chain n-alkane in the forage. In this experiment two forages were consumed by the steers and these forages had different concentrations of C₃₁. In cases where animals are grazing adjacent monocultures or a mixed sward with different forages having different n-alkane concentrations, a single hand plucked forage sample that is not separated by forage species may not contain the same proportion of each forage that the animal consumed. Or, if diet composition is not estimated and an average of C₃₁ in the two forages is used, the resulting estimation may not accurately reflect DMI if animals have a partial preference for one forage. In such cases DMI may be under or over estimated if n-alkane profiles of the forages vary. In the present study DMI was calculated with different ratios forage C₃₁ concentrations of tall fescue and alfalfa to examine possible differences in DMI estimation.

The diet composition, in terms of proportion of each forage species consumed, was estimated by using the non-negative least squares procedure of Dove and Moore (1995) with the 'EatWhat' computer program. The software compared forage marker profile to fecal marker concentrations and determined the proportion of each forage species in the diet. The software algorithm minimized the squared deviations between the marker concentration in the fecal sample and the concentration profile that came from the diet composition estimation as follows:

$$\sum_{alc: 1 \dots n} [F_i - xA_i + yB_i + zC_i]^2$$

Where:

F_i = the concentration of the marker i in the feces

x, y and z = amounts of diet components A, B , and C

A_i, B_i , and C_i = respective concentration of i in A, B , and C

(Dove and Mayes, 1991)

Fecal alkane concentrations were adjusted for incomplete recovery based on the values derived by Mayes et al. (1986). Long chain alcohol concentration in the feces was adjusted for a recovery of 80% for all chain lengths based on the values of Ashton (1998). Diet composition was estimated by 4 combinations of the n-alkanes (C_{27} , C_{29} , C_{31} , and C_{33}) and LCOH (C_{26} , C_{28} , and C_{30}). Lewis et al. (2003) determined that use of C_{27} and C_{29} n-alkanes for diet composition analysis provided estimations that did not match the actual diet composition measured in penned sheep fed pelleted ryegrass and alfalfa diets ad libitum. This was due to the similarity in the concentrations of C_{27} and C_{29} n-alkanes of the two feeds in that study. When this occurs or when the concentrations are low, they will not likely add any additional characterization of the plants to differentiate them from one another in determining diet composition. In the

present study the concentrations (Table 5-1) of C₂₇ were similar between forage types and both it and C₃₃ were around the 50 mg kg DM⁻¹ level consider low for use in intake calculations (Laredo et al., 1991). Therefore, 4 different combinations of n-alkanes and LCOH were analyzed by 'EatWhat'. These combinations were as follows: 4 n-alkanes + 3 LCOH = n-alkanes: C₂₇, C₂₉, C₃₁, C₃₃ and LCOH: C₂₆, C₂₈, C₃₀; 2 n-alkanes + 3 LCOH = n-alkanes: C₂₉, C₃₁ and LCOH: C₂₆, C₂₈, C₃₀; 4 n-alkanes = C₂₇, C₂₉, C₃₁, C₃₃; and 2 n-alkanes = C₂₉, C₃₁. Angular transformation of diet composition data was conducted after 'EatWhat' analysis (Parsons et al., 1994). The angular transformed percentage of alfalfa and tall fescue in the diet was then analyzed to determine if differences existed in the diet composition estimation method using the four combinations.

The experimental unit for all analyses was the paddock (3 steers) because individuals within a group are not considered statistically independent (Rook and Penning, 1991). Data were then analyzed using SAS (version 9.1.3, Cary, NC). Least squares means are reported for all variables with means separated by Tukey's adjustment. A significance level of $\alpha \leq 0.05$ was set for all analyses with trends defined as $0.10 > \alpha > 0.05$.

Diet composition was analyzed using the MIXED procedure and the compound symmetry (cs) covariance structure. The repeated measure was day within yr. The model included yr, method (n-alkane and LCOH combinations) and their interaction. Repeated measure was day within yr. Analysis methods found to be similar were then used in further analysis of the data with a model including yr, day, and their interaction.

Estimations of DMI were analyzed using the MIXED procedure and the compound symmetry (cs) covariance structure. The repeated measure was day within yr. The model included yr, method (C₃₁ ratios of tall fescue and alfalfa at 25:75, 30:70, 50:50, 70:30, and 75:25) and their interaction. Repeated measure was day within yr. Analysis method determined to be similar to diet composition analysis was then used in further analysis of the data with a model including yr, day, and their interaction.

Alkane and LCOH profiles were analyzed using the MIXED procedure and the compound symmetry (cs) covariance structure. The model included yr, forage type (alfalfa or tall fescue), and their interaction. Repeated measure was day within yr.

Forage nutritive value and sward measurement data were analyzed using the MIXED procedure. The model included yr, forage type (alfalfa or tall fescue), and their interaction. The experimental unit was the paddock within yr.

RESULTS AND DISCUSSION

Forage and sward measurements

Crude protein and lignin were higher ($P < 0.0001$) in alfalfa than tall fescue while NDF and ADF were lower ($P \leq 0.0008$) in alfalfa (Table 5-2) than tall fescue. The nutritive value of alfalfa was greater in Yr 2 than Yr 1 with higher CP ($P = 0.01$) and lower NDF ($P = 0.007$) and ADF ($P = 0.003$). Tall fescue was mostly similar between years except for higher ($P = 0.006$) NDF and lignin in Yr 2. Sward height in Yr 1 was similar between alfalfa and tall fescue (31.2 and 25.1 cm, respectively; $P = 0.61$, SEM =

3.4), but differed ($P = 0.006$) between the forages in Yr 2 (39.5 and 20.7 cm, respectively; SEM = 3.0). No difference in forage mass between years ($P = 0.12$) or forage types ($P = 0.37$) was observed. Forage mass of alfalfa was 2,702 kg ha⁻¹ in Yr 1 and 2,467 kg ha⁻¹ in Yr 2 (SEM = 312). Forage mass of tall fescue was 2,236 kg ha⁻¹ in Yr 1 and 1,932 kg ha⁻¹ in Yr 2 (SEM = 267).

