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Estimation of feed intake and apparent digestibility of equines and cattle grazing on heathland vegetation communities using the *n*-alkane markers

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Abstract

The application of *n*-alkanes as faecal markers to estimate feed intake and apparent digestibility (DMD_{ap}) of equines and cattle was studied. Additionally, the effect of using different data on diet composition, known proportions of the diet components (DC1) and those estimated using the alkane markers (DC2), on the accuracy of intake and DMD_{ap} estimates was evaluated. Six mature horses, divided in two groups of three animals (H1 and H2), and three adult non-lactating cows of Asturiana de los Valles breed (*C*) were housed in individual stalls. H1 and *C* groups were fed on a diet composed of *Lolium perenne* L. (70%) and heather (30%) and H2 received *L. perenne* (40%), heather (30%) and *Ulex gallii* Planchon (30%). The dietary component heather represented the field proportions of different plant species of heathland, namely *Erica umbellata* L., *Erica cinerea* L. and *Calluna vulgaris* L., at this experimental period. All animals received a daily dose of paper pellets containing C_{24}, C_{32} and C_{36} *n*-alkanes as external markers with the purpose of using different *n*-alkane pairs of adjacent chain length for feed intake estimations. The results indicated that a period of 3 and 5 days was sufficient for these external markers to reach a steady concentration in faeces of cattle and equines, respectively. In contrast to the results obtained in cattle, the alkane faecal recovery in equines was unrelated to the carbon chain length. Diet composition only affected the faecal recovery of the alkanes C_{24} ($P<0.05$), C_{31} ($P<0.05$), C_{32} ($P<0.05$) and C_{36} ($P<0.01$) in the faeces of the equines, suggesting a different dispersion of the synthetic *n*-alkanes in the digesta. In equines, DMD_{ap} estimates were not affected by the *n*-alkane (C_{27}, C_{29}, C_{31} and C_{33}) used in the calculations, contrasting with the significant ($P<0.001$) effect observed in cattle. In both animal species, the data on diet composition (DC1 or DC2) used in the calculations did not affect DMD_{ap} estimates. Feed intake estimates were affected by the alkane pair used in the calculations in H1 ($P<0.05$), H2 ($P<0.001$) and *C* ($P<0.001$). The data on diet composition used in the intake calculations affected the resultant estimates in H1 ($P<0.05$) but not in H2 and *C*. The differences from the known intake values were lower when using C_{31}:C_{32} alkane pair, overestimating intake in only an average of 4.5, 13.0 and 1.3% in H1, H2 and *C*, respectively, using DC1 or DC2. The results obtained in this study confirm the accuracy of the *n*-alkane markers to estimate simultaneously feed intake, apparent digestibility and diet composition of equines and cattle grazing these type vegetation communities.

Keywords: Equines; Cattle; *n*-alkanes; Feed intake; Apparent digestibility; Diet composition

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1. Introduction

In many mountain and marginal areas around the world equines graze together with sheep, cattle and goats and also with the wild herbivores in a multi-species system, with different levels of competition between them. It seems that these levels of competition are high in the case of cattle and equines even under different vegetation conditions. In savannah conditions, Lechner-Doll et al. (1995) recorded grazing times in different vegetation components and observed that the overlapping level between equines and cattle grazing areas was around 70%. Aldezabal (2001) also estimated around 70% of similarity between equines and cattle diet components, in the Pyrenees. Nevertheless, Osoro et al. (2005) in heathland communities, in which 20% of the area is covered by improved pasture (perennial ryegrass-white clover), observed that horses' grazing time was longer than cows' although their percentages of grazing time on different vegetation covers were similar, grazing around 90% of the time on the improved pasture.

The strategies adopted by these animal species to survive in an environment in which feed resources vary in quality and quantity are quite different. Large grazing ruminants, as cattle, optimize the utilization of fibrous feeds through longer retention times of feed particles, leading to a more efficient utilization of slow digestive cell wall components (CWC), at the expense of food intake (Van Soest, 1996). Although the utilization of CWC in equines is less efficient (Lechner-Doll et al., 1995) they may compensate a lower digestibility by higher intakes and throughput rates. In contrast to ruminating animals, the clearance of undigested feed particles from the digestive tract slightly limits intake in equidae (Lechner-Doll et al., 1995). Thus, these animals can ingest much higher amounts of feed rich in CWC than ruminants (Cymbaluk, 1990; Duncan et al., 1990), being feed intake limited only by the time available for grazing or by the maximum intake rate (Lechner-Doll et al., 1995). Menard et al. (2002) also suggests resource partitioning by the use of different plant species as another coexistence mechanism.

The alkane markers were initially proposed to estimate feed intake and, additionally, have been used to estimate diet composition. Most of the research has been carried out in ruminants (Mayes et al., 1986; Hendricksen et al., 2002, 2003; Brosh et al., 2003; Elwert et al., 2004). However, very few studies have been conducted in equines (Ordakowski et al., 2001; Stevens et al., 2002; Peiretti et al., 2006) and none of them with diets composed of browse and herbaceous feeds.

The alkane technique should be validated to estimate diet intake and also apparent digestibility (DMD_{ap}) in equines and cattle grazing on grass and shrub components, enhancing the knowledge of the level of competition between herbivores for the sustainable management of the large mountain and marginal areas where horses are grazing together with cattle, sheep and goats.

The objective of this work was to evaluate the accuracy of alkane technique to estimate feed intake and apparent dry matter digestibility of equines and cattle grazing on upland vegetation communities. Additionally, the usefulness of the *n*-alkane markers to estimate simultaneously diet composition, feed intake and apparent digestibility was evaluated.

