

Faecal N excretion as an approach for
estimating organic matter intake by
free-ranging sheep and cattle

Animal Research Paper

Cite this article: Kozloski GV *et al.* (2018). Faecal N excretion as an approach for estimating organic matter intake by free-ranging sheep and cattle. *The Journal of Agricultural Science* **156**, 443–449. <https://doi.org/10.1017/S0021859618000412>

Received: 24 November 2017
Revised: 23 April 2018
Accepted: 16 May 2018
First published online: 11 June 2018

Key words:
Cattle; intake; pasture; sheep; techniques

Author for correspondence:
G.V. Kozloski, E-mail: gilberto.kozloski@ufsm.br

G. V. Kozloski¹, E. M. C. Zilio¹, F. Ongarato¹, B. C. Kuinchtner¹, A. G. Saccol¹,
T. C. M. Genro², L. Oliveira³, B. M. Faria³, I. M. Cezimbra³ and F. L. F. Quadros¹

¹Departamento de Zootecnia, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil;

²Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Pecuária Sul, Bagé, RS 62010-970, Brazil and

³Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS 91540-000, Brazil

Abstract

The current study aimed to test whether organic matter intake by free-ranging ruminants could be estimated from the amount of nitrogen (N) excreted in faeces and to compare this approach to conventional techniques. An equation describing the relationship between excreted N and nutrient intake was developed in indoor digestibility trials conducted with male sheep ($n = 36$) and cattle ($n = 24$) housed in metabolism cages and solely fed hay harvested from a local rangeland. Faecal N excretion was linearly related to organic matter (OM) intake without a significant animal species effect. To evaluate the linear equation, data from free-ranging trials conducted with sheep and cattle were used. The faecal N approach was compared with either *in situ* digestibility plus external marker ($n = 123$) or *n*-alkanes ($n = 272$) to estimate OM intake and digestible OM intake. Estimates obtained through the faecal N approach did not closely fit those obtained with either conventional technique for any variable. Averaging all individual values, the supply of metabolizable energy (ME) estimated through faecal N was similar to the required level, whereas both the *in situ* and *n*-alkanes techniques overestimated ME supply. In conclusion, OM intake by free-ranging sheep and cattle can be estimated based on the amount of N excreted in faeces with some advantages over conventional techniques: knowledge about herbage attributes is not required and it accounts for individual variability on selectivity and digestion processes.

Introduction

In several regions of the world, most sheep and cattle are raised on natural grasslands. The nutrient intake by these animals cannot be measured directly, so estimating their nutritional status throughout the year remains a challenge. A commonly used technique for estimating the nutrition of free-ranging ruminants is based on measuring both faecal excretion and herbage digestibility. Faecal excretion can be measured individually with bags attached to the animals or by using external markers, and herbage digestibility is usually measured by *in vitro* incubation of herbage samples collected by hand clipping, which simulates grazing behaviour. However, the main flaws of this technique are a failure to account for individual variability in digestibility values expected for grazing ruminants and limited accuracy of the *in vitro* assay for estimating the actual *in vivo* digestibility (Peyraud, 1997). These flaws are probably more problematic when estimating the diet quality of free-ranging animals, which encounter a heterogeneous canopy and ingest forage selectively. To overcome some of the limitations of the *in vitro* technique, Mayes and Lamb (1984) proposed the use of *n*-alkanes as both external (dotriacontane, C₃₂) and internal markers (odd-chain *n*-alkanes) to simultaneously estimate both faecal output and herbage digestibility with greater accuracy than conventional markers (Dove and Mayes, 1996). However, the *n*-alkanes technique does not solve the problem of obtaining representative samples of the herbage ingested by free-ranging ruminants.

Alternatively, a strong correlation has been reported between organic matter (OM) intake and faecal nitrogen (N) excretion (Lancaster, 1949; Peripolli *et al.*, 2011), and linear equations have been proposed to estimate OM intake by sheep based on the amount of N excreted in the faeces (Azevedo *et al.*, 2014; David *et al.*, 2014; Kozloski *et al.*, 2014). However, these equations were developed with data obtained in trials conducted with animals fed only one type of forage or forage supplemented with concentrate feedstuffs, and no information on the reliability of this technique on estimating nutrients intake by free-ranging ruminants was provided. Furthermore, all previous studies have focused only on estimating total OM intake, whereas the animal performance is more a consequence of the digestible OM intake.

