Validation of the double η-alkane technique to estimate the dry matter intake of red deer fed fresh ryegrass-based pasture or plantain

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ABSTRACT

The accuracy of the double η-alkane technique to estimate dry matter intake (DMI) of red deer (*Cervus elaphus)* fed fresh cut perennial ryegrass-based pasture (*Lolium perenne* cv Nui) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) was determined in a cross-over experiment involving five adult castrated stags housed in metabolism cages. A strong positive linear relationship between actual and estimated DMI was obtained for pasture when using both C_{31} and C_{33} (C_{31} R^2 = 0.94*;* P < 0.01; C_{33} R^2 = 0.92; P < 0.01) and plantain for C_{31} (R^2 = 0.80*;* P < 0.04), but not for C_{33} (R^2 = 0.49; P < 0.19). However, the predicted DMI of deer using the η-alkane technique was underestimated up to 27.6% for deer fed ryegrass-based pasture and overestimated up to 17.2% for deer fed plantain when compared with actual DMI. The discrepancies between actual and predicted DMI could have been partly due to a difference of 26% between faecal recovery rates of odd and even-chain alkanes with pasture feeding and 12% with plantain feeding. This suggests that the η-alkane technique may not be appropriate for estimating DMI of ruminants grazing New Zealand fresh forages, especially when comparisons between forage species are required.

Keywords: alkane technique; feed intake; faecal recovery rates; plantain; pasture.

INTRODUCTION

Forage feeding value is a function of voluntary feed intake (VFI) and associated digestive processes (Ulyatt *et al.,* 1980; Waghorn & Clark, 2004) with potentially up to 70% of the variation observed in feeding value attributable to differences in VFI (Ulyatt *et al.,* 1980). Ruminant methane emissions described in the New Zealand (NZ) Greenhouse Gas Inventory (Anon, 2003), required by the Kyoto Protocol, are expressed per unit feed intake. Therefore, accurate determination of VFI is critical for current research investigating forage feeding value, providing valid measurements of methane production and evaluating methane mitigation technologies.

Determining the VFI of grazing animals is notoriously difficult. Traditional methods are based upon the ratio between faecal output and forage digestibility. This relies on total faecal collections or estimates of total faecal output using internal or external markers. However, markers are often not ideal and faecal recoveries are variable (Dove & Mayes, 1996). A further difficulty is that an *in vivo* or *in vitro* estimate of digestibility must be applied to the forage consumed and may not take into account individual animal variability in digestibility, diet selection and changing forage chemical composition.

The double η-alkane technique, using saturated long-chain hydrocarbons of plant cuticular wax (alkane) has been proposed as a reliable alternative to estimate dry matter intake (DMI) (Dove & Mayes, 1996). This technique has been reported to be used in NZ to estimate VFI of grazing sheep, cattle and deer, often in conjunction with rumen controlled release capsules to administer the even-chain alkanes (Ulyatt *et al.*, 2002; Swainson, 2004). However, recent use of the double η-alkane technique in red deer grazing ryegrass-based pasture (*Lolium perenne*), chicory (*Cichorium intybus*) or plantain (*Plantago lanceolata*) questioned the accuracy of the intake estimations due to unrealistically high values obtained for chicory (Swainson, 2004).

Advantages of the double η-alkane technique are firstly that alkane faecal recoveries need not be complete, provided samples accurately represent faecal alkane content, and secondly, this method allows for individual variation between animals as it does not require a common digestibility value to be applied to all animals (Dove & Maye, 1996).

Limited reports describing validation studies of the double η-alkane technique with ruminants, let alone deer, fed fresh pasture or forages are available in the literature, particularly when coupled with use of rumen controlled release devices. Therefore, the primary objective of this study was to validate the double η-alkane technique as used under grazing conditions for estimating DMI of deer. A secondary objective was to compare the release rate of even-chain alkanes from rumen controlled release capsules administered to adult red deer fed either fresh ryegrass-based pasture or plantain.