2. Materials and methods

This study was conducted at Carbayal Research Station, situated at 900–1000 m above sea level, at San Isidro's Mountain, Asturias, Spain (longitude −6° 53′, latitude 43° 21′), where the vegetation is dominated by a mosaic of gorse-heathland interspersed with areas of improved pastures (*Lolium perenne* L.). The natural vegetation consists mainly of short heaths, dominated by heather species (*Erica umbellata* L., *Erica cinerea* L. and *Calluna vulgaris* L.) and gorse (*Ulex gallii* Planchon) a thorny and woody legume. There are also sparse tall heathlands (*Erica australis* L. and *Erica arborea* L.)

2.1. Animals and diets

Six mature crossbreed mares (399±61 kg live weight), divided in two groups of three animals (H1 and H2), and three adult non-lactating cows (499±36 kg live weight) of Asturiana de los Valles breed (*C*) were housed individually in metabolic crates. H1 and *C* were fed on a diet composed of ryegrass (70%) and heather (30%) and H2 received a mixture of ryegrass (40%), gorse (30%) and heather (30%). During the study animals were offered a daily total amount of 1.0 kg dry matter (DM)/100 kg live weight (LW) and were fed twice daily.

Freshly cut vegetation components (ryegrass and green shoots of heather and gorse) were harvested daily from random sites within the experimental field and were offered in individual mangers along the day in an attempt to prevent loss of diet components and to measure individual intakes. The botanical composition of the vegetation component called "heather" represents the field proportions of different plant species of heathland, namely *E. umbellata* L., *E. cinerea* L. and *C. vulgaris* L. *Erica* spp. and *C. vulgaris* were considered as a single dietary group due to their similar alkane profile (Celaya et al., in press).

The experiment (11 days) comprised a 7-day period for adaptation of the animals to the diets and experimental conditions followed by 4 days of collection of samples of faeces used for subsequent feed intake and apparent digestibility estimations. During the study, daily total faecal output and feed intake were recorded individually.

Samples of each of the diet components were collected daily for chemical and alkane analysis (from day 1 to 11). For the determination of total faecal output, faeces were removed from the floor, retained in individual containers, weighted and homogenised and sub-sampled for alkane analysis.

During the experiment, animals were dosed orally, using a specific designed applicator, once-daily (08:30 h) a paper pellet containing 1033.7±2.79 mg of the *n*-alkane C_{24} (*n*-tetracosane), 1011.8±2.73 mg of the *n*-alkane C_{32} (*n*-dotriacontane) and 914.3±2.47 mg of the *n*-alkane C_{36} (*n*-hexatriacontane). These synthetic *n*-alkanes (>99% pure, Sigma-Aldrich Corp., St. Louis, MO, USA) were absorbed into shredded paper (Whatman No 1 filter paper) following the procedure of Mayes et al. (1986).

To assess the pattern of the *n*-alkanes excretion and the time needed for faecal concentrations of the synthetic alkanes to reach constancy, one sample of faeces was collected daily throughout the experiment (day 2 to 11).

2.2. Chemical analysis

Samples of the diet components and faeces were immediately dried on the day of collection, using a forced-air oven at 60 °C for 72 h, for dry matter determination. The samples for alkane analyses were immediately frozen at −20 °C and then freeze-dried and milled through a 1 mm screen.

Ground samples were analysed for ash (ID 942.05) and nitrogen (ID 954.01) according to the methods proposed by AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed by the method of Van Soest et al. (1991) and Robertson and Van Soest (1981). Hemicellulose and cellulose were calculated as the difference between NDF and ADF and ADF and ADL, respectively.

2.3. Alkane analysis

Alkane content of individual samples of diet components and faeces were analysed in duplicate according to the methods of Mayes et al. (1986) modified by Oliván and Osoro (1999).

The *n*-alkane content of the dosed pellets was determined on 5 pellets randomly selected during the trial. Five replicates of 1 g were extracted in a Soxhlet extractor using 100 mL of petroleum spirit (B.P. 60–80 °C, Merck, Germany) after weighing 100 mg of C_{22} and C_{34} (>99% pure, Sigma-Aldrich Corp., St. Louis, MO, USA) as internal standards. A 20 mL aliquote of the extract was evaporated to dryness, redissolved in 500 μL heptane and submitted to the same analytical procedures as the dietary and faecal extracts.

Gas chromatographic method was calibrated with a standard solution containing a mixture of synthetic alkanes (from C_{23} to C_{36}) with concentrations similar to those found in extracts. The response factors for individual alkanes were calculated from peak areas and the known concentrations. The detector response to different concentrations of each solute was linear throughout the range of concentrations found in the injected samples. Alkane concentrations were quantified relative to known amounts of the internal standards C_{22} (*n*-docosane) and C_{34} (*n*-tetratriacontane), added at the beginning of the extraction process. The use of two internal standards enabled the evaluation of the effectiveness of the extraction process and the correction of the peak areas for any discrimination detected during the solvent-extraction step (Oliván and Osoro, 1999).

2.4. Calculations

The faecal recovery of each *n*-alkane (AFR) was calculated for each animal as the proportion of alkane consumed in the diet (natural) and paper pellet (dosed) which was recovered in the faeces (Eq. (1)):

$$\mathrm{AFR}_i = \frac{F^*F_i}{R^*R_i + H^*H_i + G^*G_i + C_i} \quad (1)$$

where AFR_i is the faecal recovery rate of *i* alkane, *F* is the faecal DM output, F_i the faecal concentration of the *i* alkane. *R*, *H* and *G* are the mean amounts of the diet components *R* (ryegrass), *H* (heather) and *G* (gorse) consumed over the entire period and R_i, H_i and G_i the *i* alkane concentrations in *R*, *H* and *G*, respectively. C_i is the amount (mg) of the synthetic *n*-alkanes (C_{24}, C_{32} and C_{36}) in the paper pellets.

