Estimating dry matter digestibility and intake in wapiti (Cervus elaphus canadensis) using the double n-alkane ratio technique

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Abstract

We estimated dry matter digestibility and intake (DMI) in wapiti hinds using the double n-alkane method. We also assessed intra-ruminal controlled-release devices (CRD) for administering n-alkane markers C32 and C36. Nine wapiti hinds were offered alfalfa-based compounded diet for ad libitum consumption and actual DMI was measured daily (mean: 9.2 ± 1.6 kg DM/day). The DMI estimate for C33:C32 (9.4 ± 1.6 kg DM/day, +2%) was slightly more accurate (P > 0.05) than that for C31:C32 (9.8 ± 1.8 kg DM/day, +6%), whereas C31:C32 was slightly more precise (P > 0.05) than C33:C32 in predicting intake (C31:C32 — R² = 0.84; C33:C32 — R² = 0.71). Feed digestibility, estimated using dosed C36, was 63%. Level of intake did not significantly affect n-alkane marker excretion rate, feed digestibility, nor n-alkane fecal recovery (P > 0.05). This offers the advantage of concurrent use of the same CRD for predicting DMI in animals over a range of intakes. The manufacturer’s in vitro release rate (58.5 mg/day) was slightly higher than estimates of C32 (52 ± 6) (P < 0.01) and C36 (50 ± 6) (P > 0.05) based on fecal output. There was more variation among days than among animals in excretion rates of C32, but not C36. The double n-alkane ratio technique provides reliable assessment of DMI in wapiti feeding on alfalfa-cubes and may prove useful for determining intake of free-ranging ruminants. Furthermore, CRD eliminate daily dosing, thus minimizing disturbance of normal grazing patterns. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Indigestible compounds as markers to determine herbage digestibility and intake have met varying degrees of success. For example, the widely used chromic oxide (Cr₂O₃) technique presents problems that emanate from applying a single digestibility value to all animals in a group, unless suitable internal markers are used (Dove et al., 1989, 1990; Dove and Mayes, 1991). Problems also arise from internal markers with variable digestibilities that produce erroneous estimates of DMD, and therefore, DMI (Langlands, 1975).

Long-chain n-alkanes (components of plant cuticular wax) have minimal absorbability in ruminants (Oro et al., 1965) and are relatively easy to analyze. Use of these indigestible plant components offers several advantages over other methods for herbage digestibil-
ity and intake determination in grazing ruminants (Mayes et al., 1986). Naturally occurring $n$-alkanes of plant species are predominantly odd-chain-length in the range C$_{25}$–C$_{35}$ (Hawke, 1973). Dotriacontane (C$_{32}$) and hexatriacontane (C$_{36}$) may be ideal external markers, as they can be easily and inexpensively obtained in pure form and are present in very low concentrations in herbage.

The double $n$-alkane ratio technique involves dosing known quantities of $n$-alkanes of a chain-length adjacent to those naturally occurring in high concentrations in herbage. This minimizes errors in digestibility and intake estimation due to among-animal variations in faecal $n$-alkane recovery could be eliminated if such faecal recoveries were concurrently estimated in the same animal. Although this paired-marker technique has shown considerable promise in several domestic ruminants, it must be thoroughly tested before it can be confidently used with mixed and selective feeders, such as cervids, that have relatively smaller rumens and more rapid rates of passage.

Most marker methods require single or twice daily dosing, which is labor intensive and can result in diurnal variation of marker release and disruption of animal grazing patterns. An intra-ruminal controlled-release device (CRD) can eliminate these problems (Laby, 1981; Ellis et al., 1981), a particularly important issue with wild ruminants. Although earlier CRD investigations with Cr$_2$O$_3$ had limited success (Parker et al., 1990; Buntinx et al., 1992), recent work has shown more promise (Momont et al., 1994). With the exception of studies by Kelly et al. (1985) on fallow deer (Dama dama) and Parker and Ataja (1990) on young red deer stags (Cervus elaphus elaphus), testing of CRD has largely been restricted to sheep and cattle, and only preliminary evaluation has involved $n$-alkanes (K.J. Ellis, personal communications).

The objectives of this study were to evaluate the double $n$-alkane ratio technique for estimating digestibility and DMI in wapiti (Cervus elaphus canadensis). We also assessed the use of intra-ruminal controlled-release devices for administering $n$-alkane marker.

2. Materials and methods

In April, 1998, a pen trial was conducted at the Ministik Research Station in central Alberta. Nine wapiti hinds (mean weight: 295 ± 18 kg; mean age: 10 ± 3) in their last trimester of gestation were confined to a 50 × 15 m enclosure void of all vegetation. Hinds were given ad libitum access to a cubed alfalfa-based (Medicago sativa) diet (“El-Kube”, Moore’s Auctioneering, Alder Flats, AB) for three weeks in a Pinpointer 4000B automatic feed monitoring system (Microcomputer Controls, Nashville, TN). This system records individual daily feed intake of each animal.