The objectives of the present study were (i) to analyse whether there is a strong relationship between OM intake and faecal N excretion in sheep and cattle fed hay from a rangeland

Table 1. Descriptive variables of indoor digestibility trials with sheep and cattle fed hay from a rangeland pasture

Animal	Variable ^a	<i>n</i>	Mean	Minimum	Maximum	S.D.
Sheep	Body weight (kg)	36	25	15	38	6.0
	OM intake (g/day)	36	324	161	575	112.6
	Faecal OM (g/day)	36	189	108	367	61.1
	Faecal N (g/day)	36	3.0	1.8	5.2	0.89
Cattle	Body weight (kg)	24	180	128	205	23.2
	OM intake (g/day)	24	2446	537	4455	1164.2
	Faecal OM (g/day)	24	1263	347	2728	627.1
	Faecal N (g/day)	24	21	6	42	11.2

^aOM, organic matter.

pasture and (ii) to evaluate the validity of using the amount of N excreted in the faeces as an index to estimate both the total and the digestible OM intake in free-ranging sheep and cattle.

Materials and methods

Locality and rangeland description

The study was undertaken in Southern Brazil at the Universidade Federal de Santa Maria, Santa Maria, RS (29°4'S, 53°5'W, 151 m alt.) and at the Centro de Pesquisa Pecuária Sul (EMBRAPA/CPPSUL), Bagé, RS (31°2'S, 54°1'W, 212 m alt.). The rangelands of Southern Brazil belong to the Pampas Biome, an ecosystem that encompasses an area of 750 000 km² known as the 'Rio de la Plata Grasslands' (Bilenca and Miñarro, 2004). The dominant forage species present in the grazing trials were *Andropogon lateralis*, *Axonopus affinis*, *Paspalum notatum* and *Eragrostis plana* (Cezimbra, 2015; Faria, 2015; Saccol, 2015; Kuinchtner, 2016).

Indoor digestibility trials (equation development)

The digestibility trials were conducted with male sheep (two trials, each one with six animals throughout three periods) and cattle (two trials, one with six animals throughout one period and other with six animals throughout three periods) housed in metabolism cages and fed only forage. The forage offered in either trial was hay containing, on average, the following (all as g/kg dry matter (DM)): 935 g OM, 780 g neutral detergent fibre, 420 g acid detergent fibre, 68 g sulphuric-acid lignin, 13 g total N, 5.7 g neutral detergent insoluble N and 2.9 acid detergent insoluble N. The hay was obtained during the summer season (i.e. December–March) from a local rangeland by cutting the pasture approximately 3 cm above ground level. In all trials, the experimental treatments were three levels of forage allowance (i.e. 15 g/kg body weight (BW), 25 g/kg BW or *ad libitum*), and the experimental periods varied from 15 to 21 days, with 10–14 days for adaptation and 5–7 days for data and sample collection. In all trials, the hay was offered twice a day (08.00 h and 17.00 h) and the animals had free access to water and a mineral salt containing the following (g/kg): calcium, 100; phosphorus, 45; sulphur, 4.1; sodium, 205; cobalt, 0.025; copper, 0.450; iron, 1.5; iodine, 0.05; manganese, 1.0; selenium, 0.009; zinc, 2.52 and fluorine, 0.45. The feed offered and refused and the faeces were weighed, recorded and sampled daily during the collection periods. All samples were dried at 55 °C in a forced-air oven, ground through

a 1-mm screen and pooled by the animal within each experimental period for analysis. General descriptions of the relevant variables are shown in Table 1.