Dry matter intake (DMI) was estimated from the faecal ratio of naturally occurring and dosed *n*-alkanes (C_{23}:C_{24}, C_{25}:C_{24}, C_{31}:C_{32}, C_{33}:C_{32}, C_{35}:C_{36}) in the diet and faeces samples, according to the equation proposed by Mayes et al. (1986):

$$\mathrm{DMI(kgDM/day)} = \frac{C_j}{\frac{F_j}{F_i}^*D_i - D_j} \quad (2)$$

where C_j is the amount (mg) of the synthetic *n*-alkanes (C_{24}, C_{32} and C_{36}) in the paper pellets, F_i and D_i are the

respective concentrations (mg/kg DM) of the natural alkanes (C_{23}, C_{25}, C_{31}, C_{33} and C_{35}) in faeces and in the consumed diet and F_j and D_j are the respective concentrations (mg/kg DM) of C_{24}, C_{32} and C_{36} in faeces and consumed diet.

The concentration of alkanes (C_{23}, C_{24}, C_{25}, C_{31}, C_{32}, C_{33}, C_{35} and C_{36}) in the consumed diet ($D_{i\ or\ j}$) were calculated for each animal (Eq. (3)), based on 1) the known proportions of the vegetation components (DC1) and 2) the estimated proportions using the alkanes C_{23}–C_{36} (except the dosed *n*-alkanes C_{24}, C_{32} and C_{36}) corrected with the mean treatment faecal recoveries (DC2), as follows:

$$D_{i\ or\ j} = r^*R_{i\ or\ j} + h^*H_{i\ or\ j} + g^*G_{i\ orj} \quad (3)$$

where r, h and g are the known proportions of components R (ryegrass), H (heather) and G (gorse) in the diet and $R_{i\ or\ j}$, $H_{i\ or\ j}$ and $G_{i\ or\ j}$ are the concentrations of alkane i or j in the vegetation components R, H and G.

The individual diet composition estimates were obtained using an optimization procedure which minimises the sum of squared discrepancies between the actual (A) alkane proportions in faeces (adjusted for the incomplete faecal recoveries, using the mean recovery rate of the dietary treatment that the animal belonged to) and the estimated (E) proportions (different combinations of diet components), as follows (Oliván et al., 1999; Salt et al., 1994):

$$\sum_{i=1}^{n} [A-E]^2 = \sum_{i=1}^{n} \left[\frac{\mathrm{Fi}}{\mathrm{Ft}} - \frac{r^*\mathrm{Ri} + h^*\mathrm{Hi} + g^*\mathrm{Gi}}{r^*\mathrm{Rt} + h^*\mathrm{Ht} + .g^*\mathrm{Gt}}\right]^2 \text{minimal} \quad (4)$$

where r, h and g are the proportions of components R, H and G in the diet; Fi, Ri, Hi and Gi are the concentrations of alkane i in faeces (corrected for incomplete faecal recovery) and components R, H and G; Ft, Rt, Ht to Gt are total alkane concentrations in faeces and components R, H and G. In the diet composition calculations it was assumed that all animals had potential access to all vegetation components (*L. perenne*, heather and *U. gallii*). The comparison of the known proportions of the vegetation components and those estimated by the alkane markers was performed using the parameter *distance* (D) suggested by Elwert and Rodehutscord (2005a,b). D was calculated as the square root of the sum of the squared differences of estimated from known proportions of each vegetation component in the diet.

Apparent *in vivo* dry matter digestibility (DMD_{ap}) was calculated from total faecal collection using the equation $DMD_{ap} = (I-F)/I$, where I is the total intake and F the total faecal output. DMD_{ap} was also estimated using the *n*-alkanes C_{27}, C_{29}, C_{31} and C_{33} as internal markers using the equation $DMD_{ap} = (1 - C_i/C_f)$, where C_i and C_f are the respective concentrations (g/kg DM) of the *n*-alkanes C_{27}, C_{29}, C_{31} and C_{33} in the diet and faeces. C_i concentrations were calculated for each animal using Eq. (3) and with different data on diet composition (DC1 and DC2).

2.5. Statistical analysis

Statistical analyses were carried out using the JMP program (2003). The *n*-alkane excretion was studied by comparing the concentration of external markers (C_{24}, C_{32} and C_{36}) in the total faeces samples obtained each day with the mean for subsequent days, using analysis of variance. The effect of diet composition (*L. perenne* + heather or *L. perenne* + heather + *U. gallii*) on the recovery of each alkane in the faeces of equines was examined by one-way analysis of variance (ANOVA).

The effect of using different alkanes (C_{27}, C_{29}, C_{31} and C_{33}), different data on diet composition (DC1 and DC2) and their interaction on the estimates of the apparent *in vivo* dry matter digestibility (DMD_{ap}) of each diet were analysed by two-way analysis of variance (ANOVA). Within each group (H1, H2 and *C*), all the estimates of feed intake were compared using the Tukey test.

The effect of using different alkane pairs (C_{23}:C_{24}, C_{25}:C_{24}, C_{31}:C_{32}, C_{33}:C_{32}, C_{35}:C_{36}), different data on diet composition (DC1 and DC2) and their interaction on the estimates of feed intake of each diet were analysed by two-way analysis of variance (ANOVA). Within each group (H1, H2 and *C*), all the estimates of feed intake were compared using the Tukey test.

The relationship between the known/estimated intake and the faecal recovery of the natural/dosed alkane was assessed by linear regression.

