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RESEARCH

Diet Composition and Dry Matter Intake of Beef Steers Grazing Tall Fescue and Alfalfa

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ABSTRACT

Alkanes are a noninvasive method to estimate dry matter intake (DMI) of grazing herbivores. Other cuticular wax components such as long chain fatty alcohols (LCOH) have been used for estimation of diet composition of ruminants eating mixed diets. This study estimated diet composition using n-alkanes and LCOH and estimated DMI using naturally occurring and dosed n-alkanes. Beef steers (*Bos taurus*) (16 mo. old, 358 ± 9 kg) grazing vegetative adjacent monocultures of tall fescue (*Festuca arundinacea* Schreb.) and alfalfa (*Medicago sativa* L. subsp. *sativa*) were used in this study, which also evaluated diet preference. The LCOH (C_{26}, C_{28}, and C_{30}) added additional characterization of the forages, but diet composition estimates were not different ($P \geq 0.22$) than when estimated using four n-alkanes (C_{27}, C_{29}, C_{31}, and C_{33}). Diet composition estimation indicated that steers consumed similar ($P = 0.13$) diets of 79 and 70% alfalfa in Year 1 and Year 2, respectively, corresponding to previous work showing a partial preference for legumes. Dry matter intake in Year 2 was lower ($P = 0.0002$, 4.7 kg d^{-1}) than Year 1 (9.2 kg d^{-1}), likely due to hot weather in Year 2. 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 to estimate diet composition could reduce labor and expenses by eliminating additional laboratory analyses.

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Abbreviations: ADF, acid detergent fiber; BW, body weight; CP, crude protein; CRC, controlled release capsule; DM, dry matter; DMI, dry matter intake; LCOH, long chain fatty alcohols; NDF, neutral detergent fiber; NNLS, nonnegative least squares; SEM, standard error of the mean.

Grazing ruminants prefer to eat a mixed diet of grasses and legumes although they are often restricted from doing so by being placed in forage systems with grass monocultures (Rutter, 2006). The establishment of mixed pasture systems by including legumes and forbs, however, has gained interest as many seek to increase biodiversity (Rook et al., 2004). In such diverse landscapes, the ability to estimate diet composition and dry matter intake (DMI) of free-ranging livestock 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., 1986, 1995).

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Alkanes occur naturally in the cuticular waxes of plants, with the highest concentrations occurring in odd-numbered C chain lengths. These odd-numbered C chain length n-alkanes can be used along with dosed artificial even-numbered C chain length n-alkanes to estimate DMI of animals by comparing their relative concentrations in both the forage and animal feces (described in more detail in the Materials and Methods).

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 on a distinct difference in the n-alkane profiles of the forages being consumed by the animal (Bugalho et al., 2002). Another important consideration is the number of different plant species consumed. 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, 2004). Long chain fatty alcohols (LCOH), which occur in plants primarily in even-numbered C chain lengths, can be used to provide better distinction among plants. Experiments using individually housed animals in which actual intake and diet composition can 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 estimate diet composition. Use of n-alkanes and LCOH is a successful method of determining diet composition in grazing trials by providing additional characterization of the diet compared with estimations based solely on n-alkanes (Kelman et al., 2003; Ali et al., 2005).

Grazing time alone cannot be used to determine DMI or diet composition of grazing animals because intake rate also must be considered (Rutter, 2006). To evaluate the preferred diet composition of grazing animals it is necessary to spatially separate the forages being evaluated to eliminate the constraints that occur within an intimately mixed sward (Parsons et al., 1994). Therefore, an intake trial was conducted to evaluate the diet of steers grazing adjacent monocultures of tall fescue and alfalfa. The objectives of this study were to determine diet composition using naturally occurring n-alkanes and LCOH and to estimate the DMI of beef steers grazing adjacent monocultures of tall fescue and alfalfa using naturally occurring and dosed n-alkanes. Diet composition estimations were made using different combinations of naturally occurring n-alkanes and LCOH to examine the impact of diet composition calculations in DMI estimations using the n-alkane method.