Grazing trials (equation evaluation)

The free-range trials were conducted from 2011 to 2014 with sheep (two trials, one with 12 animals throughout three periods, and other with six animals throughout eight periods) and cattle (two trials with heifers, one with ten animals throughout four periods and other with 21 animals throughout seven periods, and one trial with 16 steers throughout eight periods). The experimental periods varied from 28 to 90 days, and sampling and data collection were performed in each period throughout the trials. The BW change (BWc, g/day) was calculated as BW at the end minus BW at the beginning of each experimental period divided by the number of days between BW measurements. The description of the animal variables is shown in Table 2. In both trials with sheep, the total amount of excreted faeces was collected for five consecutive days in each experimental period using bags fixed to the animals with harnesses, and the faeces of each animal was weighed and sampled daily. All faecal samples were dried in a forced-air oven at 55 °C, ground to pass through a 1-mm screen and pooled by animal and experimental period for analysis. In one of the trials with heifers (i.e. that conducted with ten animals throughout four periods), chromium oxide (Cr₂O₃) was used as an external marker for estimating faecal excretion. The Cr₂O₃ was offered once daily (5 g/day) in 1-g capsules (i.e. five capsules/day) for 10 days in each experimental period. The capsules were mixed with 0.2 kg of rice bran, which was offered in individual feeders to each animal. Ingestion of the supplement, which occurred within a few minutes, was monitored by an observer to ensure that all capsules were actually ingested. Ground and coloured polyethylene particles (20 g) were also mixed with the rice bran from days 5 to 9 and used as a marker to identify the excreta of individual animals in the field (i.e. each heifer received different-coloured particles). From days 8 to 10, faecal samples were collected daily from faeces excreted in the field and containing the coloured particles and then dried in a forced-air oven at 55 °C, ground (1-mm screen) and pooled by animal in each period for analysis. In the other trial with heifers and in the trial with steers, C₃₂ *n*-alkane was used as an external marker for estimating faecal excretion. For 10 days in each experimental period, animals were dosed orally twice daily with approximately 150 mg of C₃₂ *n*-alkane (i.e. 300 mg/day), which was impregnated in 2-g cellulose pellets.

Table 2. Description of the animal variables of trials with free-ranging sheep and cattle where conventional techniques were used for estimating organic matter intake

Animal	Technique	Source	Variable	N ^a	Mean	Minimum	Maximum	s.d.
Sheep	Total faecal collection	Saccol (2015); M Gindri, personal communication, 2016	Body weight (kg)	83	43	18	67	18.1
			Body weight change (g/day)	83	5	-116	191	56.6
Cattle	Cr ₂ O ₃ marker	Kuinchtner (2016)	Body weight (kg)	40	208	127	312	43.9
			Body weight change (g/day)	40	211	-178	655	181.1
Cattle	<i>n</i> -alkanes marker	Cezimbra (2015); Faria (2015)	Body weight (kg)	272	281	163	508	57.6
			Body weight change (g/day)	272	167	-1036	1189	529.6

^aAnimal/period number.

Faecal samples were then collected directly from the rectum twice daily from days 5 to 10, just before marker dosing, dried in a forced-air oven at 55 °C, ground (1-mm screen) and pooled by animal in each period for analysis. In all trials, parallel to the faecal sampling, the herbage was sampled daily using the hand-clipping procedure, which simulated the grazing behaviour of the animals. All samples were dried in a forced-air oven at 55 °C and ground (1-mm screen) for analysis. In the sheep trial conducted with 12 animals and in the heifer trial conducted with ten animals, the herbage samples were pooled by pasture plot and period for analysis.

Chemical and *in situ* digestibility analysis

Dry matter content was determined by drying the samples at 105 °C overnight. Ash was determined after combustion at 600 °C for 3 h, and OM was calculated by the difference in mass. Nitrogen concentration was assayed by the Kjeldahl method (Method 984.13; AOAC 1997), and the chromium concentration in the faeces samples was analysed by atomic absorption spectrophotometry after acid digestion as described by Czarnocki *et al.* (1961). The concentration of *n*-alkanes was analysed in the herbage and faeces samples by gas chromatography (GC-2010, Shimadzu Corp., Japan) using the extraction and analysis protocols of Dove and Mayes (2006). A mixed standard (Sigma-Aldrich Corp., St. Louis, MO, USA) containing known concentrations of C₇ to C₄₀ *n*-alkanes was used for equipment calibration. To measure the *in situ* herbage digestibility, approximately 1 g of dried and ground samples were weighed in 5 × 5 cm² polyamide bags (40 μ porosity) and incubated in the rumen of a grazing steer for 48 h (Derमारquilly *et al.*, 1969). Afterward, the bags were removed from the rumen, washed with tap water, oven dried at 110 °C overnight and weighed. Ash was then determined after combustion at 600 °C for 3 h, and OM was calculated by the difference in mass. The herbage *in situ* OM digestibility was calculated as: (incubated OM (g) – residual OM (g))/incubated OM (g).

