The use of n-alkane markers to estimate the intake and apparent digestibility of ryegrass and Kikuyu by horses

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Abstract

The n-alkane marker (dosed marker, dotriacontane, C32, herbage markers C31, C33 and C35) technique was evaluated for use in feed intake and digestibility studies with horses. The mean retention time of digesta in the digestive tract was determined in horses following a single dose of C32. The n-alkane technique was then employed to estimate dry matter intake and digestibility in horses fed fresh perennial ryegrass (*Lolium perenne*), fresh Kikuyu grass (*Pennisetum clandestinum*, Hochst) and Kikuyu hay. The mean retention time of the C32 marker in the horses was estimated to be 27.9 h. On average the C32 had a slightly greater faecal recovery of 0.89 than the odd-chain alkanes (0.80-0.85), though the differences were not significant. The alkane technique, using the n-alkane markers, gave good estimates of dry matter intake, e.g. for fresh ryegrass the measured intake was 8.86±0.23 kg and the estimated intakes from the C31:C32 ratio, 7.9±1.9 kg and from the C32:C33 ratio, 8.3±1.4 kg. However, the effect of the higher recovery of the dosed marker needs further investigation. The estimates of apparent dry matter digestibility corresponded well with measured values, provided the factor for the incomplete faecal recovery of the internal alkanes was included in the calculation. It was concluded that the alkane technique is suitable to estimate feed intake under grazing conditions. However, the proportion of dietary alkanes recovered in the faeces has to be known to obtain an accurate estimate of apparent digestibility. This would be a problem under grazing conditions when faecal grab samples are taken for measuring faecal alkane concentrations.

Keywords: *Lolium perenne,* ryegrass, n-alkane markers, *Pennisetum clandestinum,* Kikuyu, intake, digestibility
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Introduction

The horse, a non-ruminant herbivore, is capable of extracting nutrients from herbage by both intestinal enzymic digestion and bacterial fermentation in its greatly enlarged caecum and colon. Natural and cultivated pastures, therefore, represent a substantial food resource for the horse industry. However, pastures appear to be under-utilised by the industry, largely due to a lack of information on the contribution of the pastures to the nutrient supply of horses. In order to utilise pastures efficiently, both the quality of the herbage and the quantity consumed by horses must be known. Many different markers have been used to estimate feed intake by horses under controlled feeding situations (Orton *et al*., 1985). However, information on the intake of horses on pastures is limited because of a lack of reliable methods for estimating intake in the grazing situation. Initial estimates of the pasture intake of horses were done by means of the chromium oxide marker technique (Gallagher & McMeniman, 1988; Martin, 1993). This technique has lost favour amongst animal nutritionists due to concern over the accuracy of the procedure (Dove & Mayes, 1991). The fact that concentrate feeds usually contain very low concentrations of n-alkanes (Dove & Mayes, 1991) which can be accounted for in the intake equation, suggests that these markers could be used to estimate forage intake when horses receive concentrates while grazing.

The natural-occurring odd-chain alkanes of plant cuticular wax are used as herbage markers, and even-chain alkanes as dosed markers (Dove & Mayes, 1996). This technique was developed initially for estimating food intake by ruminants. Only a few studies have been conducted on the potential use of the technique for estimating the feed intake in horses (Gudmundsson & Thorhallsdottir, 1998; Ordakowski *et al*., 2001). The aim of this study was to evaluate under controlled conditions the potential of n-alkanes as markers to

estimate the intake and apparent digestibility of forages by horses to serve as a guide for implementing the technique under grazing situations.

Materials and Methods

To measure the mean retention time of the external marker (dotriacontane, C32) in the digestive tract of horses, three thoroughbred horses (two colts and a filly) were given a single dose of the marker at the onset of the test. The horses were stabled at night but allowed to graze Kikuyu (*Pennisetum clandestinum,*Hochst) grass during the day. Each horse received an exact quantity (between four and six kg per d, depending on the horse) of a commercial concentrate meal in two separate meals (morning and afternoon) and had access to lucerne hay during these feeding periods. The marker was mixed into the concentrate fed in the morning. Faecal grab samples were collected then at four-hourly intervals for a period of 72 h.

