Use of N-alkanes to Estimate Intake and Digestibility by Beef Steers

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ABSTRACT : The objective of the study was to evaluate the use of n-alkanes to estimate DM intake and digestibility by beef cattle. Six steers were blocked (3 blocks, 2 animals/block) according to the body weight (279±19 kg) and randomly allotted within blocks to two diets (3 steers/diet). A second trial was conducted with the same animals (321±18 kg) after 36 days (d), using a switch back design. The diets consisted of two types of chopped sun-cured hay, alfalfa (*Medicago sativa* L) hay, or fescue (*Festuca arundinacea* Schreb) and alfalfa mixture, which were fed in equal amounts to steers. Animals were dosed with C_{32} and C_{36} alkanes, employing an intra-ruminal controlled-release device at the beginning of each trial. Hay intake per animal was measured from d 6 to 12 and sub samples were taken for chemical analysis. Rectal samples of feces were taken from each animal once/daily from d 8 to 14, freeze dried, and ground prior to alkane analysis. Alkanes were extracted from ground hay and feces. Feed intake was calculated from the dose rate of C_{32} alkane and, the herbage and fecal concentrations of adjacent odd (C_{33} or C_{31}) and even (C_{32}) chain length alkanes. Crude Protein, NDF, ADF, ash concentrations and *In vitro* dry matter digestibility (IVDMD) were 17.7, 42.2, 28.4, 7.9 and 71.7 for alfalfa, and 12.4, 56.5, 30.4, 6.9 and 69.1% for fescue/alfalfa mixture, respectively. For both diets, intake estimated from C_{33}:C_{32} ratio was not different from the measured intake, but intake estimated from C_{31}:C_{32} ratio was lower ($p<0.05$), than the measured intake for both diets. The average estimated forage intake from C_{33}:C_{32} ratio was 4.86 and 0.69% below than the measured intake for alfalfa and, fescue/alfalfa mixed diets, respectively. The respective estimates with C_{31}:C_{32} ratio were 9.59 and 11.33% below than the measured intake. According to these results, alkane C_{33}:C_{32} ratio is better than alkane C_{31}:C_{32} ratio for the estimation of intake by beef steers. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1564-1568)***

Key Words : Alkane, Controlled-release Capsules, Feed Intake, Beef Steers

INTRODUCTION

Use of indigestible plant components as internal markers for estimating intake and digestibility in grazing ruminants gives potential advantages over external markers or other methods. The compounds, n-alkanes are saturated aliphatic hydrocarbons of plant cuticular wax, which have been used as internal markers to estimate the feed intake of grazing ruminants (Dove and Mayes, 1991; Mayes et al., 1994) and the digestibility of herbage mixtures (Dove, 1992). These n-alkanes are predominantly odd-chain in the range of C_{25} to C_{35} and substantially indigestible. They can be used in combination with orally dosed even-chain n-alkanes to estimate feed intake by grazing ruminants (Mayes et al., 1986a).

Mayes et al. (1986a) developed a double alkane procedure for estimating intake where animals were dosed with a known quantity of an even-chain alkane. These synthetic alkanes can be administered to grazing ruminants in different ways and one such method is the intra-ruminal controlled release devices (CRD), which release alkanes in the rumen continuously (Dove et al., 1991).

Research testing n-alkanes as internal markers using beef cattle is limited. Therefore, the objective of the present study was to evaluate the use of n-alkanes to estimate DMI and digestibility of forages by beef cattle.

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MATERIALS AND METHODS

Two types of sun-cured hay, alfalfa or fescue and alfalfa mixed (1:1) hay were fed to beef steers (Angus crossing) in individual pens. Hay was chopped (2 cm) so that selection was minimized. Six steers were blocked (3 blocks; large, medium and small) according to body weight (279 kg) (SD = 19) and randomly allotted within blocks (2 animals/block) to the two diets (3 animals per diet). Diets were changed and a second trial was conducted with the same animals after 36 d (322 kg) (SD = 19) using the switch back design. At the end of two trials, number of replicates per treatment was 6. Steers were housed in pens (2.4 m×3.4 m) and water was made available at all the times. A trace mineralized salt (Na, Cl, Zn, Fe, Mn, Cu, I and Co) block was provided to each animal. Adjustment period for the diet was 7 d. Animals were dosed with even-chain alkanes (C_{32} and C_{36}), employing an intra-ruminal controlled-release capsule (CRC, Captec Ltd., Auckland, New Zealand) (4,150 and 4,000 mg of C_{32} and C_{36} per capsule, respectively) at the beginning of each trial (d 1). Hay intake per animal was measured from d 6 to 12 (7 d). Equal amounts of hay (6.32 kg DM) were fed in the morning at 0900 so that there were no refusals (restricted feeding). Hay samples were taken for