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, VA, from 9 to 22 Aug. 2006 (Year 1) and 2 to 15 Aug. 2007 (Year 2). Elevation of the site is approximately 540 m above sea level. Soils at the site are the Unison-Braddock series (fine, mixed, semiactive, mesic Typic Hapludults), which are old, deep, well drained soils with gently sloping to steeply sloped topography (25 to 65% slope). Pastures were established in 2004 when all pastures were sprayed with Roundup (9 L ha^{-1}, glyphosate 41%; Monsanto) in May to eliminate existing pasture species. Two weeks after spraying, foxtail millet [*Setaria italica* (L.) P. Beauv.] (34 kg ha^{-1}) was planted as a suppression crop. In late July, the millet was harvested for hay and 2 wk later the pastures were sprayed again with Roundup (2 L ha^{-1}) for weed suppression. Diammonium phosphate (18–46–0) was applied to tall fescue and alfalfa pastures (168 kg ha^{-1}), and 112 kg ha^{-1} of potash (0–0–60) was applied to the alfalfa pastures. On 2 and 3 September, pastures 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 tall fescue pastures at a rate of 34 kg ha^{-1} in October and then again in March 2005 to promote vigorous growth and tillering. All alfalfa pastures were sprayed with 37 mL ha^{-1} of Harmony GT (thifensulfuron-methyl 75%; Dupont) and 2 L ha^{-1} Poast Plus (sethoxydim 13%; BASF) with 1 L ha^{-1} of surfactant in April 2005. All tall fescue pastures were sprayed with 1 L ha^{-1} of 2–4D (2,4-dichlorophenoxyacetic acid 46.6%; Helena Holding Co.) and 0.6 L ha^{-1} Banvel (3,6-dichloro-0-anisic acid 48.2%; Micro Flo) in April 2005.

Pastures were grazed by nontrial stocker steers during the summer grazing season of 2005. 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 pastures were sprayed with Harmony GT in April 2007 for control of thistle and with Baythroid (β-cyfluthrin 12.7%; Bayer) for insect control in July 2007 according to manufacturer recommendations.

Animals and Fecal Sampling

Twenty-four Angus-crossbred steers (12 steers yr^{-1}, Year 1 initial body weight [BW] = 392 ± 8 kg, Year 2 initial BW = 323 ± 9 kg, 16 mo old) grazed in groups of three allotted to group by initial BW. Each group was then randomly allotted to pastures (four pastures total). Pastures were 0.2 ha of tall fescue monoculture adjacent to 0.2 ha of alfalfa monoculture. Within each treatment pasture, areas of tall fescue and alfalfa were contiguous so that steers could move freely between each forage type. Water troughs and mineral blocks with poloxalene as an antibloat agent (Bloat Guard Pressed Block; Sweetlix Livestock Supplement System) were provided ad libitum and located at the midpoint of the pasture where the two forage types converged. This ensured that the steer's need to drink and consume mineral did not influence into which forage area the steer would travel.

During each year, the experimental period was 13 d (day –7 to day 6). Steers were maintained on similar pastures for 10 d before the experiment and entered experimental pastures

on day –7. Just before entering the pasture, steers were dosed on day –7 (0730 h) with an intra-ruminal n-alkane controlled release fecal marker capsule (controlled release capsule [CRC]) for 300 to 650 kg cattle (Captec Ltd.). Each capsule contained 8 g of n-dotriacontane (C_{32}) and 8 g of n-hexatriacontane (C_{36}) with a release rate of 400 mg d^{-1} (per manufacturer). The period from day –7 to 0, before fecal collection, allowed time for the dosed even-chain alkane concentration in the feces to stabilize. After the stabilization period, two fecal samples were collected daily from each steer at 0730 and 1630 h from day 0 to 6. Fecal samples from each steer were collected in the field either by rectal grab sampling or immediately after the steer defecated on the ground. Samples from the ground were carefully collected to avoid contamination by foreign matter such as soil or plant material. One sample from each steer of approximately 500 g was stored individually in a plastic bag and packed on ice for transport and subsequently frozen at –20°C for storage before analyses in the laboratory.

Total fecal collection was performed with three steers from Year 1 to determine release rate of the CRC. Steers were halter-broken 3 mo before the experiment. Two weeks before steers were dosed with the CRC, the three steers were acclimated to wearing fecal collection bags. This process began by putting the harness and collection 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). During the experiment, fecal collection bags were weighed empty and placed on the steers at 1630 h on day –1. At 0730 and 1630 h on day 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. Steers were moved back to their pasture (separate from the other steers) after each sample collection. Fecal collection bag samples were stored similarly to grab samples.