Four mature geldings (average weight 490 kg) were housed individually in stables in which the floors were kept clean to facilitate accurate faecal collection. The horses were fed fresh perennial ryegrass (*Lolium perenne*), fresh Kikuyu grass and Kikuyu hay for three consecutive trials of 10 d each. The trial was conducted in the autumn and this sequence of feeding was followed instead of a change-over design to ensure that the quality of a growing grass changes as little as possible over a collection period. Between each trial the animals were returned to a Kikuyu pasture. The fresh grass was cut daily and stored in a cold room for the day. It was offered in excess of voluntary intake at 8:00, 12:00 and 17:00. Prior to the morning feeding each horse was dosed with grass pellets (see below) containing the dosed marker. Daily dosing commenced four days prior to a six-day faecal collection period. Orts, feed intake and total faecal output were recorded and representative samples of feed and faeces were collected, dried and stored at room temperature pending chemical analyses. In a further study, faecal grab samples were taken directly from the anus of each horse when the fresh grasses were fed. They were collected for four d at each mealtime, pooled and dried as described before. A salt/mineral lick was made available with all diets and the horses had free access to water.

The dosed marker (C32) was coated onto dried, milled (1 mm) Kikuyu at a concentration of 9 g/100 g of grass by means of the solvent evaporation procedure described by Marais *et al.* (1996). The coated grass was compressed into pellets of *ca.* 2 g, using a hand press, and contained 88±7.1 g C32 /kg DM. The pellets were weighed before the daily dosing and selected to supply 600 mg C32 per dose.

Feed and faecal samples were dried in a forced-draught oven at 70 ^{0}C, and milled to pass a 1 mm sieve. The concentrations of n-alkanes in the feed and faeces were obtained by gas chromatography, as described by Marais *et al.* (1996). The ash, crude protein, calcium and phosphorus concentrations of the feed were determined using standard AOAC (1990) procedures. Neutral detergent fibre and acid detergent fibre concentrations in the grass and hay were determined according to the procedure of Robertson & Van Soest (1981).

1. Mean retention time of the dosed marker was calculated using the following equation of Graham & Williams (1962):

Mean retention time (h^{-1}) = 1 / total marker $\sum$[1/2 n (t' + t)]

Where:

n = concentration of C32 collected between times t and t', and (t'- t) = hours.

$\sum$ = the sum of n collected between time intervals until n = 0.

2. Indigestibility (faecal recovery) of marker =

Faecal n-alkane (mg/kg) x faecal output (kg) / Ingested n-alkane (mg/kg) x feed intake (kg)

3. Estimated DM intake:

Measured intakes were compared with estimates of intake, based on the C31:C32 and C32:C33 n-alkane concentrations in the feed and faeces, using the following equation of Mayes *et al*. (1986):

Herbage intake (kg DM/day) = ($^{Fi}/_{Fj}$ x Dj) / (Hi - $^{Fi}/_{Fj}$ x Hj)

where:

Hi and Fi = the respective concentrations of natural odd-chain n-alkanes in the herbage and faeces (mg/kg

DM).
Hj and Fj = the respective concentrations of even-chain n-alkane per herbage intake and faeces (mg/kg DM).
Dj = the amount of n-alkane j dosed by pellet (mg/day).

4. Apparent DM digestibility was calculated using the following equations:
Measured DM digestibility = (DM in - DM out) / DM in
Estimated DM digestibility = 1- (Ha / Fa)
Where:
Ha = concentration of internal n-alkane in herbage
Fa = concentration of that alkane in faeces (corrected for incomplete recovery).

Statistical comparisons on the data were performed using an ANOVA on the computer program, Genstat (1992).

Results and discussion

The chemical composition and n-alkane concentration of the experimental grasses are presented in Table 1. The chemical composition of the fresh grasses corresponds well with values reported by Bredon *et al*. (1987) for these species of grass at similar growth stages. The n-alkane concentrations in the ryegrass compare well with values of the same species published by Dove & Mayes (1991).

Table 1 Nutrient and n-alkane composition (± s.d.) of the forages (dry matter basis)

	Ryegrass (fresh) g/kg	Kikuyu (fresh) g/kg	Kikuyu (hay) g/kg
Crude protein	117 ± 23.3	157 ± 6.4	92 ± 23.5
Ash	75 ± 3.0	84 ± 1.0	60 ± 19
Neutral detergent fibre	619 ± 8.8	640 ± 33.5	706 ± 41.9
Acid detergent fibre	365 ± 8.2	301 ± 8.4	387 ± 10.4
Calcium	8.8 ± 0.4	6.5 ± 0.6	5.4 ± 0.1
Phosphorus	2.3 ± 0.1	2.8 ± 0.3	1.8 ± 0.8
n-Alkanes	mg/kg	mg/kg	
C31	212 ± 30.4	121 ±2.2	-
C32	6 ±1.8	6 ±1.5	-
C33	116 ±10.7	272± 17.7	-
C35	12 ±1.6	212 ±16.6	-

The mean retention time of the marker in the digestive tract of the horses was 27.9±0.59 h. This relatively constant retention time was measured even though the proportion of concentrate to roughage intakes might have differed between horses. The retention time corresponds well with the retentions of between 18 and 38 h measured and quoted by Orton *et al*. (1985) from trials where a variety of roughages were fed and different markers, other than n-alkanes, were used. Pearson & Merritt (1991) measured mean retention times of between 30 and 39 h for hay fed to ponies and donkeys.