3. Results and discussion

The chemical composition and the n-alkane profile of the feeds used in this trial are presented in Table 1. Cell walls (NDF) represented the highest fraction in the two feeds, 532, 681 and 628 g/kg DM for *L. perenne*, *U. gallii* and heather, respectively. *U. gallii* and heather showed high levels of ADL (217 and 341 g/kg DM, respectively). The woody vegetation components (*U. gallii* and heather) presented quite different ($P<0.001$) levels of CP (93 and

Table 1
Chemical composition (g/kg DM) and *n*-alkane concentrations (mg/kg DM) of diet components consumed by the animals in the experiment

	L. perenne	*U. gallii*	Heather
Organic matter	934±4.1	975±1.5	976±8.3
Crude protein	163±11.2	93±6.7	53±4.6
NDF	532±24.8	681±23.5	628±37.1
ADF	269±15.5	520±19.9	519±23.9
ADL	53±8.3	217±6.4	341±64.3
Cellulose	205±26.3	303±20.9	178±57.9
Hemicellulose	263±17.5	161±11.5	121±7.5
C_{23}	3.2±0.55	0.9±0.25	5.5±0.83
C_{24}	2.2±0.33	1.7±0.35	3.6±0.68
C_{25}	11.3±1.05	3.3±0.62	9.9±1.37
C_{26}	2.8±0.54	2.3±0.31	6.4±0.73
C_{27}	19.3±1.51	20.8±2.47	32.9±6.55
C_{28}	6.5±0.83	11.0±1.41	17.0±6.25
C_{29}	78.2±6.35	55.4±5.54	108.9±19.70
C_{30}	10.1±1.46	8.0±0.83	27.2±4.38
C_{31}	161.4±10.69	106.1±10.29	654.2±123.65
C_{32}	7.5±1.16	3.0±0.33	54.8±5.54
C_{33}	93.0±9.68	4.4±1.34	518.7±84.71
C_{35}	10.8±1.58	0.4±0.24	13.8±5.87
C_{36}	1.4±0.14	1.2±0.18	1.5±0.23
Total	411.6±24.52	218.4±20.52	1454.2±162.51
Total odd-chain	380.8±22.39	191.3±18.19	1343.9±152.74

53 g CP/kg DM, respectively). These values reveal the advanced growth stage of the vegetation components with higher values for the fibrous fractions, when compared to the data (533 and 583 g NDF/kg DM for heather and gorse, respectively) presented by Ferreira et al. (2005, in press) studying the same plant species in earlier (May–June) stages of growth.

The alkane profile of the plant species used in the experimental study (Table 1) is similar to that reported elsewhere (Dove and Mayes, 2005; Ferreira et al., 2005, in press), predominating the odd-chain ones, representing over 87% of the total alkane content). The hydrocarbons with chain lengths adjacent (C_{23}, C_{25}, C_{31}, C_{33} and C_{35}) to the dosed synthetic *n*-alkanes (C_{24}, C_{32} and C_{36}) had quite different concentrations and varied with the vegetation components. The natural alkanes frequently used in the estimation of feed intake (C_{31} and C_{33}) presented high concentrations in the three feeds (>93 mg/kg DM), except for C_{33} in *U. gallii* (4.4 mg/kg DM). The other natural alkanes (C_{23}, C_{25} and C_{35}) were present in very low concentrations in *L. perenne* (3.2, 11.3 and 10.8 mg/kg DM, respectively), *U. gallii* (0.9, 3.3 and 0.4 mg/kg DM, respectively) and heather (5.5, 9.9 and 13.8 mg/kg DM, respectively). The very low concentration of these alkanes could limit their use for *n*-alkane recovery and/or calculations of feed intake and DMD_{ap} as the result of higher measurement errors associated with their analytical determination (Brosh et al., 2003; Dove and Mayes, 2005). As stated by Hameleers and Mayes (1998), a small error on the estimation of their concentrations could have a large effect on the estimate of feed intake.

As with other type of markers, the collection of representative samples of faeces can limit the use of alkanes as faecal markers, due to the variations in their faecal concentrations, within and between days. In this study, a possible diurnal variation in the markers faecal concentration would not have a significant effect since all of the daily faecal output was collected, mixed and sub-sampled (Dove and Mayes, 2003).

In order to detect the minimum dosing time required to reach a steady excretion pattern, the evolution of the faecal excretion (Fig. 1) of dosed *n*-alkanes (C_{24}, C_{32} and C_{36}) was studied along the dosing period. As calculated by Molina et al. (2004) this required time was attained when the concentrations of external markers in the faeces in one day were not significantly different

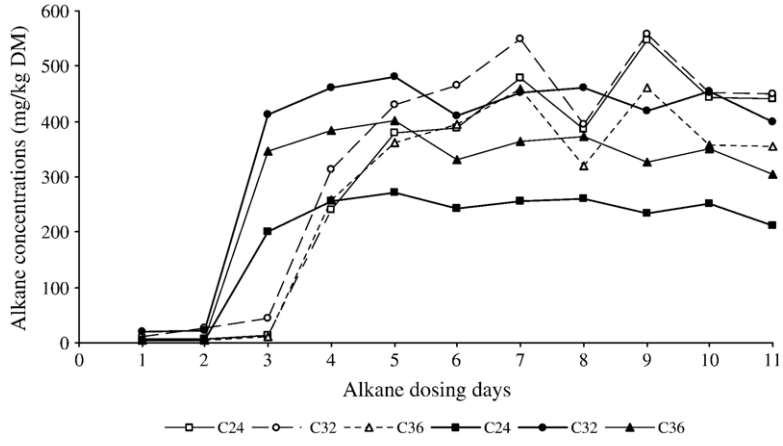

Fig. 1. Evolution of synthetic *n*-alkanes (C_{24}, C_{32} and C_{36}) along the dosing period, in samples of total faeces of equines (□, ○ and △) and cattle (■, ● and ▲).

from the mean concentrations in the following days. In the equine groups, the synthetic even-chain alkanes C_{32} and C_{36} reached a steady state on day 4 as faecal alkane concentrations on day 4 vs. days 5 to 11 did not differ. The synthetic alkane with lower carbon chain length (C_{24}) needed 5 days to reach equilibrium in the same animals. A shorter period (3 days) was necessary for the same markers to reach a steady concentration in the faeces of cattle, as faecal alkane concentrations on day 3 vs. days 4 to 11 did not differ.