Marker concentrations

The n-alkane profiles of tall fescue and alfalfa (Table 5-1) were similar to those previously reported (Bovolenta et al., 1994). Effects of yr ($P \leq 0.03$) within forage type were observed for all chain lengths except for C₂₇ for which there tended to be a yr effect ($P = 0.07$) in alfalfa, and no effect of yr ($P = 0.22$) for tall fescue. The n-alkane C₃₅ was not detected in either species. The higher concentrations of n-alkanes in Yr 2 may have been due to the hot and dry conditions in that year (Table 5-3). Cuticular wax deposition can increase in plants during drought as a moisture conservation strategy (Shepard and Griffiths, 2006).

The LCOH profile of tall fescue was also similar to previous data (Bughalo et al., 2004); however information on the LCOH profile of alfalfa was not available (Table 5-4). A yr effect for C₃₀ ($P = 0.003$) was observed and there tended to be a yr effect ($P \leq 0.09$) for both C₂₆ and C₂₈, but the pattern of effect (whether an increase or decrease in concentration) varied between forage type and chain lengths.

Proportion of forages in the diet

Diet composition data calculated by 'EatWhat' is presented in Figure 5-1. There was an effect of analysis method on the estimated proportions of tall fescue and alfalfa.

When using only the n-alkanes C₂₉ and C₃₁, diet composition was different ($P \leq 0.0004$) from all other analysis methods. Diet composition calculations including all three LCOH and n-alkanes concentrations was similar ($P \geq 0.22$) to that estimated when using all LCOH + 2 n-alkanes or all 4 n-alkanes together. This indicates that even though concentrations of n-alkanes C₂₇ and C₃₃ were low, they did provide additional discrimination between the forages when only n-alkanes were considered. When only C₂₉ and C₃₁ n-alkanes were used, over 66% of the diet composition estimates were calculated as either 100% alfalfa or 100% tall fescue. Using only the 2 n-alkanes did not seem to provide enough discrimination between the 2 forage species for 'EatWhat' to estimate diet composition.

Diet composition values using the 3 similar marker methods were used to further analyze effects of yr and day. Diet composition did not differ ($P = 0.13$) between yr of the trial and was 79 and 70% alfalfa in Yr 1 and Yr 2, respectively. No effect of day ($P = 0.15$) on diet composition was observed, with proportion of alfalfa in the diet numerically declining from d 1 to 7 (Figure 5-2). The decline, although not significant, in proportion of alfalfa over time may be due to depletion of leaves in the alfalfa sward.

This experiment was conducted during the time between to the Novelty and Experience treatment periods reported in the previous chapter. The steers used here were the same animals used in that experiment (along with 3 additional steers in each year from that had been managed under the same grazing protocols). Although grazing behavior was not analyzed concurrently with intake determination, it could be assumed that grazing behavior would not differ greatly since conditions of the sward and meteorological conditions were also similar. The proportion of time spent grazing alfalfa

was 86 and 63% in Yr 1 and Yr 2, respectively. In both yr, there was a difference of 7% between proportion of alfalfa in diet and time spent grazing. This would indicate that intake rate was likely similar between yr, and that time spent grazing alfalfa overestimated proportion of alfalfa in diet by 7%. Proportion of alfalfa in the diet of these steers is very similar to previous reports of other adjacent monoculture studies of a legume and a grass (Table 5-5).

Dry matter intake estimation

Dry matter intake estimations comparing C_{31} calculated at different ratios of tall fescue to alfalfa are presented in Figure 5-3. The ratio of forages used in the DMI calculation did have an effect on the estimated values. Using a ratio of 50:50 the forage C_{31} concentrations resulted in a different ($P < 0.0001$) DMI estimation from all other ratio estimations. The lack of difference between the 70:30, 75:25 ($P = 0.45$) or 25:72, 30:70 ($P = 0.84$) ratios indicated that when forage n-alkane ratios were within $\pm 5\%$ of actual intake proportions the estimates of DMI were similar.

Based on the diet composition analysis using 'EatWhat', it appears the steers were consuming between 79.6 and 65.8% alfalfa (Figure 5-1). Since the 70:30 and 75:25 ratio estimations of DMI fall between those values, it can be assumed that meanDMI of steers was near 7.1 and 7.0 kg d⁻¹ for the two years. However, there was a difference ($P = 0.002$) observed between yr for DMI (Table 5-6). The steers in Yr 1 consumed more than steers in Yr 2 (9.4 and 4.5 kg d⁻¹, respectively). This is probably due to air temperatures in Yr 2 being higher than in Yr 1 (Table 5-3). As air temperatures increase cattle will decrease grazing time (Findlay, 1958) and DMI

(Mitlohner et al., 2001). Other reports of DMI of cattle grazing adjacent monocultures of tall fescue and alfalfa were not available. However, some data on mixed swards has been reported. The DMI of steers in Yr 1 was similar to that reported by Scaglia et al. (2005) of steers grazing a mixed sward of tall fescue and alfalfa (9.9 kg d⁻¹). Seman et al. (1999) estimated that cattle grazing mixed swards of tall fescue and alfalfa (33 or 67% alfalfa content) removed between 5.3 and 6.3 kg of forage from those swards each day, respectively.