A prerequisite for accurate intake estimates using the n-alkane technique is that the faecal recoveries of the herbage and dosed n-alkane markers are equivalent (Dove & Mayes, 1991). This allows for the errors due to their incomplete recoveries to be cancelled out in the numerator and denominator of the intake equation. Results presented in Table 2 show that the dosed alkane (C32) had a slightly higher faecal recovery than the adjacent odd-chain alkanes. This may be due to the tendency of C32 to be associated more with the liquid phase than with the particulate phase of the digesta (Mayes *et al*., 1988). The mean recoveries for the alkanes, C31, C32 and C33, were slightly lower (84%) than recoveries of 89% reported by Gudmundsson & Thorhallsdottir (1998)

and O'Keefe & McMeniman (1998), while Ordakowski *et al*. (2001) reported recoveries for C31 and C33 of between 76 and 90%.

Table 2 Faecal recovery of n-alkanes for each grass diet (C32 data refer to natural+dosed)

Diets	n-alkanes							
	C31		C32		C33		C35	
	Mean	Sem[1]	Mean	sem	Mean	sem	Mean	sem
Ryegrass (fresh)	0.73[a]	0.06	0.86[b]	0.05	0.76[a]	0.03	0.76[a]	0.12
Kikuyu (fresh)	0.79[ab]	0.07	0.87[bc]	0.02	0.84[bc]	0.07	0.91[bc]	0.12
Kikuyu (hay)	0.88[b]	0.10	0.93[b]	0.05	0.86[b]	0.08	0.88[b]	0.08
Mean	0.80[b]	0.06	0.89[b]	0.04	0.82[b]	0.05	0.85[b]	0.05

[1]Standard error of the mean
[a,b,c] Values in columns and across rows bearing different superscripts differ significantly ($P < 0.05$)

Initial studies indicated that faecal recoveries of n-alkanes from the horse, although incomplete, did not differ with chain length (Dove & Mayes, 1996; O'Keefe & McMeniman, 1998). However, Gudmundsson & Thorhallsdottir (1998) showed that the faecal recovery of n-alkanes did change and tended to decrease as the carbon chain length increases. A similar observation was made by Ordakowski *et al*. (2001). Differences in faecal recoveries in the present trial (Table 2) are statistically non-significant. However, arithmetically, the recovery of C32 (based on 16 observation) is consistently greater than either the C31 and C33 recoveries. Dove & Mayes (1996) calculated that for every one percentage unit difference in recovery between an alkane pair an error of 1.25% incurs in the estimated intake. Therefore, although the mean estimated intakes of animals, based on the C31:C32 and C32:C33 alkane pairs compare well with the mean actual intake of forage (Table 3), except for the estimate obtained using C31:C32 as marker pair for Kikuyu hay ($P < 0.05$), further studies are required to substantiate the relative recoveries of these alkanes. Estimates based on the C31:C32 pair appear to be more erratic than for other pairs, as evident from the higher standard errors. The estimates of DM intake of the fresh grasses compare well with measured values where the alkane concentrations in the faecal grab samples are used to estimate DM intake (Table 4). Again, the variation of estimated values is high, which would affect the reliability of the n-alkane technique to estimate feed intake. Under grazing conditions daily feed intake would probably vary substantially more between days than what occurred under the more controlled feeding conditions in the present study. If faecal grab samples are collected under grazing conditions, longer collection periods than four d may be required to average out the effects of daily fluctuations in feed intake.