Following a one-week adjustment to the diet, pen and feed monitoring system, a CRD (Captec (NZ), Auckland, New Zealand) was administered per os into the rumen of each hind. Capsules were standard cattle CRD, modified with longer wings when it was discovered in preliminary trials that they were inconsistently retained in the wapiti rumen. The CRD was designed and rated to release 58.5 mg/day each of C$_{32}$ and C$_{36}$ for approximately 15 days. Rumen $n$-alkane equilibrium was achieved within five days of dosing and trial duration was two weeks. A small sample of freshly voided feces was collected daily from each animal commencing on day five. Fecal samples were freeze-dried at −60°C for 72 h, ground through a 20-mesh screen in a Wiley mill, and $n$-alkanes extracted.

$n$-Alkanes were extracted from freeze-dried fecal and feed samples by adding 10 ml hexane (HPLC grade) and 5 ml distilled water, transferring separated hexane layer to a 20 ml scintillation vial, then repeating extraction with another 8 ml hexane. Pooled hexane layers were evaporated to approximately 500 μl and passed through a silica gel column (70–230-mesh) to separate lipids from $n$-alkanes. Scintillation vials were rinsed twice with 2 ml hexane which was also added to the silica gel column. Approximately 1 ml of remaining solution is transferred to a 1 ml glass gas chromatography (GC) vial. Analysis was conducted on a Varian 3400 Capillary GC equipped with a Varian 8100 Autosampler and EzChrom GC Data System (Version 3.1). Capillary column was an Rt$_{t-1}$, 30 m × 0.25 mm ID × 0.25 μ df with helium as the carrier gas. The initial column temperature was set at 80°C, held for 0.04 min, then programmed to rise 20°C/min to a maximum of 280°C for a four minute holding time. Septum programmable injector temperature commenced at 90°C and increased to 280°C at a rate of 150°C/min, at which it was held for twelve minutes. Flame ionization detector tem-
perature was 280°C. Individual n-alkanes were identified from their retention times on the column, and peak areas on the printout converted to concentrations of n-alkanes (in ppm) by reference to the internal standard C34 (tetratriacontane).

The relative concentration of naturally occurring (C31) and dosed (C32) n-alkanes in both herbage and faeces samples were used to calculate the herbage intake (in kg) of organic matter per day. DMI was calculated using two pairings of adjacent n-alkanes (C31:C32, C33:C32):

Herbage intake (DMI) (kg DM/day)

\[ \frac{(D_{32} \times F_n/F_{32})}{H_n - (F_n/F_{32}) \times H_{32}}, \]

where \( D_{32} \) is the excretion rate (mg/day) of the dosed n-alkane (C32), \( F_{32} \) and \( H_{32} \) are the respective concentrations (mg/kg DM) of C32 in the feces and herbage, and \( F_n \) and \( H_n \) are the concentrations (mg/kg DM) of the natural (i.e., non-dosed) n-alkanes (either C31 or C33) in feces and herbage, respectively.

Fecal recovery of n-alkanes increases with carbon chain-length (Dove and Mayes, 1991). Therefore, C36 was used to determine herbage digestibility (%) since it was the most indigestible n-alkane. Following Heydon et al. (1993), digestibility was calculated

Feed digestibility (fraction)

\[ 1 - \frac{[(0.96 \times D_{36})/F_{36}]}{DMI}, \]

where \( D_{36} \) is the excretion rate (mg/day) of the dosed C36, \( F_{36} \) is the fecal concentration (mg/kg DM) of C36, and 0.96 represents a correction factor to account for partial digestibility of C36 (Heydon et al., 1993). DMI represents actual intake (kg) electronically recorded by the automatic feed monitoring system.

Marker excretion in individual wapiti was predicted (e.g., for C32):

Marker excretion

\[ = \left( \frac{FO \times F_{32}}{((H_{32} \times DMI + 58.5) \times DIG)} \right) \times 100\%, \]

where FO is the calculated faecal output which is equal to DMI*(1 – (DIG/100))(mg/day), \( F_{32} \) the C32 faecal concentration (mg/kg), \( H_{32} \) the C32 herbage concentrated C32 (mg/kg), DMI the actual DMI (measured by pinpointer) (mg/day), 58.5 the manufacturer’s C32 release rate (corrected with our endpoint determination, mg/day) and DIG is the calc. feed digestibility (see Eq. (2)).