chemical analysis every day from d 6 to 12 and stored in polyethylene bag separately in the refrigerator. Rectal samples of feces from each animal were taken once/daily at 0800 from d 8 to 14 (7 d). Feces samples were frozen (-20°C) immediately in double lined polyethylene bags.

The research was conducted under an Animal Care and Use Protocol approved by the Virginia Tech Animal Care and Use Committee.

Hay samples were ground (0.5 mm screen) without drying, whereas, feces samples were freeze dried and ground (0.5 mm screen) prior to alkane analysis because measurement and extraction of n-alkanes were easier with smaller particle size (Berry et al., 2000). Hay samples were used to measure the n-alkane concentrations of consumed hay and to correct fecal concentrations of dosed alkanes for the small amounts of C_{32} and C_{36} present in hay so that actual intake measurements could be compared with the alkane procedure. Fecal samples from the rectum were taken from d 17 to 23 after CRC dosing, to measure the release rate of C_{32} and C_{36} (Personnel communications with Captec Ltd., Auckland, New Zealand).

Alkanes were extracted from ground hay and fecal samples using the methods described by Mayes et al. (1986a) with minor modifications (Ordakowski et al., 2001). A sample of feces (0.1 g) or hay (0.3 g) was placed in a Pyrex tube fitted with a screw cap prior to the addition of 0.1008 mg of C_{34} internal standard (Sigma Chemical, St. Louis, MO) and 7 ml of 10% ethanolic KOH. Tube contents were mixed thoroughly, placed in a water bath (90°C) for 3 h, and mixed thoroughly after every 30 min. After cooling of samples, 7 ml distilled water and 7 ml of heptane were added and tube contents were mixed. The organic extract was removed, applied to a column consisting of silica gel (Sigma chemical, St. Louis, MO) contained in disposable Oxford pipette tips (200 µl) with glass wool stoppers, and the effluent was collected in 20 ml scintillation vials. Approximately 10 ml of heptane was used to rinse the column. The total eluent was allowed to evaporate overnight in a fume hood. The dried sample was re-dissolved with 1 ml of heptane before injection of 1 µl onto a 30 m×0.52 mm i.d., 1.5 µm fused silica film thickness capillary column (Supelco Inc., Bellefonte, PA) in a 'Varian' (Vista 6000) gas chromatography fitted with a flame ionization detector. The chromatograph column oven temperature was programmed (240°C for 2 min; 3°C/min to 288°C; 2°C/min to 298°C, where it was held for 3 min), and the carrier gas, He, had a flow rate of 9 to 9.25 ml/min. The temperatures of the detector and injection ports were 300°C. The flow rates of H_2 and air were 40 ml/min and 400 to 450 ml/min, respectively. The area under the peak for each n-alkane was determined using an integrator (Camag-SP4270). The ratio of the peak areas of the analyzed n-alkanes to that of internal standard (C_{34}) was used to calculate n-alkane amounts in the sample. Identification of the different n-alkanes was made based on the relative retention times of known standards.

All samples were analyzed for DM, ash, CP (AOAC, 2000), NDF (Georing and Van Soest, 1970), and ADF (Van Soest, 1967, modified by Georing and Van Soest, 1970). *In vitro* DM digestibility (ANKOM Daisy $II^{200/220}$) was determined in hay samples.

Feed intake was calculated from the dose rate of C_{32} alkane and the herbage and fecal concentrations of adjacent odd (C_{33} or C_{31}) and even (C_{32}) chain length alkanes (Dove and Mayes, 1991) using the following equation:

$$HI = [D_{32}\times(F_{33}/F_{32})]/[H_{33}-((F_{33}/F_{32})\times H_{32})]$$

where HI is the herbage intake (kg DM/d), D_{32} is the amount of dosed C_{32} alkane (mg/d), F_{33} and F_{32} are fecal concentrations of C_{33} and C_{32} alkanes (mg/kg DM), H_{33} and H_{32} are the herbage concentrations of C_{33} and C_{32} alkanes (mg/kg DM) respectively. When intake was calculated using C_{31} alkane, C_{33} values in the above equation were replaced by C_{31} values. Intake calculated from alkane method was compared with the values obtained from measured intake.