In preparation for analyses, fecal samples were partially thawed in a walk-in refrigerator at 5°C and then brought to room temperature (22°C). A composite sample for each steer from each day was used since it has been shown that there is no difference in DMI estimation using fecal samples from morning- and afternoon-collected samples and daily composite samples (Stewart, 2006). The composite was prepared by weighing an aliquot of 10 g of the morning sample and 10 g of the afternoon 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 0.5 mm (Cyclotec 1093 Sample Mill; Foss Tecator).

Forage Sampling and Analyses

Forage mass and sward height measurements were taken on the day before and the day after each experimental period. Six quadrants of 0.25 m^2 were selected from each adjacent monoculture of tall fescue and alfalfa (three in each forage type, six per treatment area). Sixty measurements of sward height were taken with a ruler within each pasture to characterize sward conditions. Clippings were collected at 2.5 cm above ground level and forage was placed in cloth bags and 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 days –1, 3, and 6. Samples were collected (one composite forage sample per forage type per pasture) while walking along a cross-section of the pasture (each diagonal was approximately 70 m) and grabbing a sample every 3 to 4 m 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) and then ground through a 1-mm screen using a Wiley mill (Laboratory Mill Model 4, Arthur H. Thomas Co.). Samples were then analyzed for chemical composition by NIRS (Foss NIRSystems 6500; Foss Tecator) (AOAC, 2000) at a commercial laboratory (Dairy One, Ithaca, NY). Calibration statistics for NIRS are as follows: dry matter (DM), 1566 samples, R^2 = 0.852, min. = 81.68, max. = 97.76, and mean = 91.40; crude protein (CP), 4704 samples, R^2 = 0.986, min. = 1.99, max. = 32.95, and mean = 13.87; neutral detergent fiber (NDF), 4642 samples, R^2 = 0.969, min. = 22.47, max. = 89.17, and mean = 54.71; acid detergent fiber (ADF), 4589 samples, R^2 = 0.912, min. = 14.30, max. = 68.10, and mean = 35.69; and lignin, 2822 samples, R^2 = 0.592, min. = 1.42, max. = 20.7, and mean = 6.69. Tall fescue subsamples were further ground to 0.5 mm (Cyclotec 1093 Sample Mill; Foss Tecator) and analyzed for alkaloid concentration by enzyme-linked immunosorbent assay at a commercial laboratory (Agrinostics Ltd. Co., Watkinsville, GA) by the method of Adcock et al. (1997) to ensure they were at low endophyte levels. Mean alkaloid level in Year 1 was 111 ng g^{-1} (standard error of the mean [SEM] = 18) and in Year 2 was 51 ng g^{-1} (SEM = 5), below levels that have been shown to induce fescue toxicosis (Parish et al., 2003).

Hand-plucked samples of each forage type for analysis of n-alkanes and LCOHs were taken from day –2 to day 4. Forage sampling began 2 d before fecal sampling based on the assumption of a 48-h retention time of consumed forage in the gastrointestinal tract (Burns et al., 1991). Samples were collected, stored, dried, and ground similarly to nutritive value samples. Subsamples were further ground to 0.5 mm (Cyclotec 1093 Sample Mill; Foss Tecator) in preparation for n-alkane and LCOH marker extraction.

n-Alkane and Long Chain Fatty Alcohols Analyses

The protocol of Dove and Mayes (2006) was used for analyses of n-alkanes and LCOH in forage and fecal samples. Samples from both years were prepared and analyzed at the same time. Analyses were conducted on a gas chromatograph with a flame ionization detector, autosampler, and integrator (GC6890, 7683; Agilent Technologies). Mixed reference standards were prepared and analyzed at the beginning of every run and after every 10 injections along with a blank injection. The n-alkanes standard included the following chain lengths: C_{21} to C_{36}. 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: C_{18} to C_{28} and C_{30}.