The 75:25 DMI results were analyzed to evaluate effect of day which was found to be significant ($P = 0.0006$). With both yr combine, DMI decreased from d 1 to 7 (7.3 to 6.0 kg d⁻¹, respectively). Looking at the data by year there was a decline in values from d 1 to 7 in Yr 2 with DMI being greater on d 1 than 7 (Figure 5-4). These results may be related to minor disruptions in normal grazing behavior due to fecal sample collections in the field.

CONCLUSIONS

The results of this study confirmed that beef steers grazing adjacent monocultures of tall fescue and alfalfa have a partial preference for alfalfa over tall fescue ranging from 79 to 70% of consumed forage and maintained that ratio of intake over a 7 d period. This supports the theory that cattle will select a diet with a larger proportion of legume than grass regardless of the forage species. These results indicate the importance of knowing the diet composition of grazing animals if DMI of mixed species paddocks is going to be estimated. The impact of heat stress on DMI was shown as well, and interestingly the steers in each year consumed equivalent

proportions of alfalfa to tall fescue in their diet even when DMI was lower in Yr 2. The comparison of different combinations of n-alkanes and LCOH as markers for determining diet composition illustrates how selecting markers that adequately discriminate between forage species is important. While having more markers generally means that greater characterization of the forages will occur, it may not always be necessary if the forage species are distinct enough from one another. This will vary between experiments, but could save analysis costs if additional analyses are not needed. Preliminary GC analysis of n-alkane concentrations of forages being grazed should be analyzed with software such as EatWhat first to ensure that their profiles are distinct enough for differentiation if additional markers such as LCOH are not going to be analyzed.

Table 5-1. Least squares mean concentrations (mg kg DM⁻¹) of n-alkanes of forages grazed as adjacent monocultures.

Forage	Year ¹	n-alkanes				
		C ₂₇	C ₂₉	C ₃₁	C ₃₃	C ₃₅
Alfalfa	1	48.8	89.8 ^{bx}	169.2 ^{bx}	39.9 ^{bx}	0
	2	61.5 ^x	140.9 ^{ax}	306.7 ^{ax}	61.9 ^{ax}	0
Tall Fescue	1	42.9	53.8 ^{by}	106.3 ^{by}	50.6 ^{by}	0
	2	48.5 ^y	87.3 ^{ay}	165.7 ^{ay}	80.9 ^{ay}	0
SEM		2.9	5.3	13.3	2.1	-

¹1 = 2006, 2 = 2007

^{ab}Different superscripts within column within forage type indicate difference P < 0.05

^{xy}Different superscripts within column within year indicate difference P < 0.05

Table 5-2. Nutritive value of forages being offered to beef steers as adjacent monocultures.

Forage	Year ¹	CP	NDF	ADF	Lignin
		-----% DM-----			
Alfalfa	1	22.1 ^b	38.1 ^c	28.6 ^b	7.7 ^a
	2	26.7 ^a	31.4 ^d	23.9 ^c	7.5 ^a
Tall Fescue	1	17.1 ^c	50.1 ^b	29.4 ^a	4.1 ^d
	2	14.7 ^c	57.0 ^a	31.5 ^a	5.5 ^c
SEM		1.0	1.3	0.8	0.2

¹1 = 2006, 2 = 2007

^{abcd} Different superscripts within column indicate difference $P < 0.05$

Table 5-3. Meteorological conditions during the intake determination periods of steers grazing adjacent monocultures of tall fescue and alfalfa.

Year	Day	Air temperature (°C)	
		High	Low
2006 (Yr 1)	1	28	15
	2	28	18
	3	29	15
	4	27	14
	5	29	12
	6	30	15
	7	27	18
2007 (Yr 2)	1	31	19
	2	34	20
	3	34	19
	4	33	19
	5	31	18
	6	32	19
	7	31	14

Table 5-4. Least squares mean concentrations (mg kg DM⁻¹) of long chain alcohols (LCOH) of forages grazed as adjacent monocultures.

Forage	Year ¹	LCOH		
		C ₂₆	C ₂₈	C ₃₀
Alfalfa	1	168.6 ^y	29.5 ^y	928.7 ^{ax}
	2	155.5 ^y	39.7 ^y	1397.9 ^{bx}
Tall fescue	1	651.7 ^x	107.0 ^{ax}	1.8 ^y
	2	617.1 ^x	77.6 ^{bx}	1.1 ^y
SEM		13.1	4.6	64.2

¹1 = 2006, 2 = 2007

^{ab}Different superscripts within column within forage type indicate difference P < 0.05

^{xy}Different superscripts within column within year indicate difference P < 0.05

Table 5-5. Diet preference of animals offered adjacent monocultures of two forages at a 50:50 ground area ratio¹

Animal species	Physiological state	Herbage choice ²	% Legume	Reference
Sheep	Lactating	PG/WC	79.7	Parsons et al. (1994)
Sheep	Lactating	PG/WC	71.6	Penning et al. (1995)
Dairy sheep	Lactating	AG/Sulla	74.0	Rutter et al. (2005b)
Dairy cows	Lactating	PG/WC	70.0	Rutter et al. (1999)
Dairy cows	Lactating	PG/WC	78.0	Rutter et al. (2001)
Dairy cows	Lactating	PG/WC	73.8	Rutter et al. (2004a)
Sheep	Dry	PG/WC	65.8	Parsons et al. (1994)
Sheep	Dry	PG/WC	71.0	Harvey et al. (1996)
Sheep	Dry	PG/WC	91.8	Newman et al. (1994)
Sheep	Dry	PG/WC	88.4	Harvey et al. (1997)
Sheep	Dry	PG/WC	66.8	Harvey et al. (2000)
Sheep	Dry	PG/WC	70.0	Cosgrove et al. (2001)
Sheep	Dry	PG/WC	60.0	Rook et al. (2002)
Dairy heifers	Dry	PG/WC	65.0	Cosgrove et al. (1996)
Dairy heifers	Dry	PG/WC	68.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/WC	70.0	Torres-Rodriguez et al. (1997)
Dairy heifers	Dry	PG/Lotus	63.9	Rutter et al. (2004b)
Beef heifers	Dry	PG/WC	60.0	Rutter et al. (2005a)

¹Adapted from Rutter (2006).