Our results confirm the length of the adaptation periods (5 to 7 days) observed by Mayes et al. (1986) and Dove et al. (2002) in sheep fed on perennial ryegrass and a mixed diet of ryegrass and barley-based concentrate, respectively, when using paper pellets (Mayes et al., 1986; Dove et al., 2002) or controlled release devices (CRD) (Dove et al., 2002). Similar results were also observed by in cattle fed on *Panicum maximum* grass (Molina et al., 2004), meadow hay (Ferreira et al., 2004) and lucerne hay (Oliván et al., in press), when using gelatine capsules, CRD or paper pellets, respectively. Therefore, a length of the adaptation period of 5 days will be enough to study intake in equines and cattle grazing heathland communities.

The *n*-alkane faecal recoveries obtained in each experimental group are presented in Table 2. Some alkanes presented recoveries higher than 100%, especially in equines but also in cattle for the alkanes C_{33} (1.14) and C_{35} (1.18). These values are biologically unexplainable, since to date no study provided any evidence of alkane synthesis in the digestive tract of both ruminant and non-ruminant species. These "erroneous" results could be due to the very low concentrations of the alkanes in the feeds and faeces, resulting from, as stated before, a higher measurement error associated with their analytical determination (Hameleers and Mayes, 1998; Brosh et al., 2003; Dove and Mayes, 2005).

Table 2
n-alkane faecal recoveries (mean±standard deviation) observed in equines (H1 and H2) and cattle (*C*)

Alkanes	H1	H2	*P*	SEM	*C*
C_{23}	1.18±0.02	1.24±0.03	0.056	0.015	0.69±0.04
C_{24}	1.03±0.06	0.87±0.01	0.012	0.026	0.56±0.02
C_{25}	1.11±0.07	1.08±0.02	0.459	0.028	0.81±0.02
C_{26}	1.06±0.06	1.10±0.04	0.400	0.030	0.97±0.04
C_{27}	1.04±0.06	0.99±0.04	0.289	0.030	0.89±0.01
C_{28}	1.02±0.05	1.03±0.05	0.919	0.030	0.89±0.06
C_{29}	0.99±0.04	0.95±0.02	0.210	0.016	0.89±0.02
C_{30}	1.03±0.03	0.99±0.03	0.215	0.018	0.98±0.03
C_{31}	1.02±0.02	0.95±0.03	0.024	0.014	0.96±0.03
C_{32}	0.99±0.07	0.85±0.04	0.044	0.033	0.96±0.03
C_{33}	1.08±0.05	0.99±0.05	0.092	0.028	1.14±0.03
C_{35}	1.07±0.08	1.00±0.04	0.229	0.035	1.18±0.03
C_{36}	0.99±0.03	0.81±0.03	0.001	0.015	0.91±0.04

SEM standard error of mean.

In the horses' groups the alkane faecal recovery seemed to be independent of the carbon chain length. The lack of the effect of carbon chain length on the recovery of *n*-alkane markers was also reported by Gudmundsson and Thohallsdottir (1998), Ordakowski et al. (2001), Stevens et al. (2002) and Peiretti et al. (2006). Similar observations were made in previous studies carried out with other non-ruminants species such as sows (Wilson et al., 1999; Sehested et al., 1999), mountain hares (Hulbert et al., 2001) and pigeons (Hatt et al., 2001). These results contrast with the positive relationship between the length of the carbon chain of alkanes with their faecal recovery observed in the cattle group (Table 2), increasing from a minimum of 0.56 (C_{24}) to a maximum of 1.18 (C_{35}).

Similar findings were reported in previous studies performed in cattle (Brosh et al., 2003; Oliván et al., in press) and also in other ruminant species as sheep (Elwert et al., 2004; Ferreira et al., in press) and goats (Ferreira et al., 2005), and indicate that the *n*-alkane markers behave differently in the gastro-intestinal tract of ruminant and non-ruminant species, suggesting that the disappearance of the shorter alkanes in the gut of ruminants occurs in a greater extent than in non-ruminants.

The comparison of the alkane recovery data obtained in the two equine groups does not suggest an effect of diet composition. Only the recovery of the dosed alkanes C_{24}, ($P=0.012$), C_{32} ($P=0.044$) and C_{36} ($P=0.0014$) and of the natural *n*-alkane C_{31} ($P=0.024$) differed between diets. The different faecal recoveries between diets of the synthetic *n*-alkanes (C_{24}, C_{32} and C_{36}) could be the result of an unequal dispersion of these alkanes in the different phases of the digesta. Mayes et al. (1988) suggested that between 30–40% of the synthetic alkanes remained associated with the liquid phase. In our opinion, (1) the level of this association may vary between diets and (2) the absorption of the alkanes may differ according to the phase they are associated to. Dove and Mayes (1991) stated that generally the synthetic alkanes present higher faecal recovery than the adjacent ones, suggesting that the alkanes associated with the liquid phase of the digesta are less prone to absorption. The diet composition effect on the recovery of alkanes in faeces is still unclear, as it is likely to be situation dependent and may be confounded with other diet characteristics (digestibility for example, Ferreira et al., 2005). Also Elwert and Dove (2005) related the effect of diet composition on the alkane faecal recoveries to the

Table 3
Apparent dry matter digestibility (mean±standard deviation) calculated using *n*-alkanes C_{27}, C_{29}, C_{31} and C_{33} and different data on diet composition (DC)