MATERIALS AND METHODS

Experimental design and animals

An experiment of cross-over design was conducted at the deer metabolism facility, Massey University's Deer Research Unit, September to October 2003. During Period one, five adult castrated red deer (*Cervus elaphus)* (mean age ± SD = 8.7 ± 6.1 years;

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mean live weight ± SD = 146.6 ± 55.6 kg), with rumen fistulae were randomly assigned to one of two forage diets, either fresh cut pasture comprising 94-96% perennial ryegrass (*Lolium perenne* cv Nui), (pasture) or narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) fed twice daily at 0800 and 1600 hours. Forage treatments were reversed for Period two. Each period lasted for three weeks consisting of: week one, animals grazing their assigned forages; week two, animals housed individually indoors whilst adapting to housing and harvested forage; and week three, measurement.

Voluntary feed intake

Dry matter allowance was determined during week two of Period one by feeding 1.2 times the previous days DM allowance until intakes stabilised, and set at this level to individuals thereafter. Voluntary feed intake was measured directly (feed offered less feed refused) and also calculated from the double η-alkane technique described by Dove *et al.* (2002) with the following adaptations. Rumen controlled release capsules were used to administer the dosed alkanes and manufactured for growing cattle (Captec, Nufarm Ltd., NZ), containing 4 g of η-dotriacontane (C_{32}) plus 4 g η-hexatriacontane (C_{36}) and inserted via the rumen cannulae on the first day of week two of each period and removed on the last day of each period. The alkane capsules were attached to a nylon string tied to the rumen cannulae to allow daily removal at 1300 h for estimation of alkane release rate by measuring the matrix disappearance rate in mm/day which was converted to mg per alkane per day based upon the linear density of the matrix given by the supplier (65.56 mg/mm for C_{32} and 69.34 mg/mm for C_{36}). Dry matter intake was estimated, using Equation 1, from the daily dose rate (D_j) and the herbage and faecal concentrations (H_j and F_j, respectively) of the dosed even-chain alkane (C_{32}), and the adjacent natural odd-chain alkane (C_{31} and C_{33}) (H_i and F_i, respectively).

Equation 1

$$Intake = \frac{F_i}{F_j} D_j \Big/ \left(H_i - \frac{F_i}{F_j} H_j \right)$$

Total faecal output, required to determine alkane recovery rate, was weighed daily at 0900 h, mixed and a 10% sub sample taken and stored frozen to be later pooled, within animal for each period, for DM determination. Faecal samples for alkane analysis were collected once daily (0900 h) during the measurement period from individual defecations occurring during this period. This was to represent faecal samples obtained by rectal sampling as undertaken if deer were in a grazing situation, where although up to two samples can be taken daily with other ruminant species (Ulyatt *et al.,* 2002), once a day sampling should be undertaken with deer due to handling difficulties and associated effects of stress on VFI in the grazing situation (Ru *et al.,* 2002; Swainson, 2004).

Forage sampling and laboratory analyses

Triplicate 200 g samples of feed offered and refused of each animal were taken daily for DM determination, 200 g samples of each feed offered and refused were taken daily for alkane analysis and another 200 g sample taken for chemical analysis. Faecal, feed offered and refused samples for alkane determination were oven dried for 48 h at 60°C and ground to pass a 1 mm sieve (Willey Mill, USA). Content of η-monotriacontane (C_{31}) to η-hexatriacontane (C_{36}) were determined by gas chromatography as described by Dove *et al.* (1996), with the following modifications. Industrial heptane was used instead of analytical grade and saponification took place in an oven rather than on heating blocks. Feed samples for chemical analysis were freeze dried and ground as above. Samples were analysed for organic matter (OM), hot water-soluble carbohydrates (HWSC), acid and natural detergent fibre (ADF and NDF), and lignin, as described in detail by McWilliam *et al.* (2004).

Statistical analysis

Data were analysed by SAS (SAS, 1998) using the general linear model (PROC GLM) with simple ANOVA or the regression model (PROC REG) for linear regression analysis. Type of forage (pasture or plantain), period, methods of intake measurement and their interactions were fixed effects of the model. Significance was declared at $P \leq 0.05$, and a trend was reported if $0.05 < P \leq 0.10$. All mean comparisons were by Fisher's least significant difference method after a significant main effect was detected.