²PG = perennial ryegrass (*Lolium perenne* L.), WC = white clover (*Trifolium repens* L.), AG = annual ryegrass (*Lolium multiflorum* L.), Sulla = *Hedysarum coronarium* L., Lotus = *Lotus corniculatus* L.

Table 5-6. Dry matter intake of steers grazing adjacent monocultures of tall fescue and alfalfa.

Ratio ¹	2006 (Yr 1)	2007 (Yr 2)	2006 (Yr 1)	2007 (Yr 2)
	-----kg d ⁻¹ -----		-----g kg BW ⁻¹ -----	
75:25	9.3 ^a	4.6 ^b	23.2 ^a	14.2 ^b
70:30	9.5 ^a	4.7 ^b	23.7 ^a	14.6 ^b
SEM	0.5		1.2	

¹ Alfalfa to tall fescue n-alkane C₃₁ concentration ratio used for intake calculation

^{ab} Different superscripts within ratio within measurement indicate difference $P < 0.05$

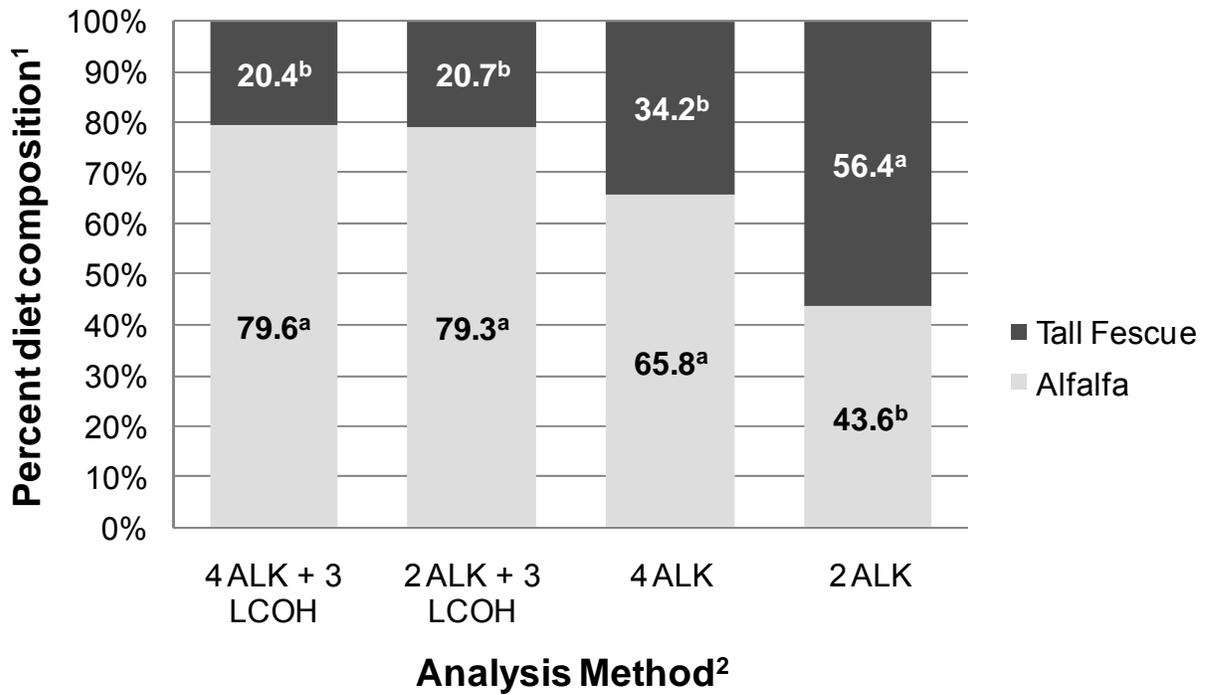


Figure 5-1. Diet composition of steers grazing adjacent monocultures of tall fescue and alfalfa using different combinations of n-alkanes (ALK) and long chain fatty alcohols (LCOH) as markers. Angular transformed SEM = 3.3

¹Non-transformed percentage units

²4 ALK + 3 LCOH = ALK: C₂₇, C₂₉, C₃₁, C₃₃ and LCOH: C₂₆, C₂₈, C₃₀; 2 ALK + 3 LCOH = ALK: C₂₉, C₃₁ and LCOH: C₂₆, C₂₈, C₃₀; 4 ALK = C₂₇, C₂₉, C₃₁, C₃₃; 2 ALK = C₂₉, C₃₁