DC	*n*-alkane (A)	H1	H2	*C*
DC1	C_{27}	0.442± 0.013a	0.394±0.003a	**0.438± 0.019d**
	C_{29}	0.407± 0.029a	0.367± 0.012a	**0.436± 0.013d**
	C_{31}	0.427± 0.036a	0.368± 0.013a	0.480± 0.011bc
	C_{33}	0.448± 0.030a	0.384± 0.008a	**0.553± 0.008a**
DC2	C_{27}	0.438± 0.009a	0.393± 0.006a	**0.438± 0.020d**
	C_{29}	0.419± 0.037a	0.371± 0.030a	**0.442± 0.016cd**
	C_{31}	0.435± 0.042a	0.371± 0.030a	0.486± 0.014b
	C_{33}	0.465± 0.031a	0.391± 0.027a	**0.562± 0.012a**
Effects (*P*)	A	0.1331	0.0956	0.0001
	DC	0.5212	0.7299	0.4016
	A * DC	0.9437	0.9883	0.9531

DC1 using known proportions of the vegetation components.
DC2 using estimated proportions using the alkanes corrected with the mean treatment faecal recoveries.
Values in the same column with different letters are significantly different ($P<0.05$).
Within each group, values marked in bold are significantly ($P<0.05$) different from *in vivo* measurements of DMD_{ap}.

characteristics of the cuticular wax layer and bond between artificial labelling (beeswax) and the labelled material.

Table 3 shows the mean values of DMD_{ap} using the *n*-alkanes C_{27}, C_{29}, C_{31} and C_{33} as internal markers and with different data on diet composition (DC1 or DC2). DMD_{ap} calculated using total faecal collection was 0.420±0.048, 0.394±0.022 and 0.503±0.023 for H1, H2 and *C*, respectively, differing significantly ($P<0.05$) from each other. As stated before (see Material and methods section), the concentrations of the alkanes used in the estimation of DMD_{ap} and feed intake (Eq. (2)) were calculated for each animal (Eq. (3)), based on 1) the known proportions of the vegetation components (DC1) and 2) the estimated proportions using the alkanes C_{23}–C_{36} (except the dosed *n*-alkanes C_{24}, C_{32} and C_{36}) corrected with the mean treatment faecal recoveries (DC2). Moreover, in the calculations of diet composition it was assumed that all feeds (*L. perenne*, *U. gallii* and heather) were available for consumption to all groups. The results (Table 4) reveal that the alkane markers were able to estimate accurately the proportions of plant species in the diet of *C* group with a mean *D* of 0.048. Assuming the threshold value of 0.07 for *D*, suggested by Elwert and Rodehutscord (2005a,b) to distinguish similar and dissimilar diet compositions, it may be considered that diet composition was estimated with lower accuracy in the equine groups (*D* of 0.118 and 0.100 for H1 and H2, respectively). This could be due to an inaccurate correction of the alkane faecal concentrations, due to the high variability between animals within the equine groups. In fact, Ferreira et al. (in press) stated that the accuracy of diet composition estimates is a reflection of the representativeness of the recovery rates used for alkane faecal corrections.

In the equine groups, the *n*-alkanes (C_{27}, C_{29}, C_{31} and C_{33}) used in the digestibility calculations as internal markers provided accurate estimates of DMD_{ap}, not differing ($P>0.05$) from the *in vivo* measurements of DMD_{ap}, and did not differ significantly ($P>0.05$) between markers. A low range of variation was observed in DMD_{ap} estimates, varying from a minimum of 0.407 (C_{29} using DC1) and a maximum of 0.465 (C_{33} using DC2) in H1, and a minimum of 0.367 (C_{29} using DC1) and a maximum of 0.394 (C_{27} using DC1) in H2. In contrast, in cattle the alkane used in the calculations affected significantly ($P<0.001$) the DMD_{ap} estimates. The alkanes C_{27}, C_{29} and C_{31} underestimated DMD_{ap} when using DC1 (0.438, 0.436 and 0.480, respectively) or DC2 (0.438, 0.442 and 0.486, respectively). On the contrary, the C_{33} marker overestimated DMD_{ap} independently of the data on diet composition used (0.553 and 0.562 using DC1 and DC2, respectively). The comparison of DMD_{ap} estimates obtained by the alkane markers to that measured by total faecal collection show

Table 4
Comparison of measured proportions of dietary components and those estimated using the *n*-alkane markers

	H1			H2			*C*		
	Ryegrass	Heather	Gorse	Ryegrass	Heather	Gorse	Ryegrass	Heather	Gorse
Measured	0.700	0.300	0	0.400	0.300	0.300	0.700	0.300	0
Estimated	0.618	0.301	0.080	0.378	0.300	0.322	0.670	0.296	0.034
D	0.118			0.100			0.048		

D distance.

Table 5
Feed intake estimates (kg DM/100 kg LW) and differences (%) between estimated and measured intake, obtained using different data on diet composition (DC) and different *n*-alkane pairs (AP)