Data Analyses

The diet composition, in terms of proportion of each forage species consumed, was estimated by using the nonnegative least squares (NNLS) procedure of Dove and Moore (1995). The NNLS procedure compared forage marker profile to fecal marker concentrations and determined the proportion of each forage species in the diet. The 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:

$$\sum \left(F_i - xA_i + yB_i + zC_i\right)^2_{alc:1\ldots n},$$

in which F_i is the concentration of the marker i in the feces, x, y, and z are the amounts of diet components A, B, and C, and A_i, B_i, and C_i are the respective concentrations 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 four 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 (*Ovis aries*) fed pelleted ryegrass (*Lolium perenne* L.) 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 1) of C_{27} were similar between forage types and both it and C_{33} were around the 50 mg kg^{-1} DM level considered low for use in intake calculations (Laredo et al., 1991). Therefore, four different combinations of n-alkanes and LCOH were analyzed by NNLS. These combinations were as follows: four n-alkanes plus three LCOH (n-alkanes: C_{27}, C_{29}, C_{31}, and C_{33} and LCOH: C_{26}, C_{28}, and C_{30}), two n-alkanes plus three LCOH (n-alkanes: C_{29} and C_{31} and LCOH: C_{26}, C_{28}, and C_{30}), four n-alkanes (C_{27}, C_{29}, C_{31}, and C_{33}), and two n-alkanes (C_{29} and C_{31}). Angular transformation of diet composition data was conducted after NNLS 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.

Table 1. Least squares mean concentrations (mg kg^{-1} dry matter) of n-alkanes of forages grazed as adjacent monocultures.

		n-Alkanes				
Forage	Year	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}
Alfalfa	1	48.8a†A‡	89.8bA	169.2bA	39.9bB	0
	2	61.5aA	140.9aA	306.7aA	61.9aB	0
Tall Fescue	1	42.9aA	53.8bB	106.3bB	50.6bA	0
	2	48.5aB	87.3aB	165.7aB	80.9aA	0
SEM§		2.9	5.3	13.3	2.1	–

†Within column within forage type means followed by the same letter are not different at $P < 0.05$.

‡Within column within year means followed by the same letter are not different at $P < 0.05$.

§SEM, standard error of the mean;

Dry matter intake was estimated using the ratio of C_{31} (naturally occurring alkane) to C_{32} (dosed artificial alkane) using the method of Dove and Mayes (1991). 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 C_{33} was near or below 50 mg kg^{-1} DM (Table 1), C_{31} 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:

$$I = [(F_j/F_i) \times D_j]/[H_i - (F_j/F_i) \times H_j],$$

in which I is dry matter intake (kg d^{-1}), i is C_{31} (naturally occurring alkane), j is C_{32} (external alkane marker), D_j is the weight of dosed j (mg d^{-1}), F_j is the concentration of j in feces (mg kg^{-1} DM), F_i is the concentration of i in feces (mg kg^{-1} DM), H_i is the concentration of i in forage (mg kg^{-1} DM), and H_j is the concentration of j in forage (mg kg^{-1} DM) (Dove and Mayes, 1991).

The method by which DMI is calculated in this equation requires the concentration of the odd-numbered C chain n-alkane in the forage. In this experiment, two forages were consumed by the steers and these forages had different concentrations of C_{31}. 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 containing a mix of all forages in the sward may not contain the same proportion of each forage that the animal consumed. One might assume that diet composition will match the proportion of forages found in the sward or the percent ground area that each forage covers in the pasture. Or, if diet composition is not estimated and an average of C_{31} in the two forages is used (an assumption of 50:50 diet composition), 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 overestimated if n-alkane profiles of the forages vary. In the present study, DMI was calculated for multiple diet composition ratios to illustrate this point with alfalfa and tall fescue at ratios of 25:75, 30:70, 50:50, 70:30, and 75:25. These calculations were made by adjusting the respective C_{31} concentrations in the DMI equation, allowing us to examine possible discrepancies in DMI estimation that may result if an estimation of diet composition is not also determined and considered in the DMI calculation. These ratios were selected for evaluation based on previous data of diet preferences for legumes and grasses in the ruminant diet (Rutter, 2006). Cattle have been shown to prefer a diet of around 70% legume and 30% grass when offered as adjacent monocultures, so ratios at and near that amount were evaluated. The reverse ratios of 25 to 30% legume to 75 to 70% grass were also included. In mixed swards of grasses and legumes, there is usually a lower percentage of legumes in the sward and these ratios of legume to grass would not be uncommon (Nolan et al., 2001). These latter ratios will represent such a case in which a single hand-plucked forage sample representative of the sward itself was taken and assumed to represent the diet of the animals for DMI calculation.