Dry matter digestibility was calculated using C_{31} or C_{33} alkanes using the following equation:

$$D\ (\%) = [1-(\text{Recovery rate of } C_{33}\times(H_{33}/F_{33}))]\times 100$$

where D is the digestibility (%), H_{33} is the amount of C_{33} alkane in feed and F_{33} is the amount of C_{33} alkane in feces (mg/kg DM). Recovery rates of 0.86 and 1.03, reported under similar conditions by Lopez-Guerrero et al. (2004) (Personal communication) for C_{31} and C_{33} alkanes respectively were used in the equation. When digestibility was calculated using C_{31} alkane, C_{33} values in the above equation were replaced by C_{31} values. Digestibility estimated using C_{31} or C_{33} alkane was compared with i*n vitro* digestibility measurements.

Statistical analysis

Data were statistically analyzed using GLM procedures of the SAS (SAS, 1989). Data were analyzed as a split plot design with animal and diet test on the whole plot error (animal×diet) and marker and day tested on the split plot error.

Variables = Diet marker diet
×Marker animal animal× Diet day

Test hypothesis = Animal error = Animal×diet ;
Test hypothesis = Diet error = Animal×diet ;
Test hypothesis = Marker error = Marker×diet ;

Table 1. Chemical composition of diets and DMI and digestibility of hay by beef steers

Item	Type of hay		SEM
	Alfalfa	Fescue and Alfalfa mixture	
Composition (%)			
CP[a]	17.7[b]	12.4[c]	0.29
NDF[a]	42.2[c]	56.5[b]	1.06
ADF[a]	28.4[c]	30.4[b]	0.64
Ash[a]	7.98[b]	6.95[c]	0.09
Alkanes (mg/kg)			
C_{31}[a]	322.3[b]	261.53[c]	11.93
C_{33}[a]	28.4[c]	53.3[b]	1.55
DMI[d] (kg/d)	6.01	6.07	0.19
In vitro DM digestibility (%)	71.7	69.1	2.23
Digestibility with markers (%)[e]	62.01	61.31	1.41

[a] DM basis.
[b, c] Within rows, means with different superscripts are different ($p<0.05$).
[d] Average of 117 values.
[e] Average of 111values.

Tukey's test was used to compare the measured intake with the intake estimated from C_{33}:C_{32} ratio or C_{31}:C_{32} ratio. Tukey's test was also used when comparing the measured *in vitro* digestibility with the estimated digestibility using C_{33} or C_{31} alkane.

RESULTS AND DISCUSSION

The chemical composition of hay used in the study is presented in Table 1. Crude protein was higher ($p<0.05$) and NDF was lower ($p<0.05$) in alfalfa hay, compared with fescue/alfalfa mixed hay.

Concentration of n-alkanes in hay varied with type of hay. Amount of C_{31} in alfalfa hay was 19% higher ($p<0.05$), compared with fescue/alfalfa mixed hay but amount of C_{33} in alfalfa was 47% lower ($p<0.05$) than that of fescue/ alfalfa mixed hay (Table 1). Several workers have reported that alkane concentration in plant material depends on many factors such as plant species (Mallossini et al., 1990; Dove and Mayes, 1991; Dove and Mayes, 1996), plant parts (Dove and Mayes, 1996; Genro et al., 2001), season (Vulich et al., 1993), and maturity (Vuilich et al., 1993). The difference in species may have affected the amount of alkanes in different types of hay used in this study. In a review of n-alkanes as markers, Dove and Mayes (1991) suggested drying method affects herbage n-alkane concentration in feed and feces. During oven drying, the high temperature may subject hentriacontane (C_{31}) to either marker degradation or chemical reactions that make extraction incomplete. Sandberg (1998) studied the effect of drying method on n-alkane in feed and feces and concluded that oven drying may decrease the amount of n-alkane extracted from feces. According to these workers, it would be advisable to use freeze drying instead of oven drying at sample preparation. Presence of C_{32} in both hays was very low (<2 mg/kg DM). Amounts of C_{31} and C_{33} in alfalfa and fescue/alfalfa mixed hay are in agreement with previous works (Baker and Klein, 1994; Ordakowski et al., 2001).