The accuracy of the double n-alkane ratio method was assessed by regressing estimated against actual intake. Further comparisons were made using non-parametric Mann-Whitney tests (Conover, 1980). One-way analysis of variance and Bonferroni’s pairwise multiple comparisons were used to compare herbage and fecal n-alkane concentrations and n-alkane fecal recovery (SPSS, 1998). Simple linear regression analysis evaluated uniformity of marker excretion in feces, and tested effects of intake level on recovery and excretion of n-alkanes in feces and feed digestibility. Means are reported with standard errors, and probabilities of \( \alpha < 0.05 \) were accepted as significant. Statistical computations were performed using SPSS Base 8.0 (1998).

3. Results

DMI was estimated in eight gestating wapiti, as one animal regurgitated its CRD early in the trial. Actual intake was plotted against estimated DMI using simple linear regression (Fig. 1). The C31:C32 pairing demonstrated a better fit (slope = 1.09, \( p < 0.001 \); x-intercept = −0.20, \( p \geq 0.05 \); \( R^2 = 0.84 \)) than C33:C32 (slope = 0.84, \( P < 0.01 \); x-intercept = 1.71, \( P > 0.05 \); \( R^2 = 0.71 \)).

Intake calculations were based on the mean of eight consecutive days from day 5 to day 12 (dosing day = day 0), to ensure that n-alkane release was at equilibrium. Mean measured intake was 9.18 ± 1.56 kg DM/day (range: 6.99–12.53 kg DM/day). Both n-alkane pairings slightly overestimated actual intake (C31:C32 — 9.79 ± 1.84 kg DM/day; C33:C32 — 9.38 ± 1.55 kg DM/day). Biases of DMI (difference between estimated and actual intake, expressed as a percentage of actual intake) based on C31:C32 and C33:C32 estimates were 6.1% and 2.2%, respectively.

Nonparametric Mann-Whitney tests implied no difference between estimates using n-alkanes and actual intake for both adjacent pairs (C31:z = 0.63, C33:z = 0.32, Z0.025 = 1.96, \( P > 0.05 \)). Estimates of intake based on C31:C32 and C33:C32 were not significantly different (\( z = 0.42, Z_{0.025} = 1.96, P > 0.05 \)).

Mean digestibility of the alfalfa-based diet offered to wapiti (calculated from dosed C36) was
Simple linear regression was used to test for effect of feeding level on digestibility, and no significant effect was found ($P > 0.05, R^2 = 0.13$). The most abundant $n$-alkane found in the cubed feed offered to wapiti was C31, followed by C29. C36 occurred in very low quantities in feed, while only traces of C35 could be detected. Differences of recovery rates were not significant among the specific $n$-alkanes used to determine digestibility and DMI (Table 1, $P > 0.05$). With the exception of C33, fecal recovery increased with carbon chain-length. Recovery (%) of C32 (95.3 ± 13.7) more closely resembled that of C31 (94.5 ± 15.7) than C33 (92.9 ± 16.0), although this difference was not significant ($P > 0.05$). Each $n$-alkane was tested for the effects of intake level on fecal recovery using simple linear regression, and no significant effects were found ($P > 0.05$).

Mean excretion rates (mg/day) of dosed $n$-alkanes, C32 and C36, based on fecal output were 51.8 ± 4.4 and 50.4 ± 6.2, respectively. There was more variation among days than among animals in excretion rates of C32, but not for C36. Predicted marker excretion in individual wapiti (across days) was closely associated with actual release for C32 ($P < 0.01, R^2 = 0.84$), but not for C36 ($P > 0.05, R^2 = 0.15$). Averaging animals across days had the same effect for C36 ($P > 0.05, R^2 = 0.15$) but exhibited a much weaker relationship for C32 ($P > 0.05, R^2 = 0.18$).

The manufacturer rated the CRD used in this study at a daily marker excretion rate of 58.5 mg of both C32 and C36 over a period of 15 days (K.J. Ellis, unpublished data). Comparison of DMI (kg) and $n$-alkane

![Graph](image)

**Fig. 1.** Relationship between actual dry matter intake (DMI) of wapiti hinds ($n = 8$) and that estimated using (a) C31:C32 and (b) C33:C32 adjacent $n$-alkane pairings. Straight lines represent the least-squares regressions ((a) C31:C32 — $y = 1.09x - 0.20, p < 0.001, R^2 = 0.84$; (b) C33:C32 — $y = 0.84x + 1.71, p < 0.01, R^2 = 0.71$). Curved lines on either side of regression lines represent 95% confidence limits.