		Intake (mean ± SD)			Diff.[a]		
DC	AP	H1	H2	C	H1	H2	C
DC1	C_{23}:C_{24}	1.15 ± 0.050a	1.43 ± 0.027a	1.22 ± 0.072b	+14.8	+43.1	+21.6
	C_{25}:C_{24}	1.08 ± 0.055a	1.25 ± 0.010ab	1.44 ± 0.038a	+8.1	+24.8	+43.3
	C_{31}:C_{32}	1.04 ± 0.067a	1.13 ± 0.024b	1.01 ± 0.038c	+4.1	+12.9	+0.7
	C_{33}:C_{32}	1.11 ± 0.117a	1.18 ± 0.058b	1.22 ± 0.039b	+11.7	+17.9	+21.7
	C_{35}:C_{36}	1.09 ± 0.060a	1.23 ± 0.077ab	1.31 ± 0.028ab	+8.7	+23.5	+30.8
DC2	C_{23}:C_{24}	1.21 ± 0.059a	1.46 ± 0.143a	1.25 ± 0.097b	+21.2	+46.3	+24.7
	C_{25}:C_{24}	1.15 ± 0.016a	1.29 ± 0.147ab	1.47 ± 0.069a	+15.2	+28.5	+47.1
	C_{31}:C_{32}	1.05 ± 0.035a	1.13 ± 0.036b	1.02 ± 0.043c	+5.5	+13.2	+1.8
	C_{33}:C_{32}	1.14 ± 0.060a	1.19 ± 0.036b	1.25 ± 0.053b	+14.3	+18.9	+24.3
	C_{35}:C_{36}	1.17 ± 0.018a	1.28 ± 0.092ab	1.35 ± 0.062	+16.8	+27.4	+35.1
Effects	AP	0.0198	0.0001	0.0001			
	DC	0.0284	0.3856	0.1672			
	AP * DC	0.8328	0.9919	0.9910			

DC1 using known proportions of the vegetation components.
DC2 using estimated proportions using the alkanes corrected with the mean treatment faecal recoveries.
Values in the same column with different letters are significantly different ($P<0.05$).
[a] Diff. = (Estimated / Measured − 1) * 100.

that only C_{31} marker provided accurate estimate of DMD_{ap} in cattle (Table 3). As for other internal markers, the accuracy of digestibility estimates depends on the quantitative faecal recovery of the markers, being under or overestimated as a result of a faecal recovery lower or higher than 1.0, respectively. For that reason, some authors (Ordakowski et al., 2001) suggest the adjustment of alkane faecal concentrations with the proper recovery data prior to their application. According to Dove and Mayes (2003) in practice, the errors due to assumptions relating to faecal recovery corrections are usually less than 3%. In this study, the assumption of a faecal recovery of 1.0 for all *n*-alkane markers (C_{27}, C_{29}, C_{31} and C_{33}) lead to a mean error of +3.6, −3.6 and −4.8% in DMD_{ap} for H1, H2 and *C*, respectively, using DC1 and DC2.

Previous studies carried out in our laboratory with goats (Ferreira et al., 2005) and sheep (Ferreira et al., in press) indicated a significant increase of alkane faecal recovery with the decrease of diet's digestibility. Thus, it would be expected that the accuracy of digestibility estimates obtained using the alkanes increased with the decrease of diet digestibility. Due to the generally observed increase of faecal recovery with carbon chain length in ruminants it is expected that only the long chain alkanes achieve high levels of faecal recovery and can provide accurate estimates of digestibility. In contrast, more alkanes can be used as digestibility markers in equines, as the alkane faecal recoveries seemed to be unrelated to the chain length. Their utilization is only limited by the low concentration in the feeds and faeces.

As a consequence of the accurate estimation of diet composition in *C* group (Table 4) the DMD_{ap} estimates were not significantly affected by the data on diet composition (DC1 and DC2) used in the calculations. Similar findings were observed in the equine groups, although diet composition was estimated with lower accuracy (Table 4).

The results on feed intake estimation (Table 5) show that the alkane pair used in the calculations affected the estimation of feed intake in H1 ($P<0.05$), H2 ($P<0.001$) and *C* ($P<0.001$). All alkane pairs overestimated intake, ranging from a minimum of +4.1% (C_{31}:C_{32} in H1 using DC1) and a maximum of +46.3% (C_{23}:C_{24} in H2 using DC2) in equines, and a minimum of +0.7% (C_{31}:C_{32} using DC1) and a maximum of +47.1% (C_{25}:C_{24} using DC2) in cattle. Quantitatively, the best agreement between known and estimated intakes was obtained using the C_{31}:C_{32} alkane pair, overestimating intake in only 4.1, 12.9 and 0.7% in H1, H2 and *C*, respectively, using DC1 and 5.5, 13.2 and 1.8% in H1, H2 and *C*, respectively, using DC2.

A consistent overestimation of intake was also observed by Gudmundsson and Thohallsdottir (1998) in Icelandic geldings fed on hay. However, Stevens et al. (2002) indicated over (2–10%) or underestimation (5–28%) of feed intake in horses fed on fresh perennial ryegrass (*L. perenne*), fresh Kikuyu (*Pennisetum clandestinum Hochst*) grass or Kikuyu hay, using the alkane pairs C_{31}:C_{32} and C_{33}:C_{32}.

In a study carried out with in Brahman cross steers fed on buffel-grass and lucerne hays, dosed with a controlled-

release capsule (CRC), Hendricksen et al. (2002) observed an underestimation of intake using the *n*-alkane pairs C_{31}:C_{32} (a minimum of −14% to a maximum of −31%) and C_{33}:C_{32} (a minimum of −5% to a maximum of −34%). Also Molina et al. (2004) observed an underestimation of intake in Holstein lactating cows fed on *P. maximum* pasture, using the same *n*-alkane pairs, dosing the synthetic *n*-alkane C_{32} via gelatine capsules (mean of −13%) or CRC (mean of −5%). Using non lactating beef cows of "Asturiana de los Valles" breed fed on lucerne hay at two feeding levels, Oliván et al.(in press) observed that the calculations based on the *n*-alkane pair C_{25}:C_{24} overestimated significantly intake (mean of +11%) and those based on the *n*-alkane pairs C_{31}:C_{32} and C_{33}:C_{32} underestimated intake (mean of −25 and −22%, respectively).