Calculations (free-range trials)

Faecal excretion (DM or OM, g/day) was estimated from external markers (i.e., chromium or C₃₂ *n*-alkane) as: dosed marker (mg/day)/faecal marker (mg/g of DM or OM). The faecal N excretion (g/day) was estimated as faecal DM (g/day) × faecal N (mg/g DM). In the sheep trials and in the trial with heifers in which chromium was used as an external marker, the herbage

intake (OM, g/day) was calculated as: faecal OM (g/day)/(1 – *in situ* OM digestibility). In two of these trials, the herbage samples were pooled by pasture plot and periods for *in situ* incubation and, thus, the same *in situ* digestibility value was used for all animals kept in the same plot. In trials where *n*-alkanes were used as markers, OM intake (g/day) was calculated as: ((faecal C₃₃ (mg/kg OM))/(faecal C₃₂ (mg/kg OM) – herbage C₃₂ (mg/kg OM)) × dosed C₃₂ (mg/day))/herbage C₃₃ (mg/kg OM) × 1000 (De-Stefani *et al.*, 2013). Furthermore, the herbage OM digestibility in these trials was calculated as: 1 – (herbage C₃₃ (mg/g OM)/faecal C₃₃ (mg/g OM)). The concentration of C₃₂ and C₃₃ in herbage and in faeces samples were (mean ± standard deviation), respectively, 13 ± 6.0 and 206 ± 69.7, and 124 ± 48.9 and 408 ± 182.3 mg/kg DM. For calculations, values were expressed on an OM basis. The digestible OM intake (g/day) was then calculated as OM intake (g/day) × OM digestibility. All intake values were then expressed per kg of BW. Alternatively, OM intake was also calculated in all trials from the amount of N excreted in the faeces using the linear equation generated with data from indoor trials. The digestible OM intake (g/day/kg BW) in the faecal N technique was then calculated as: OM intake (g/day/kg BW) – faecal OM (g/day/kg BW). The general description of the estimates generated with either technique is shown in Table 3.

The metabolizable energy (ME) intake (kJ/day/kg BW) of the animals was calculated as: digestible OM intake (g/day/kg BW) × 18.41 × 0.82 (Fox *et al.*, 2004) and the ME required for maintenance (ME_m, kJ/day/kg BW) was calculated considering the basal metabolism (i.e. 403 kJ/kg BW^{0.75} for sheep and 500 kJ/kg BW^{0.75} for cattle) plus an additional 15% which was required for activity (Fox *et al.*, 2004; Tedeschi *et al.*, 2010). Additionally, the ME associated with BWc (ME_c, kJ/day/kg BW) was also calculated using the appropriate equations described by Cannas *et al.* (2004) for sheep and by Fox *et al.* (2004) for cattle. The total ME requirement (kJ/day/kg BW) was calculated as ME_m + ME_c, where the ME_c value was negative or positive depending on whether the change in BW was negative or positive, respectively.

Statistical analysis

Indoor digestibility trials (equation development)

Data from indoor trials were analysed using a variance-covariance model, in which OM intake was the dependent variable and faecal N excretion was the independent variable. The animal species was included as a class fixed effect in the model. The PROC MIXED

Table 3. Description of nutritional variables estimated through either conventional technique or through the alternative faecal N (Nf) technique in trials^a carried out with free-ranging sheep or cattle