The experimental unit for all analyses was the pasture. Data were then analyzed using SAS (SAS Institute, 2010). 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 $0.10 > \alpha > 0.05$.

Forage nutritive value and sward measurement data were analyzed using the MIXED procedure (SAS Institute, 2010). The model included year, forage type (alfalfa or tall fescue), and their interaction. The experimental unit was the pasture within year.

Alkane and LCOH profiles were analyzed using the MIXED procedure (SAS Institute, 2010) and the compound symmetry (cs) covariance structure. The model included year, forage type (alfalfa or tall fescue), and their interaction. Repeated measure was day within year.

Diet composition was analyzed using the MIXED procedure (SAS Institute, 2010) and the compound symmetry (cs) covariance structure. The repeated measure was day within year. The model included year, method (n-alkane and LCOH combinations), and their interaction. Repeated measure was day within year. Analysis methods found to be similar were then used in further analysis of the data with a model including year, day, and their interaction.

Estimations of DMI were analyzed using the MIXED procedure (SAS Institute, 2010) and the compound symmetry (cs) covariance structure. The repeated measure was day within year. The model included year, method (C_{31} 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 year. Estimated daily diet composition was used to determine daily DMI, which was further analyzed with a model including year, day, and their interaction.

Correlation analysis, discriminate analysis, and repeated analysis of covariance were performed using PROC CORR, PROC DISCRIM, and PROC GLIMMIX programs, respectively, from Statistical Analysis Systems (SAS Institute, 2010). Analysis determined significance ($\alpha = 0.05$) of correlations among the seven different alkanes and LCOH identified for dietary analysis, the potential to differentiate between alfalfa and tall fescue using one or more of the seven chemical measurements, and the linear and quadratic effects of time and the effect of species with interactions on concentration of each chemical.

RESULTS AND DISCUSSION

Forage and Sward Measurements

Forage nutritive value and forage mass and height were evaluated to characterize grazing conditions and the forages being consumed during the experiment. 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 2) than tall fescue. The nutritive value of alfalfa was greater in Year 2 than Year 1 with higher CP ($P = 0.01$) and lower NDF ($P = 0.007$) and ADF ($P = 0.003$). Tall fescue CP and ADF were similar between years, but NDF and lignin were higher ($P = 0.006$) in Year 2. Sward height in Year 1 was similar between alfalfa and tall fescue (31.2 and 25.1 cm, respectively; $P = 0.61$, SEM = 3.4) but differed ($P = 0.006$) in Year 2 (39.5 and 20.7 cm, respectively; SEM = 3.0). The year effects on these forages were likely the result of a dry hot summer in Year 2. Tall fescue, particularly endophyte free, is not as tolerant of such conditions whereas alfalfa is more adapted to heat and drought. No difference in forage mass between years ($P = 0.12$) or forage types ($P = 0.37$) was observed. Forage mass of alfalfa was 2702 kg ha^{-1} in Year 1 and 2467 kg ha^{-1} in Year 2 (SEM = 312). Forage mass of tall fescue was 2236 kg ha^{-1} in Year 1 and 1932 kg ha^{-1} in Year 2 (SEM = 267). These levels of forage mass indicate that forage availability was not at a level that would limit DMI (Paterson et al., 1994) during the experiment.

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

Forage	Year	CP†	NDF‡	ADF§	Lignin
		g kg^{-1} dry matter			
Alfalfa	1	221b¶	381c	286b	77a
	2	267a	314d	239c	75a
Tall Fescue	1	171c	501b	294a	41c
	2	147c	570a	315a	55b
SEM#		9.7	13.1	8.2	2.1

†CP, crude protein.

‡NDF, neutral detergent fiber.

§ADF, acid detergent fiber.

¶Within column means followed by the same letter are not different at $P < 0.05$.

#SEM, standard error of the mean.