As referred by Mayes et al. (1986), in order to obtain accurate estimates of intake, the faecal recoveries of the two *n*-alkanes of each pair must be similar, so that the errors associated with their incomplete recoveries cancel out in the equation used to estimate intake (Eq. (3)). Thus, the overestimation or underestimation of feed intake referred to previously was the result of a positive or negative discrepancy between the faecal recoveries of the natural and the dosed *n*-alkane of each pair, respectively. The results obtained in this study (Fig. 2) indicate that these discrepancies are of equivalent proportion as we observed a high correlation (r=0.998) between the variations of the alkane faecal recoveries (natural/dosed alkanes) and the accuracy of feed intake estimates (known/estimate intake). For example, the overestimation of feed intake by 14.8% when using the *n*-alkane pair C_{23}:C_{24} in H1 (Table 5) was due to a faecal recovery of the natural alkane C_{23} 14.6% higher than the dosed alkane C_{24} (1.18 vs. 1.03, respectively). In the same way, the good agreement between the measured intake and that obtained using the C_{31}:C_{32} alkane pair in the cattle group (lower than 1% using DC1 data) was the result of an identical faecal recovery of the two alkanes (0.96). A similar association between the variations of the alkane faecal recoveries and the accuracy of feed intake estimates was also reported by Hendricksen et al. (2002) and Oliván et al. (in press).

In ruminants the differences in the faecal recovery of adjacent alkanes decrease with the increase of the carbon chain length (Dove and Mayes, 1991). Although we can determine alkanes with longer chain length (C_{35} and C_{36}), feed intake is generally calculated with accuracy, using C_{32} as the dosed alkane and C_{31} or C_{33} as the natural alkanes. The very low concentrations of C_{35} limit its use as internal marker in feed intake calculations as illustrated in our study by the poor estimates produced by the alkane pair C_{35}:C_{36}. In this study we observed a lack of effect of carbon chain length on the alkane faecal recovery in equines, being relatively constant across the range C_{23}–C_{36}. This could suggest that other alkane pairs can be used to obtain accurate estimates of intake in equines. Nevertheless, as referred for the alkane C_{35}, the low concentrations of some markers may limit their usefulness on the estimation of intake.

Dove and Mayes (2005) refer that one of the main advantages of using alkane markers is the possibility to estimate two important nutrition parameters (diet composition and feed intake) using only one analytical procedure. This can be achieved by using the alkane concentrations of the estimated diet as inputs for the

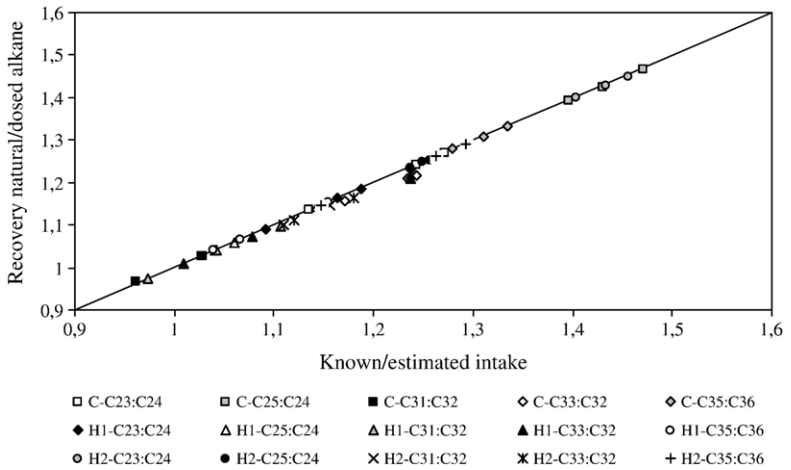

Fig. 2. Relationship between the known/estimated intake and the faecal recovery of the natural/dosed alkanes in H1, H2 and *C*. Solid line is the line of equality (y=x).

subsequent calculations of feed intake. This obviates, for example, the need of oesophageal-fistulated animals to obtain a sample of the diet being consumed.

It is obvious that the precision of feed intake and DMD_{ap} estimates will be a function of the accuracy of the resultant estimate of diet composition (Dove and Mayes, 2005). In the present study, the data on diet composition (DC1 or DC2) used in the calculations affected the feed intake estimated in H1 ($P<0.05$) but not in H2 ($P>0.05$) and C ($P>0.05$). These results were expected, as feed intake estimates obtained using DC2 could result from two possible sources of error: 1) the discrepancy between the proportions of each plant species in the diet estimated by the alkane markers and the known ones (Table 4) and 2) the discrepancy between the faecal recoveries of the natural and the dosed *n*-alkane of each pair (Table 2). On the contrary, when using the known diet composition (DC1), intake estimates could be biased only due to the discrepancy between faecal recoveries.

Nevertheless, this effect is likely to be situation dependent, and could vary according to the impact of the estimates of diet composition on the calculated concentrations of the alkanes on the diet consumed (Eq. (3)), that are then used for intake (Eq. (2)) and DMD_{ap} estimation.

4. Conclusions

The results obtained in this study show that the *n*-alkane markers can be used to estimate simultaneously feed intake, apparent digestibility and diet selection of equines and cattle grazing on heathland vegetation communities.

In equines the alkane faecal recoveries were unrelated to their chain length, suggesting that in this animal species' feed intake can be estimated accurately using different pairs of alkanes with a wider range of carbon chain length, being only limited by the low concentrations in feed and faeces of the alkanes. On the contrary, in cattle it was observed a positive association between the faecal recoveries of the alkanes and their carbon chain length.

The results reveal that a minimum of 3 and 5 days is required in order to the faecal concentrations of the synthetic even chain alkanes (C_{24}, C_{32} and C_{36}) absorbed into paper pellets reach equilibrium in cattle and equines, respectively.

Although the results obtained in this study indicate a different behaviour of the *n*-alkane markers in the digestive tract between ruminants (cattle) and non-ruminants (equines), more research is needed in order to clarify the effect of animal species on the faecal recovery of alkanes, in animals fed on the same experimental diets.

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