<table>
<thead>
<tr>
<th>$n$-Alkane</th>
<th>Concentration (mg/kg DM)</th>
<th>Faeces ($n = 8$)</th>
<th>Recovery (%) ($n = 64$)</th>
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</thead>
<tbody>
<tr>
<td>C29</td>
<td>157.9±2.4</td>
<td>226.1±4.4</td>
<td>83.8±14.5</td>
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<tr>
<td>C31</td>
<td>216.3±2.3</td>
<td>348.0±7.2</td>
<td>94.5±15.7</td>
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<tr>
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<td>21.3±2.0</td>
<td>95.3±13.7</td>
</tr>
<tr>
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<td>23.0±0.8</td>
<td>92.9±16.0</td>
</tr>
<tr>
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<td>IS**</td>
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<tr>
<td>C35</td>
<td>Trace</td>
<td>Trace</td>
<td>-</td>
</tr>
<tr>
<td>C36</td>
<td>1.9±0.4</td>
<td>16.1±3.5</td>
<td>113.2±10.9</td>
</tr>
</tbody>
</table>

*a,b,c,d* Means that do not share superscripts differ ($P < 0.05$).

*Number of samples.

**Internal standard.
4. Discussion

Most studies that have compared actual intake to n-alkane estimates have found the C_{33}:C_{32} pairing to be a more accurate predictor of DMI than C_{31}:C_{32} (sheep: Mayes et al., 1986; Vulich et al., 1991; dairy cows: Dillon and Stakelum, 1988, 1990; Dillon, 1993). The only exception is the work of Duncan (1986) with goats, which demonstrated slightly greater accuracy from C_{31}:C_{32} estimates. Dove and Mayes (1991) indicated that, as carbon chain-length increases, differences in fecal recovery between adjacent n-alkanes decrease. Therefore, if the longer chain-length n-alkane is used (i.e. C_{33} in this study), lower disparity between recoveries will lead to more accurate predictions of intake.

Mayes et al. (1986) suggested that pairing the dosed (even-chain) n-alkane with the adjacent shorter carbon chain-length n-alkane should produce a slight underestimate of herbage intake, whereas if coupled with the longer-chain n-alkane, DMI should be slightly above measured intake. In the present study, both n-alkane pairings resulted in overestimation of herbage intake. This likely emanates from the difference in fecal recovery between C_{31} and C_{32} (0.8%) that was less than that between C_{33} and C_{32} (2.4%), although, all three n-alkane recoveries were not significantly different ($P > 0.05$).

Unlike herbage intake calculations using n-alkanes (where use of adjacent pairs obviates problems of unknown fecal recovery), estimations of digestibility require correction for incomplete recovery. In the present study, a fecal recovery of 96% for C_{36} was assumed for calculation of digestibility. Based on the fecal recoveries of n-alkanes C_{29}–C_{33} found in this study, and the logarithmic response displayed in other work (n-alkane recovery against carbon chain-length) (Duncan, 1986; Dove and Mayes, 1991), this value seems a reasonable conjecture.

n-Alkane concentrations and change in concentration with increasing carbon chain-length are in agreement with other work on *Medicago sativa* (Malossini et al., 1990). Low quantities of feed C_{36} (1.9 ± 0.4 mg/kg DM) in this study may lead to inaccuracies when determining fecal recovery and may explain C_{36} recovery values exceeding 100%. Fecal recoveries of C_{31} have ranged from 63% in sheep (Dillon and Stakelum, 1990) to as high as 99% in goats (Duncan, 1986). Although quantitative marker recovery is not a requisite of this technique, dosed (even-chain) and natural (odd-chain) n-alkane pairs must have the same fecal recovery (Vulich et al., 1991).

Treatment of herbage and fecal samples following collection may influence estimates. Samples are most commonly freeze-dried, however, some have oven-dried them at 100°C (Duncan, 1986), while others have even used a different drying process for herbage than for fecal samples (Vulich et al., 1991). Herbage n-alkanes are distributed in the thin film of wax on the plant surface and exposure to excessive heat could leave behind quantities of n-alkanes as residues in the drying container. Identical treatments should be applied to all samples, and in this study, both fecal and herbage samples were freeze-dried at −60°C.

Problems with bolus regurgitation and failure of CRD to release marker have been reported (Ellis et al., 1981; Parker et al., 1990; Momont et al., 1993). In this study, 8 of 9 CRD remained functional in the wapiti rumens for the duration of the trial and exhibited reasonable fecal marker concentrations. This contrasts with preliminary trials that involved high loss rates of CRD designed for sheep.

5. Conclusion

The double n-alkane ratio technique can provide accurate and precise assessment of DMI in wapiti, at least on uniform diets. Its value on pasture depends on selecting appropriate n-alkanes and accurate knowledge of diets. DMI predictions using C_{33}:C_{32} n-alkanes did not differ significantly from those using C_{31}:C_{32}. However, C_{33}:C_{32} estimates were slightly more accurate, while those from C_{31}:C_{32} adjacent pairs were more precise. CRD for n-alkane administration provide uniform daily marker release in wapiti. They offer the advantage of eliminating daily dosing, thus minimizing disturbance to normal grazing patterns. Level of intake had no effect on n-alkane marker excretion rate, feed digestibility, nor fecal n-alkane recovery.
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References