TABLE 1: Chemical composition (mean ± SEM of both periods) of pasture and plantain offered to deer

% DM	Pasture	Plantain
Organic matter	88.8 ± 0.29	92.0 ± 0.29
ADF	24.2 ± 0.27	26.1 ± 0.26
NDF	42.5 ± 0.07	38.0 ± 0.07
Lignin	1.62 ± 0.123	7.40 ± 0.123
Pectin	1.44 ± 0.041	3.80 ± 0.041
HWSC	8.8 ± 1.26	18.3 ± 1.26
CP	21.7 ± 0.08	24.2 ± 0.08
Gross energy (MJ /kg DM)	18.9 ± 0.03	18.5 ± 0.03

RESULTS

The chemical composition of pasture and plantain are shown in Table 1. There was found to be a significant effect of forage, period and interaction of forage by period ($P < 0.05$). The alkane concentrations of both feed offered and refused of both forages are shown in Table 2. The content of C_{31} in pasture tended to be lower compared with plantain ($P < 0.06$), but the

converse was found for C_{33} and C_{35} ($P < 0.01$). The content of $C_{31,}$ C_{33} and $C_{35,}$ of the feed offered and feed refused was similar ($P > 0.9$).

Regression of the rate of alkane rumen capsule matrix disappearance with time, was found to be strongly linear for both forages with little variation around the line of best fit (pasture $y = -2.68x + 27.04$, $R^2 = 0.95$, $P < 0.01$ and plantain $y = -3.03x + 25.90$, $R^2 = 0.90$, $P < 0.01$). However, disappearance rate of the alkane matrix in animals fed pasture was lower compared with those fed plantain ($P < 0.05$).

TABLE 2: The mean η-alkane concentrations of feed offered and refused for both pasture and plantain (concentrations given are an average of both periods) fed to deer.

	η-alkane concentrations (mg/g)				
	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Feed offered					
Pasture	0.182	0.009	0.117	0.011	0.000
Plantain	0.240	0.015	0.064	0.008	0.000
Feed refused					
Pasture	0.199	0.011	0.136	0.016	nd
Plantain	0.258	0.018	0.070	0.009	nd

nd - not determined, number of samples of feed offered (n = 2) and feed refused analysed (n = 5).

The average dose rate, calculated from the matrix disappearance rates, of C_{32} and C_{36} were found to be below that of the stated manufacturer's dose rate when animals were fed pasture ($C_{32,}$ 175.82 and $C_{36,}$ 185.96, ± 5.91 (SEM) mg/day). However, the real and manufacture's dose rates ($C_{32,}$ 200 and C_{36} 211.5 mg/day) were similar for animals fed plantain ($C_{32,}$ 199.10 and C_{36} 210.58, ± 5.91 (SEM) mg/day).

The average faecal recovery rates of both natural and dosed alkanes are presented in Table 3. The recovery rates of individual alkanes differed from one another ($P < 0.003$). However, only a weak relationship was found between faecal recovery rate and increasing alkane carbon length for both pasture ($R^2 = 0.09$; $P > 0.15$) and plantain ($R^2 = 0.0009$; $P > 0.9$). The faecal recovery rates of alkanes used to calculate intake (C_{31}, C_{32} and C_{33}) differed from one another by up to 26% when animals were fed pasture and up to 12% when animals were fed plantain.

Actual and estimated DMI using both C_{31} and C_{33} for both forages are shown in Table 4. The actual DMI of pasture was similar to that of plantain ($P < 0.2$). Actual DMI was greater in Period 1 (1634 ± 50 SEM g/day) compared with Period 2 (1452 ± 50 SEM g/day) ($P < 0.01$).