Table 3 Measured and estimated dry matter (DM) intakes of forages using n-alkane pairs (C31:C32 and C32:C33) in faecal samples obtained from total faecal collections over 6 d

Diet	DM intake (kg/d)					
	Measured		Estimated			
	Measured	sem[1]	C31:C32	sem	C32:C33	sem
Ryegrass (fresh)	8.63	0.97	8.43	4.42	7.82	2.91
Kikuyu (fresh)	8.27	1.77	8.68	4.86	8.27	1.65
Kikuyu (hay)	4.72[a]	0.82	6.49[a]	2.26	4.64[a]	1.51

[1]Standard error of mean
[a, b] Values within rows bearing different superscripts differ significantly ($P < 0.05$)

Table 4 Measured and estimated dry matter (DM) intakes of fresh forages using n-alkane pairs (C31:C32 and C32:C33) in faecal grab samples collected for 4 days

Diet	DM intake* (kg/d)					
	Measured		Estimated			
	Measured	sem[1]	C31:C32	Sem	C32:C33	sem
Ryegrass (fresh)	8.86	0.287	7.90	1.973	8.30	1.370
Kikuyu (fresh)	6.53	0.752	5.05	0.516	6.52	0.940

[1]Standard error of mean *Differences were not significant

The timing of dose administration (before, during or after a meal) especially in indoor trials where discrete meals are fed, may have a significant effect on the rate of passage of the dosed marker and the extent to which the marker disperses within the digesta. Frape (1986) reported that a meal normally remains in the stomach of a horse for only a short period of time and may reach the caecum within 45 min after feeding. The tendency of digesta to move in pockets through the digestive tract of horses necessitates that the number of dosings of a dosed marker per day be increased to reduce the variation in the ratio of faecal concentrations of dosed and herbage markers. Stefanon *et al*. (1999) recorded satisfactory results when dosing the marker three times per day to horses. Although estimated and true DM intakes compare well in the present study, the variations around the means were high. Under grazing conditions more frequent dosing of the marker may improve these estimates.

Table 5 Measured and estimated apparent dry matter (DM) digestibility (means ± s.e.m.[1]) of forages in horses using internal n-alkane markers (C31, C33, C35) without and with a correction for faecal recovery of the alkanes

Diet	Apparent DM digestibility						
	Measured	Estimated					
		C31		C33		C35	
	Means	A	B	A	B	A	B
Ryegrass (fresh)	0.535[b] ± 0.087	0.397[a] ± 0.053	0.544	0.454[b] ± 0.151	0.597	0.411[b] ± 0.127	0.597
Kikuyu (fresh)	0.586 ± 0.046	0.445 ± 0.092	0.571	0.490 ± 0.054	0.583	0.523 ± 0.088	0.538
Kikuyu (hay)	0.338[b] ± 0.084	0.273[b] ± 0.045	0.316	0.212[a] ± 0.027	0.247	0.298[b] ± 0.089	0.241

[1]Standard error of mean
A Calculated form formula: Estimated DM digestibility = 1- (Ha/Fa), with Ha and Fa defined as above.
B Recalculated as in A, with Fa corrected for marker recoveries given in Table 4.
[a, b] Values within rows bearing different superscripts differ significantly ($P < 0.05$)

The estimated apparent DM digestibilities of the forages, based on C31, C33 or C35 concentrations in the feed and corrected and uncorrected recoveries in the faeces are presented in Table 5. Estimates based on uncorrected recoveries are substantially lower than those based on total faecal excretion, though differences are not statistically significant, with the exception of the estimate of the digestibility of ryegrass when using C31 as marker, and of Kikuyu hay using C33 as marker ($P < 0.05$). Our observations suggest that, when total faecal output cannot be collected and the recovery of the marker cannot be calculated, internal n-alkane markers would not give reliable estimates of the digestibility of forages. This would be a problem with grazing animals such as horses.

Estimates based on corrected recoveries compare very well with the measured DM digestibilities. The apparent DM digestibilities of the fresh Kikuyu and ryegrass by the horses also correspond well with values of similar grasses reported for ruminants (Bredon *et al*., 1987). These authors reported a CP concentration of 115

g/kg DM for Kikuyu in autumn, with a digestible organic matter (DOM) content of 0.55, compared to the 157 g CP /kg DM (Table 1) and DM digestibility of 0.59 (Table 5) in the present study. For an average quality perennial ryegrass, Bredon *et al*. (1987) published a CP concentration of 95 g/kg DM and a DOM of 0.58, compared to the 117 g CP /kg DM (Table 1) and a DM digestibility of 0.54 (Table 5) in this study.

Conclusions

Although the n-alkane technique gave good estimates of DM intake, variation around the means was large. Faecal recoveries of n-alkanes did not differ statistically between alkanes, but the recovery of C32 was arithmetically greater than those of C31 and C33. This would introduce an error in the calculation of intakes and further studies are required to substantiate this difference in faecal recovery of the different alkanes. The alkane technique estimated apparent DM digestibility well, provided a correction is made for faecal recovery of the alkanes. However, under grazing conditions estimating the digestibility of the forage would be problem because faecal recovery values of the markers are usually unknown.

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