Marker Concentrations

The n-alkane profiles of tall fescue and alfalfa (Table 1) were similar to those previously reported (Bovolenta et al., 1994). Effects of year ($P \leq 0.03$) within forage type were observed for all chain lengths except for C_{27} for which there tended to be a year effect ($P = 0.07$) in alfalfa and no effect of year ($P = 0.22$) for tall fescue. The n-alkane C_{35} was not detected in either species. The higher concentrations of n-alkanes in Year 2 may have been due to the hot and dry conditions in that year with high temperatures reaching 34°C (compared with an average high of 28°C in Year 1). An increase in deposition of cuticular wax (where n-alkanes are found) can occur as a response to elevated temperatures and during drought as a moisture conservation strategy (Shepherd and Griffiths, 2006).

The LCOH profile of tall fescue was also similar to previous data (Bugalho et al., 2004); however, information on the LCOH profile of alfalfa was not available (Table 3). A year effect for C_{30} ($P = 0.003$) was observed and there tended to be a year effect ($P \leq 0.09$) for both C_{26} and C_{28}, 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 NNLS is presented in Fig. 1. There was an effect of analysis method on the estimated proportions of tall fescue and alfalfa. When

Table 3. Least squares mean concentrations (mg kg^{-1} dry matter) of long chain alcohols (LCOH) of forages grazed as adjacent monocultures.

Forage	Year	LCOH		
		C_{26}	C_{28}	C_{30}
Alfalfa	1	168.6a†B‡	29.5aB	928.7bA
	2	155.5aB	39.7aB	1397.9aA
Tall fescue	1	651.7aA	107.0aA	1.8aB
	2	617.1aA	77.6bA	1.1aB
SEM§		13.1	4.6	64.2

†Within column within forage type, means followed by the same letter are not different at $P < 0.05$.

‡Within column within year, means followed by the same letters are not different at $P < 0.05$.

§SEM, standard error of the mean.

using only two n-alkanes (C_{29} and C_{31}), diet composition was different ($P \leq 0.0004$) from all other analysis methods. Diet composition calculation with four n-alkanes plus three LCOH was similar ($P \geq 0.22$) to that estimated when using two n-alkanes plus three LCOH or all four n-alkanes. This indicates that even though concentrations of n-alkanes C_{27} and C_{33} were low, they did provide additional discrimination between the forages when only n-alkanes were considered. When only C_{29} and C_{31} n-alkanes were used, however, over 66% of the diet composition estimates were calculated as either 100% alfalfa or 100% tall fescue, which we know was not the case based on behavioral observation (Boland et al., 2011). Using only the two n-alkanes, C_{29} and C_{31}, did not provide enough discrimination between the two forage species for NNLS to estimate diet composition.

Diet composition values using the three similar marker methods were used to further analyze effects of year and day. Diet composition did not differ ($P = 0.13$) between year of the trial, (79 and 70% alfalfa in Year 1 and Year 2, respectively). No effect of day ($P \geq 0.20$) on diet composition was observed, with the mean proportion of alfalfa in the diet numerically declining from day 1 (79.5%) to day 7 (68.1%). The decline in proportion of alfalfa consumed over time, although not significant, may be due to depletion of leaves in the alfalfa sward. Daily diet composition estimates in Year 1 ranged from 88 to 73% alfalfa and 12 to 27% tall fescue and in Year 2 were 84 to 59% alfalfa and 16 to 41% tall fescue. Preference trials using alfalfa and tall fescue adjacent monocultures have not been previously reported; however, other legumes–grass combinations have been evaluated. Proportion of alfalfa in the diet of the steers in the present study was similar to legume proportion in the diets reported previously in other adjacent monoculture studies with different forages (Table 4). Only one of those evaluated beef cattle (Rutter et al., 2005a) and they reported a 60% preference for white clover (*Trifolium repens* L.) in adjacent monocultures with ryegrass. Studies with dry dairy heifers reported legume preferences of 64 to 70% (Table 4). In a review by Rutter (2006) this repeated observance of partial preference for legumes exhibited by cattle and sheep is discussed in great detail and it has been proposed that this partial preference is not affected by the species of legume and grass being used and would likely reoccur with other multiple other legume–grass combinations. One of the driving factors behind this partial preference may have been discovered by Merry et al. (2002) who showed that a 70:30 legume:grass diet achieved an optimal level of microbial protein synthesis in artificial rumen studies.