Table 2. Comparison of DMI of steers and digestibility of hay by different methods[1]

Item	Method			SEM
	1	2	3	
DMI[2] (kg/d)	6.14[a]	5.65[b]	6.33[a]	0.28
Digestibility[3] (%)	56.8[b]	57.89[b]	70.30[a]	0.63

[1] Average of 72 values.
[2] Methods used were 1 = estimated by C_{33}:C_{32} ratio, 2 = estimated by C_{31}:C_{32} ratio; 3 = from animal feeding trial.
[3] Methods used were 1 = estimated by C_{33}, 2 = estimated by C_{31}, 3 = *in-vitro* DMD.
[a, b] Within rows, means with different superscripts are different ($p<0.05$).

According to the capsule manufacturer, mean release rate for C_{32} was 200 mg/d, and the expected time-span at a constant release rate was 20±3 days. When release rate of capsules were tested under our trial conditions, it was found that four capsules started to deteriorate on d 21, six capsules on the d 22 and, two capsules on the d 23. Therefore, based on the deterioration of the capsules given above, the following release rates were used in intake calculations for those animals respectively: 197.6 mg/d for four animals; 188.6 mg/d for six animals and 180.4 mg/d for two animals.

No significant differences were observed between alfalfa and fescue alfalfa mixed hay on DMI, *in vitro* DM digestibility or digestibility with markers (Table 1). *In vitro* DMD of alfalfa hay was higher ($p<0.11$), than that of fescue/alfalfa mixed hay.

The DM intake of steers measured by different methods is shown in Table 2. No significant difference was found between the measured intake and the intake calculated with C_{33}:C_{32} ratio however, intake calculated from C_{31}:C_{32} ratio was lower ($p<0.05$) than the measured intake (Table 2). Average estimated intakes for alfalfa and fescue/alfalfa mixed hay with C_{31}:C_{32} were 9.59% and 11.33% below than the measured intake, respectively. These results are in agreement with the other workers who reported that good estimates of herbage intake could be obtained using C_{33}:C_{32} alkane pair (Mayes et al., 1986a; Vulich et al., 1991). According to Casson et al. (1990), if estimates of forage intake are to be reliable, concentration of the natural alkane in the forage used as the internal marker should exceed 50 mg/kg DM. However, C_{33} concentration in alfalfa hay was about 28.4 mg/kg DM in this experiment but intake estimation with C_{33}:C_{32} was similar to the measured intake.

In vitro DM digestibility of hay were much higher ($p<0.05$) compared to the digestibility values estimated using C_{31} or C_{33} alkane (Table 2). In general, *in vitro* digestibility values, usually are somewhat higher than *in vivo* values (Van Soest and Robertson, 1980; Nsahlai and Umunna,

1996), due to the smaller particle size of samples. Furthermore, when calculating the digestibility from n-alkanes, recovery rates for C_{31} and C_{33} have to be included in the equation. Since total collection of feces was not done in this study, recovery rates for C_{31} and C_{33} were taken from Lopez-Guerrero et al. (2004) where similar animals, feeds and conditions were used. Mayes et al. (1986b), reported recovery rates of 0.59 and 0.81 for C_{31} and C_{33} alkanes respectively, in cattle where alkanes were given as a pellet (absorbed in shredded paper). Unal and Garnsworthy, (1999) reported a value of 0.94 for C_{33} in dairy cows when alkanes were dosed either on filter papers or as part of a specially prepared concentrate. According to literature, *in vivo* digestibility for fescue/legume mixed hay can vary from 63.4 to 67.5% (Brown et al., 1963; Pendulum et al., 1980; Bagley et al., 1983; Reid et al., 1988) whereas *in vivo* digestibility of legume hay for cattle can be around 62.8% (Reid et al., 1988). These *in vivo* digestibility values are somewhat higher compared to the values calculated from both markers in the present study (Table 2).

CONCLUSION

Intra-ruminal controlled-release capsule technology appears to be an accptable and practical way to dose C_{32} alkanes for estimating feed intake by beef cattle. Results of this study show that alkanes C_{33}:C_{32} ratio could be used for estimating intake by beef cattle satisfactorily, hence, alkanes may be used to estimate the feed intake of ruminants.

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