On average, the η-alkane technique underestimated the DMI of deer consuming pasture by 27.6% and overestimated the mean DMI of plantain by 17.2%. This contributed to a significant method of intake determination by forage interaction ($P < 0.01$). Regression analysis showed a strong linear relationship between actual and estimated DMI when using C_{31} and C_{33} for pasture (C_{31} $R^2 = 0.94$; $P < 0.01$; C_{33} $R^2 = 0.92$; $P < 0.01$) and plantain (C_{31} $R^2 = 0.80$; $P < 0.04$), but not when DMI of plantain was estimated by C_{33} ($R^2 = 0.49$; $P < 0.19$). Adjustment of the faecal alkane concentrations for the faecal recovery rates of alkanes C_{32} and C_{31} or C_{33}, used to estimated intake resulted in an improvement in the estimation of pasture, but not plantain.

TABLE 3: Faecal recovery rates of both dosed (even-chain) and natural (odd-chain) η-alkanes of deer fed pasture or plantain.

	Faecal recovery (%)				
	C_{31} [a]	C_{32} [ab]	C_{33} [a]	C_{35}	C_{36} [b]
Pasture					
Deer P	77.8	98.5	81.8	89.0	88.29
Deer A	80.8	102.6	87.7	96.6	95.31
Deer G	75.0	107.1	80.3	89.2	93.63
Deer T	84.9	106.5	93.8	105.8	101.0
Deer S	75.7	113.4	80.6	87.8	104.5
Mean ± SEM	78.8 ± 1.82	105.6 ± 2.48	84.8 ± 2.61	96.7 ± 3.39	96.5 ± 2.84
Plantain					
Deer P	135.7	111.1	158.9	172.4	89.7
Deer A	129.8	136.3	154.7	169.0	106.8
Deer G	138.8	104.8	187.0	202.4	88.5
Deer T	121.9	109.5	13.6	150.4	98.4
Deer S	116.5	123.4	12.9	141.6	114.3
Mean ± SEM	128.5 ± 4.16	117.0 ± 5.72	105.4 ± 38.03	167.1 ± 10.49	99.6 ± 4.96

[a] Alkanes used to calculate intake. [b] Dosed alkanes.

TABLE 4: Average actual and estimated DMI using the double η-alkane technique, of deer fed fresh perennial ryegrass-based pasture and plantain.

DMI (kg/day)	Pasture	Plantain	Average SEM	P-value forage
Intake technique				
Actual	1.52[a]	1.63[a]	0.089	0.3631
Estimated C31	1.10[b]	1.83[ab]	0.089	0.0001
Estimated C33	1.16[c]	1.91[b]	0.089	<.0001

[abc] Differing letters within columns denotes a significant difference between methods of intake determination ($P < 0.05$)

DISCUSSION

The present study feeding fresh forages has confirmed previous reports feeding conserved forages and concentrate rations (Gedir & Hudson, 2000; Ru *et al.,* 2002; Dove *et al.,* 2002; Lewis *et al.,* 2003) and fresh perennial ryegrass-based pasture (Vulich *et al.,* 1991; Waghorn *et al.,* 2004) of a strong positive linear relationship between actual DMI and DMI estimated using the double η-alkane technique. However, the current study found that this technique was not accurate at estimating absolute DMI of red deer fed fresh forage, with an underestimation of pasture intake and overestimation of plantain intake.