^{ab} Different superscripts within forage type indicate difference $P < 0.05$

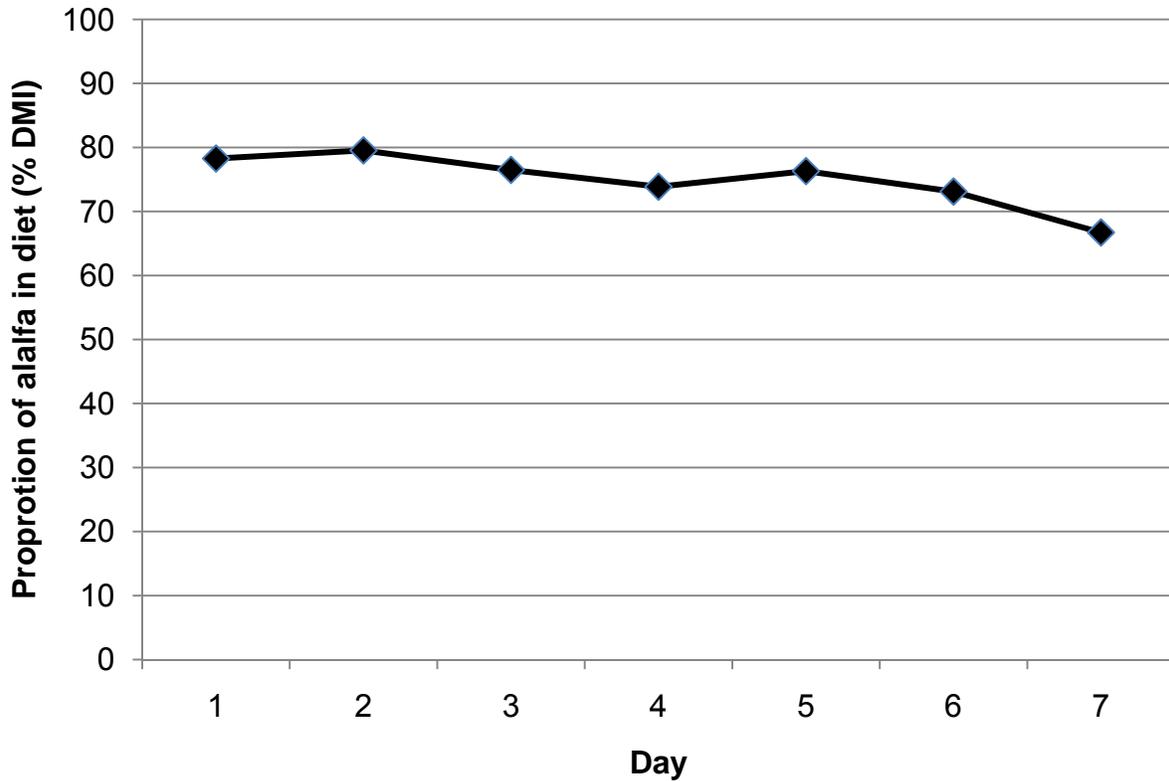


Figure 5-2. Daily diet composition^{1,2} of steers grazing adjacent monocultures of tall fescue and alfalfa. Angular transformed SEM = 3.2

¹Non-transformed percentage units

²Data calculated from the use of the three similar analysis methods: ALK + 3 LCOH = ALK: C₂₇, C₂₉, C₃₁, C₃₃ and LCOH: C₂₆, C₂₈, C₃₀; 2 ALK + 3 LCOH = ALK: C₂₉, C₃₁ and LCOH: C₂₆, C₂₈, C₃₀; 4 ALK = C₂₇, C₂₉, C₃₁, C₃₃ (where ALK= n-alkanes and LCOH = long chain alcohols)

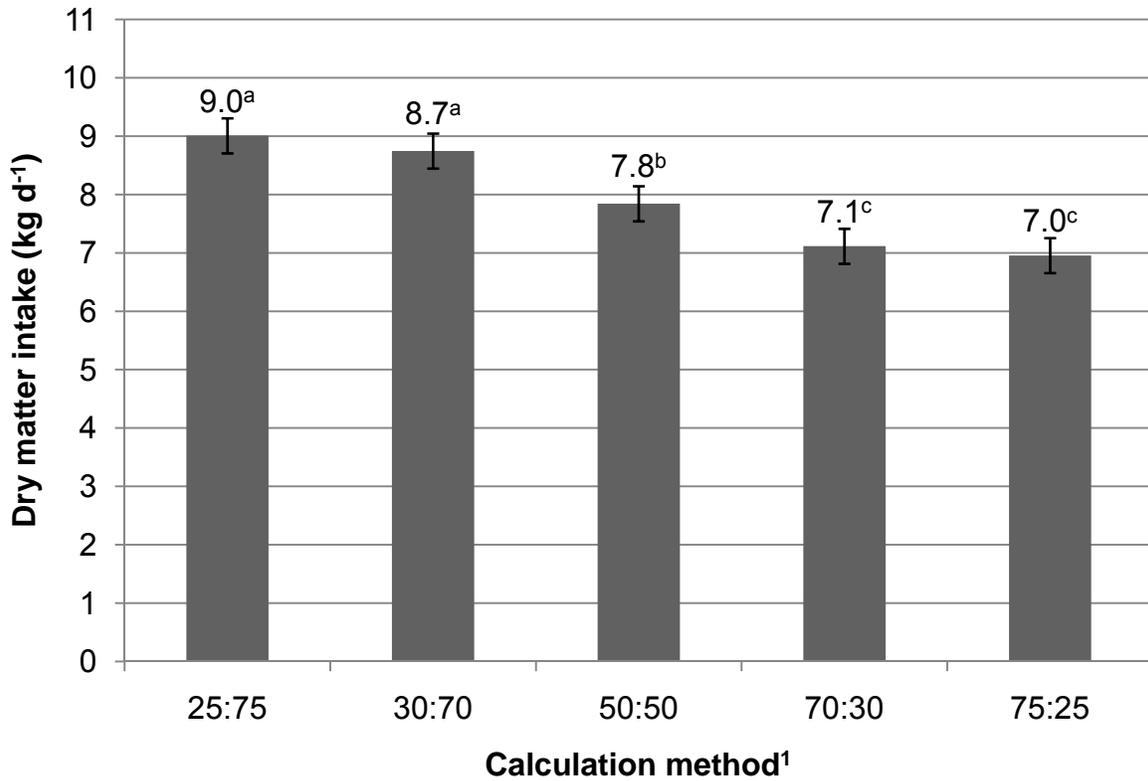


Figure 5-3. Dry matter intake of steers grazing adjacent monocultures of tall fescue and alfalfa using different ratios of forage n-alkane C₃₁ concentrations of alfalfa to tall fescue for the calculation. SEM = 0.3