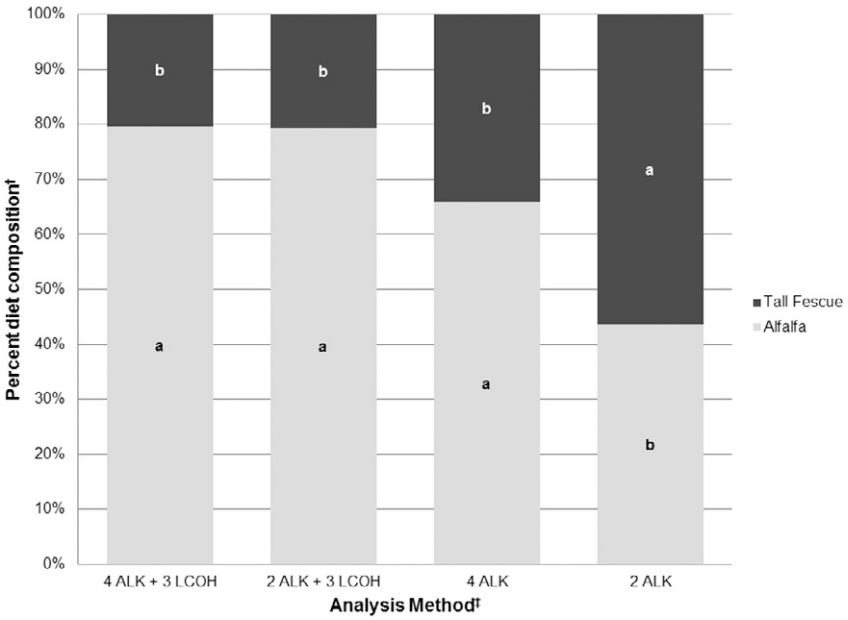

Figure 1. Diet composition of steers grazing adjacent monocultures of tall fescue and alfalfa using different combinations of n-alkanes (ALK) and long chain fatty alcohols (LCOH) as markers. Angular transformed standard error of the mean = 3.3. †Nontransformed percentage units. ‡Four ALK plus three LCOH (ALK: C_{27}, C_{29}, C_{31}, and C_{33} and LCOH: C_{26}, C_{28}, and C_{30}), two ALK plus three LCOH (ALK: C_{29} and C_{31} and LCOH: C_{26}, C_{28}, and C_{30}), four ALK (C_{27}, C_{29}, C_{31}, and C_{33}), and two ALK (C_{29} and C_{31}). Within forage type, means followed by the same letter are not different at $P < 0.05$.

Dry Matter Intake Estimation

Dry matter intake estimations comparing C_{31} calculated at different ratios of tall fescue to alfalfa are presented in Fig. 2. The ratio of forages used in the calculation did have an effect on the estimated DMI values. Using a ratio of 50:50 alfalfa:tall fescue, 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 and 75:25 ($P = 0.45$) or 25:72 and 30:70 ($P = 0.84$) ratios indicates that when forage n-alkane ratios were within ±5% of estimated diet composition, the estimates of DMI were similar. The DMI estimations assuming

25 to 30% alfalfa were also found to be different ($P < 0.0001$) than those with 70 to 75% alfalfa. This illustrates the necessity to first estimate diet composition and use that information in DMI calculation rather than basing DMI calculations only on forage samplings representative of the pasture composition or averages of the C_{31} concentrations of all forages that occur in the sward.