This study is not the first to question the accuracy of the double η-alkane technique for estimation of DMI of fresh forages by ruminants. Krause *et al.* (2002 unpublished) found DMI of sheep fed white clover was underestimated by 17% and Waghorn *et al.* (2004) found that intakes were both under and overestimated by 10% and 14%, respectively, with dairy cows in two separate experiments fed pasture. Similarly, Dicker *et al.* (1996) found the DMI of steers fed Italian ryegrass (*Lolium multiforum* cv Concord) was underestimated by 20% using the alkane technique and assuming equal recovery rates of the alkanes in the faeces. Using the η-alkane technique, non-lactating adult hinds fed chicory were estimated to consume an average of 6.0 kg DM per day, compared with hinds fed pasture that were estimated to consume an average of 2.0 kg DM per day (Swainson, 2004). Previous reports have indicated high VFI of deer fed chicory in comparison to pasture (Kusmartono *et al.,* 1997). However, it was unlikely that hinds would consume the quantity of DM suggested by the alkane technique in the study by Swainson (2004), which is equivalent to three times the energy requirements of non-lactating hinds according to Fennessy *et al.* (1981). Yet validation experiments with sheep and cattle (Dove and Mayes, 1996; Dove *et al.,* 2002) and fallow deer (Ru *et al.*, 2002) using conserved forage, frozen and thawed pasture or concentrate rations, and validations with fresh forages (Vulich *et al.,* 1991, Piasentier *et al.,* 1995) have found the alkane technique to be accurate at estimating DMI. Interestingly, Valiente *et al.* (2003) found that for sheep fed differing proportions of straw and barley grain the double η-alkane technique overestimated DMI by 3.4-12.4%, with the largest differences observed for sheep fed the higher proportions of straw.

The double η-alkane method is reliant on two main assumptions according to Dove and Mayes (1996). The first assumption is that the herbage sub-sample taken to represent feed offered has a representative η-alkane profile of the forage consumed. In the current study, the alkane contents of feed offered and feed refused were similar. Therefore, the η-alkane profiles in the herbage samples taken were considered representative of the feed consumed by the animals. However under true grazing conditions where animals are able to selectively graze, samples estimating feed offered may not be representative of animal consumption.

The second assumption for grazing animals is that the faecal recovery of η-alkane is similar for both the dosed (even-chain) alkane and the natural (odd-chain) alkane used to calculate intake, even if faecal recovery rates are not complete. Dissimilarity of recovery rates can result in an estimated error in DMI of 1.25% for each percentage difference in recovery rate between alkanes (Dove & Mayes, 1996). In this study there was up to a 26% difference for pasture and plantain in the faecal recovery of C_{32} and C_{31} and up to 11% difference between C_{32} and C_{33}. It is therefore suggested that this is a major contributor to the inaccuracy of the technique in the current study, as errors in estimating DMI associated with differential faecal recoveries may have reached up to 32.5%.

Other reports with sheep and cattle fresh pasture, white clover or alfafa (Dicker *et al.,* 1996; Krause *et al.,* 2002 unpublished; Waghorn *et al.,* 2004) and cattle fed buffel-grass and lucerne hays (Hendricksen *et al.,* 2002) have shown differences of faecal recovery rates of up to 38% between alkanes used to calculated DMI. The dissimilarity between recovery rates, of both the dosed and naturally occurring η-alkanes, suggests that η-alkanes may not be inert in the digestive tract, and may undergo digestion, absorption and/or modification (Mayes *et al.*, 1988; Ohajuruka & Palmquist, 1991; Hendrickson *et al.,* 2002).

Once a day sampling of the faeces to simulate faecal rectal sampling, may have resulted in an increase in the variability of estimated intake, if there is a within-day variation in the concentration of the alkane excreted. The within-day variation in faecal concentration of alkanes has been shown to be small for sheep when dosed daily (Dove & Mayes, 1996) and close to zero when sheep were dosed with a rumen slow release capsule for all alkanes except C_{35} (Dove *et al.,*

2002). However, the diurnal variation of alkane excretion in deer has not been reported.

The secondary objective of the current study was to compare the rumen controlled release capsule release rate of deer fed pasture with those fed plantain. Use of this technique across different forage species may be limited because dose rates for pasture and plantain fed deer differed and the calculated dose rate for pasture was deviated from the manufacture's recommendation in the present study. Hence, for future use of the double η-alkane technique, coupled with rumen slow release capsules, extrapolation of the manufacture's dose rates across species and diets is not advised, and where possible rumen fistulated animals or serial faecal sampling should be used to determine dose rates under individual experimental conditions.

In conclusion, the use of the double η-alkane technique may not be appropriate for estimating DMI of ruminants grazing New Zealand fresh pasture or forages, especially when comparisons between forage species are required. However, the technique may be useful for detecting relative differences between treatments when animals are consuming a common forage where absolute DMI is not required.

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