¹Alfalfa to tall fescue ratios of n-alkane C₃₁

^{abc} Different superscripts within forage type indicate difference $P < 0.05$

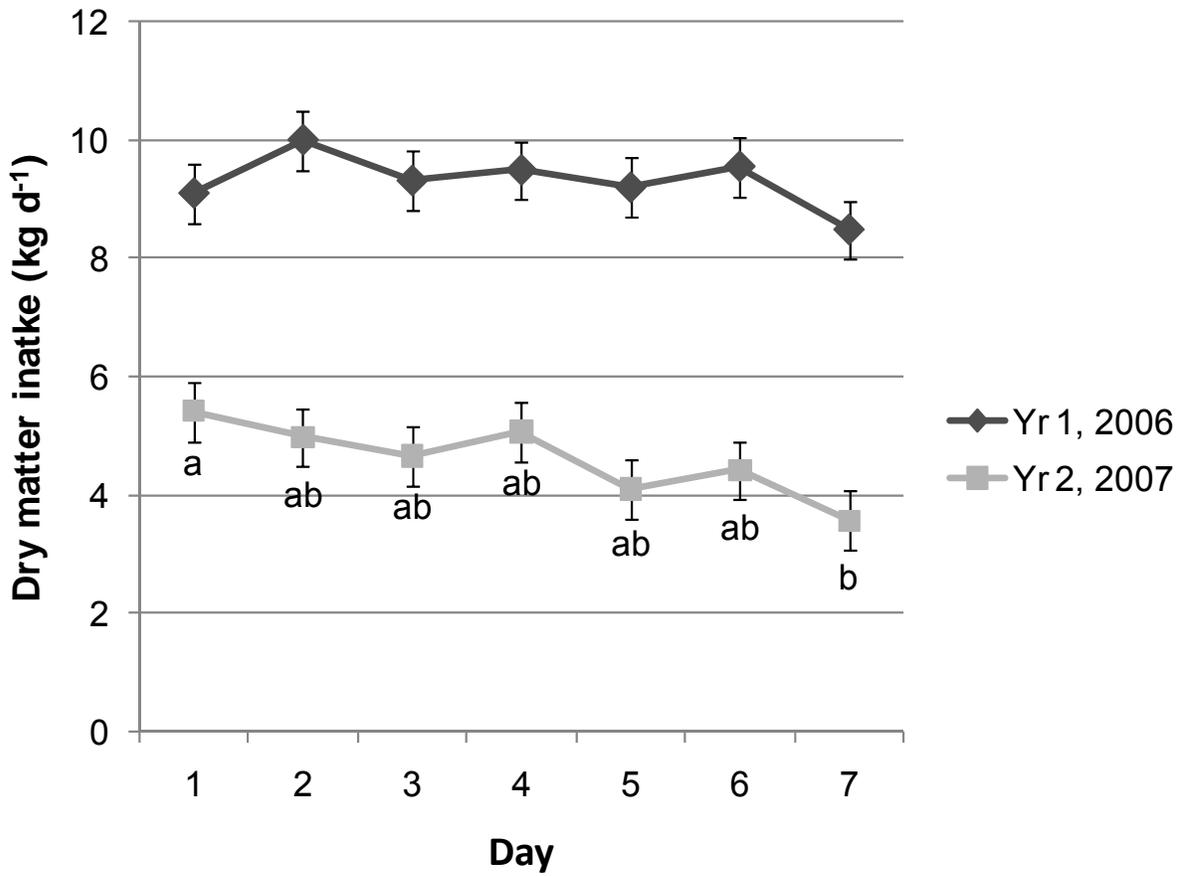


Figure 5-4. Daily dry matter intake of steers grazing adjacent monocultures of tall fescue and alfalfa.