Daily estimates of diet composition by NNLS were used to estimate daily DMI. There was a difference ($P = 0.0002$) observed between years for DMI (kg d^{-1}) but no differences ($P \geq 0.24$) were observed between days within years. The steers in Year 1 consumed more than steers in Year 2 (9.2 and 4.7 kg d^{-1}, respectively). Similarly, when DMI was analyzed by BW there was a difference ($P = 0.001$) between years (22.8 and 14.6 g kg^{-1} BW, Year 1 and 2, respectively), but no differences ($P \geq 0.11$) between days within years. These effects of year were probably due to air temperatures in Year 2 being higher than in Year 1. As air temperatures increase, cattle will decrease grazing time (Findlay, 1958) and have decreased DMI (Mitlohner et al., 2001). Other reports of DMI of cattle grazing adjacent monocultures of tall fescue and alfalfa were not available for comparison. There are only a limited number of studies available involving adjacent legume–grass monocultures of any forage species with cattle. From Table 4, the most comparable studies to the present would be those with dry dairy and beef heifers and most of those only reported percentage of time the animals were observed to be consuming legumes or grasses. Rutter et al. (2005a) estimated DMI (using n-alkanes) of Simmental × Holstein yearling heifers grazing white clover and ryegrass planted in strips of varying widths along with a mixed sward. They found no differences in DMI between the forage treatments, which ranged between 8.6 and 9.9 kg d^{-1}. Some studies of DMI on mixed swards of tall fescue and alfalfa have been reported. Scaglia et al. (2005) estimated DMI with n-alkanes and reported that steers (330 kg BW) grazing a mixed sward of tall fescue and alfalfa consumed 9.9 kg d^{-1}. Seman et al. (1999) estimated that steers (300 kg BW) grazing mixed swards of tall fescue and alfalfa (33 or 67% alfalfa content) removed 5.3 and 6.3 kg of forage from those swards each day, respectively, based on forage disappearance.

Table 4. Diet preference of animals offered adjacent monocultures of two forages at a 50:50 ground area ratio.†

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

†Adapted from Rutter (2006).

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

CONCLUSIONS

The results of this study show 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 of other researchers (Rutter, 2006) that cattle will select a diet

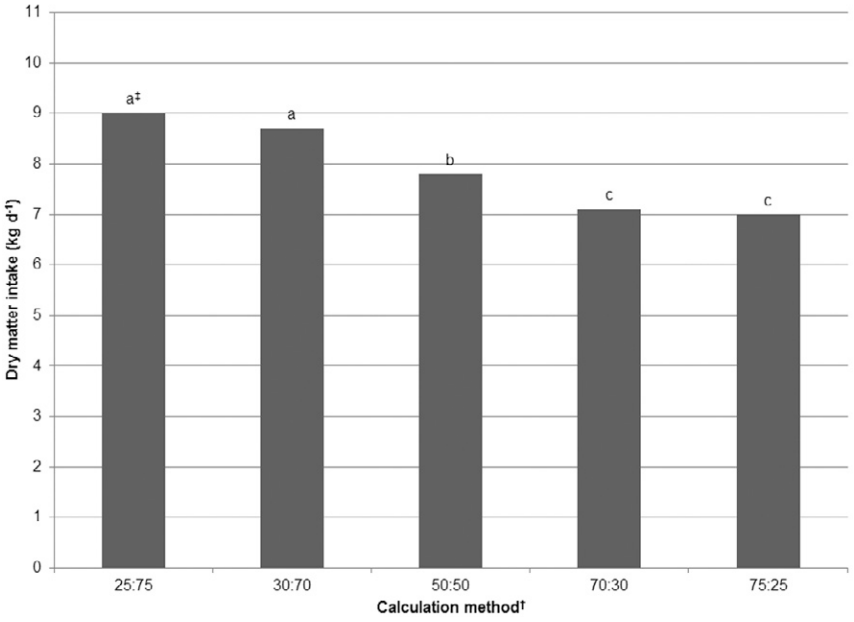

Figure 2. Dry matter intake of steers grazing adjacent monocultures of alfalfa and tall fescue using different ratios of forage n-alkane C_{31} concentrations for the calculation. Standard error of the mean = 0.3. †Alfalfa to tall fescue ratios of n-alkane C_{31}. ‡Means followed by the same letter are not different at $P < 0.05$.

with a larger proportion of legume than grass regardless of the forage species. The results reported here also indicate the importance of knowing the diet composition of grazing animals if DMI of mixed species pastures is going to be estimated with the n-alkane technique. 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 Year 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 gas chromatograph analysis of n-alkane concentrations of forages being grazed should be evaluated using the NNLS procedure of Dove and Moore (1995) first to ensure that their profiles are distinct enough for differentiation if additional markers such as LCOH are not going to be analyzed.

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