^{ab} Different superscripts within forage type indicate difference $P < 0.05$

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CHAPTER 6

SUMMARY

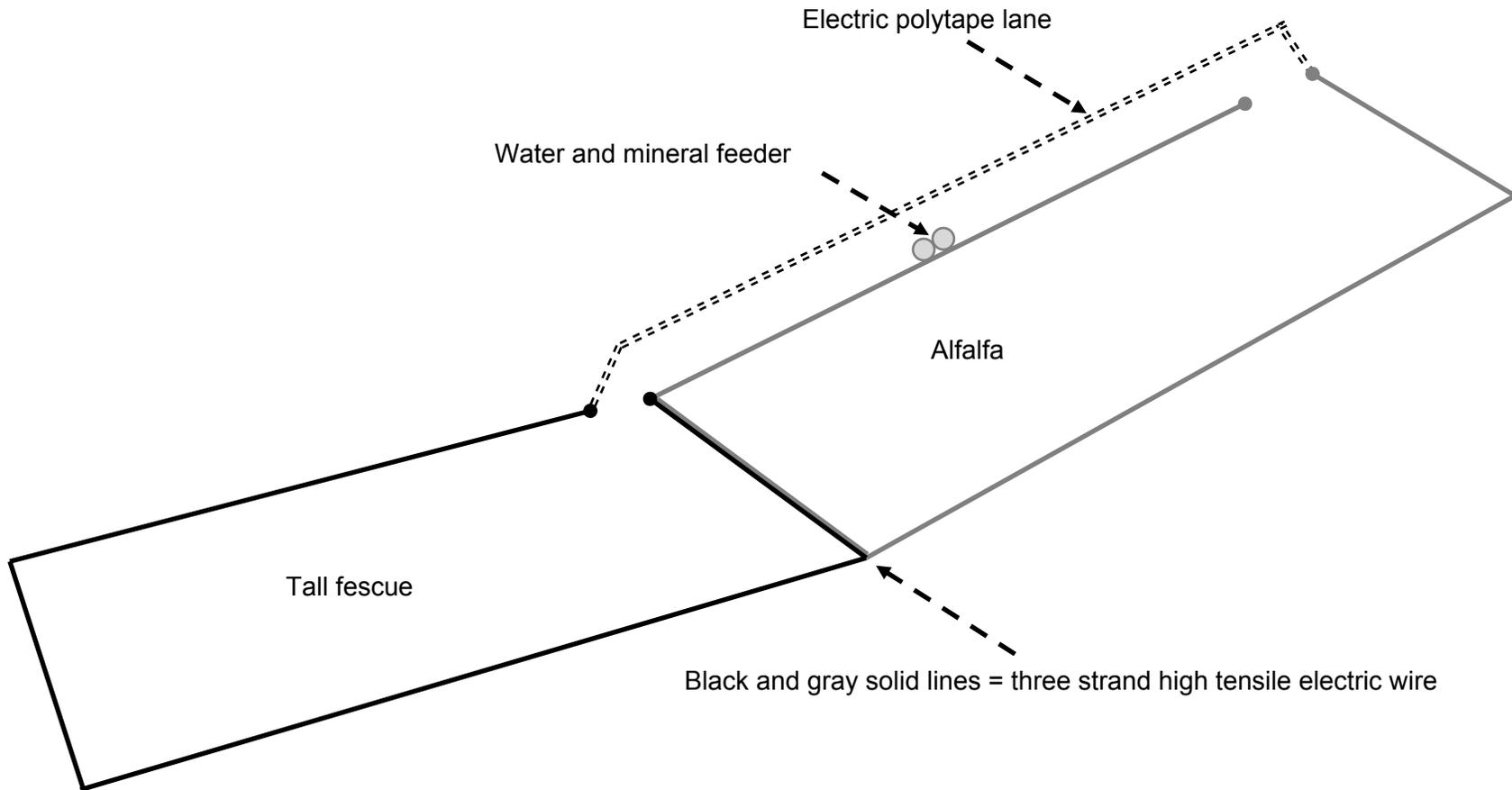
The data collected from the experiments conducted and described here add useful information to the collection of previously conducted research on the selection of mixed diets in grazing cattle. Two forage species, tall fescue and alfalfa, which had not been evaluated together previously as adjacent monocultures were studied. The theory that cattle will have a partial preference for legume over grass regardless of the forage species being evaluated was supported by this research. Steers were shown to maintain this partial preference regardless of the proportion of each forage species offered to them. This showed that steers were actively selecting what they were grazing to achieve a certain ratio of forages in their diet. The role of novelty, another factor thought to influence selection of mixed diets, was also explored. Steers responded to novelty by spending more time grazing in alfalfa compared to tall fescue when they had not previously grazed alfalfa. Previous research reported that grazing herbivores had a diurnal preference for white clover, decreasing the proportion consumed from morning to late afternoon while increasing the proportion of perennial ryegrass in the diet. This was thought to be a strategy to increase fiber intake before nightfall or a response to higher carbohydrate levels in grass in the afternoon. Steers in the present study did not decrease the proportion of time spent grazing alfalfa from morning to afternoon and did not increase grazing time on tall fescue in the afternoon. While fiber concentration was higher in the tall fescue, carbohydrate concentrations were similar, so steers were not attempting to increase fiber intake in the afternoon based on these results. Dry matter intake and diet composition were estimated in steers grazing adjacent monocultures of

alfalfa and tall fescue over two years. Diet composition of these steers was estimated using internal markers of n-alkanes and long chain alcohols (LCOH) in several different combinations. Alkanes occurring in low concentrations added characterization to the forages when only n-alkanes were used to estimate diet composition. However, when LCOH were used, those low level n-alkanes did not add to the discrimination between forages or change diet composition estimates. Depending on the forages being evaluated, laboratory analysis costs may be reduced if n-alkanes alone can adequately characterize the forages being consumed. Preliminary analysis should be conducted to determine if forage species are different enough, or if other markers such as LCOH should be analyzed. Differences in meteorological conditions impacted DMI with intake being less in hotter conditions. Even though total DMI differed between years, steers consumed the same proportions of alfalfa and tall fescue in their diet, having a partial preference for alfalfa over tall fescue. When animals are consuming two different forages as adjacent monocultures such as in the current experiments, it is important to determine the proportion of each forage in the diet before calculating DMI using odd chain n-alkanes of the forage along with a dose even chained n-alkane. This research showed how DMI can be overestimated if the proportion of the forages consumed is not estimated and accounted for in the equation. This would apply to other studies utilizing mixed swards or any diet containing multiple components that differ in concentration of the n-alkane being used for DMI estimation. Analysis of n-alkane concentration should be performed on each item in the diet and the proportion of each item in the diet estimated so that the right value can be used in the calculation. Differences in marker concentrations between years also indicate the importance of analyzing those

concentrations in the feed or forage at the time of fecal collection and not using values reported from previous research.

The data presented here lays the ground work for future research that could be more production oriented. Evaluation of adjacent monocultures in different production phases (cow/calf, stocker, forage finishing) would be beneficial based on previous work showing that physiology impacts diet selection. This information would help in the development of management tools for producers interested in adding legumes such as alfalfa into beef production systems currently using tall fescue. Future research evaluating animal performance in beef production systems on a longer term should be evaluated to determine proper stocking systems and rotation strategies in order to best utilize adjacent monocultures to their full potential. Endophyte-free tall fescue was used here; however future studies evaluating adjacent monocultures of legumes with endophyte infected or non-ergot alkaloid producing tall fescue would be useful considering their widespread use, particularly in the southeastern US. By evaluating behavior and estimating diet composition and DMI of grazing animals, and how they in turn impact the land they are grazing, we should be able to develop sustainable and profitable grazing systems. If we can determine what selection strategies are being employed by grazing animals then we will have a better understanding of the dynamic nature of these grazing systems.

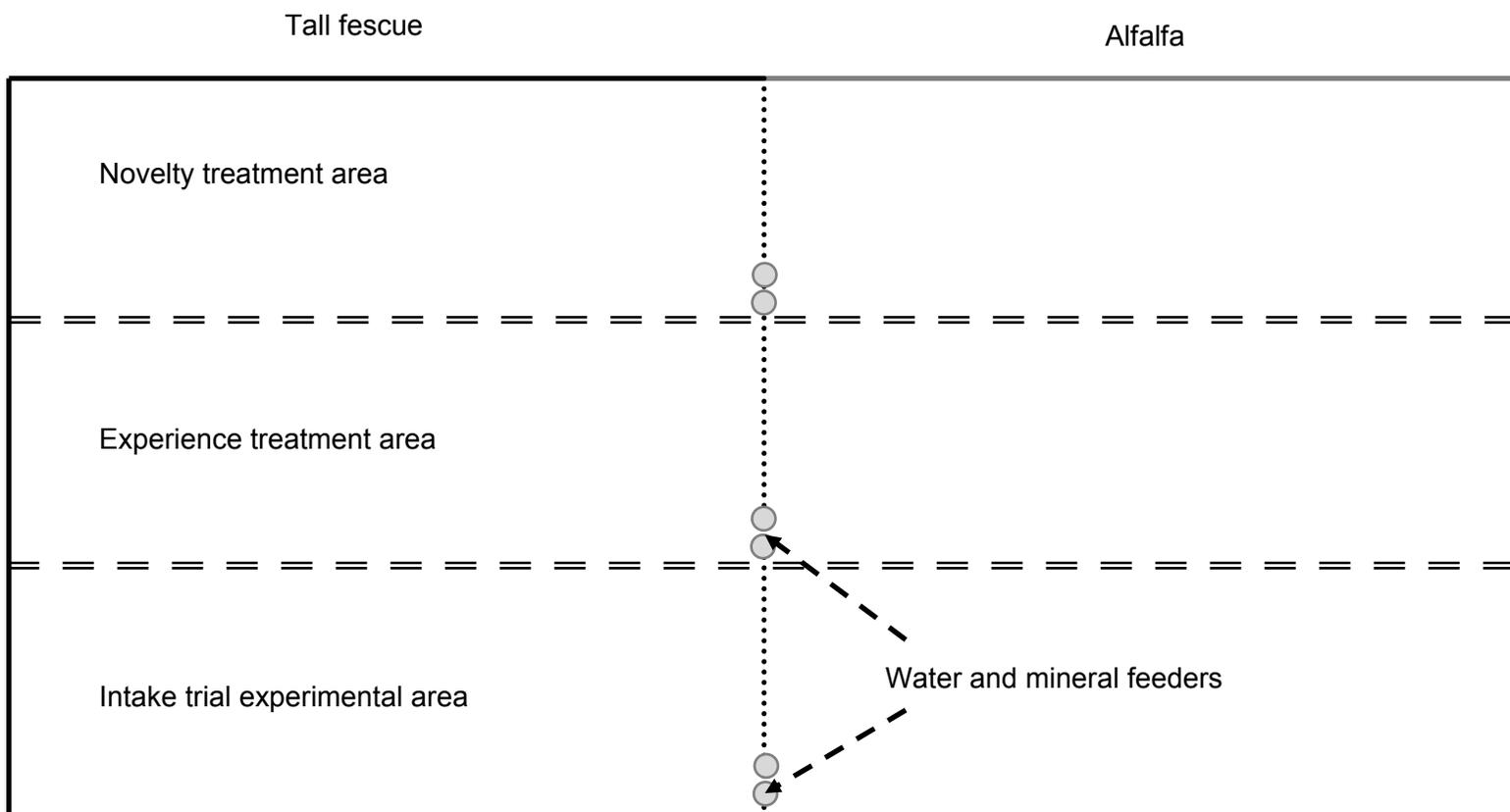
APPENDIX 1. Paddock Map for Chapter 3



Paddock layout for 50F50A treatment in Chapter 3. Other treatment paddocks were similar, varying in size based on treatment ground area specification. Lane location varied between periods and ran along the tall fescue or alfalfa paddocks an equal number of times.

APPENDIX 2. Paddock Map for Chapters 4 and 5

Paddock layout for Chapters 4 and 5. Illustration represents one replicate paddock. Three steers would graze in one strip at a time depending on the experimental periods listed below.



Black and gray solid lines = three strand high tensile electric wire

Horizontal dashed lines = Electric polytape

Vertical dotted line = bare ground approximately transition area from tall fescue to alfalfa, no fence, steers could move freely between forage types