Animal	Technique	Variable ^b	N ^c	Mean	Minimum	Maximum	s.d.
Sheep	Total faecal collection	OM intake (g/day/kg BW)	83	26	10	47	8.6
		OM digestibility	54	0.6	0.4	0.8	0.13
		Digestible OM intake (g/day/kg BW)	83	16	4	38	8.5
	Faecal N	OM intake (g/day/kg BW)	83	21	10	32	3.8
		OM digestibility	83	0.53	0.39	0.67	0.077
		Digestible OM intake (g/day/kg BW)	83	11	4	19	3.1
Cattle	Cr ₂ O ₃ marker	OM intake (g/day/kg BW)	40	24	15	35	4.1
		OM digestibility	18	0.50	0.42	0.59	0.045
		Digestible OM intake (g/day/kg BW)	40	12	6	20	2.9
	Faecal N	OM intake (g/day/kg BW)	40	24	16	36	4.4
		OM digestibility	40	0.50	0.32	0.63	0.079
		Digestible OM intake (g/day/kg BW)	40	12	6	22	3.8
Cattle	<i>n</i> -alkanes marker	OM intake (g/day/kg BW)	272	23	8	58	7.9
		OM digestibility	272	0.5	0.0	1.0	0.22
		Digestible OM intake (g/day/kg BW)	272	12	1	50	7.9
	Faecal N	OM intake (g/day/kg BW)	272	19	8	58	8.9
		OM digestibility	272	0.52	0.34	0.73	0.079
		Digestible OM intake (g/day/kg BW)	272	10	4	46	5.7

^aThe sources of trials were described in Table 1.

^bOM, organic matter; BW, body weight.

^cSome of the herbage samples were pooled by pasture plot and period.

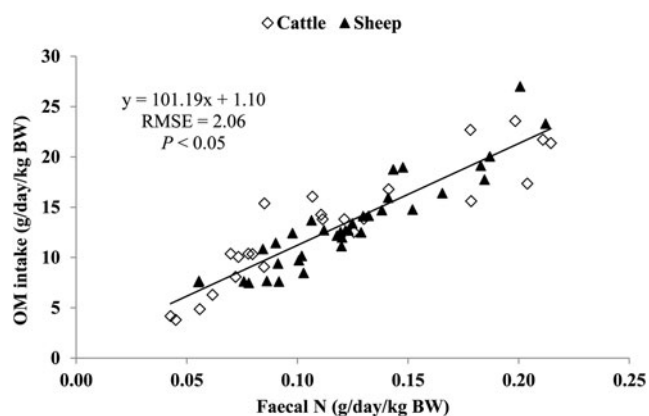


Fig. 1. Relationship between organic matter (OM) intake and faecal N excretion in cattle and sheep fed hay from a natural pasture. BW, body weight; RMSE, root mean square error; $n = 60$. The effect of animal species was not significant ($P > 0.05$).

statement in SAS (2002) was used for this analysis, which generated a linear equation.

Grazing trials (equation evaluation)

For convenience, the analyses described below were performed separately for the two groups of trials: trials that used the *in situ* technique (Group 1) and trials that used the *n*-alkanes technique (Group 2). Data on OM intake and digestible OM intake generated from the alternative faecal N technique were compared with those obtained with either conventional technique (i.e. *in situ* or

n-alkanes) using a similar variance-covariance model for indoor trials, except that trial was included as a random class effect in the model. Additionally, the relationship between the ME requirement and the ME supply estimated by either technique was also analysed with this model. When relevant, the average values obtained through either technique were also compared. Significance was declared at $P < 0.05$, and the root mean square error (RMSE) was used to determine the precision of the relationship between techniques. When appropriate, the confidence interval (95%) of the equation parameters was calculated from the standard error (s.e.) values (i.e. ± 2 s.e.) and used to evaluate the deviation of either the slope from 1 or the intercept from 0.

Results

Indoor digestibility trials (equation development)

Faecal N excretion was linearly ($P < 0.05$) related to OM intake without a significant effect of animal species (Fig. 1), and the generated equation was: OM intake (g/day/kg BW) = $1.1 \pm 0.75 + (101 \pm 5.9 \times \text{faecal N (g/day/kg BW)})$, which was then used to estimate OM intake in the free-range trials.

Free-range trials (equation evaluation)

Faecal N v. *in situ*

The OM intake and the digestible OM intake values estimated by the *in situ* technique were related linearly ($P < 0.05$) to those estimates based on faecal N excretion (Fig. 2). However, for both variables, the intercept and slope of the linear equation were

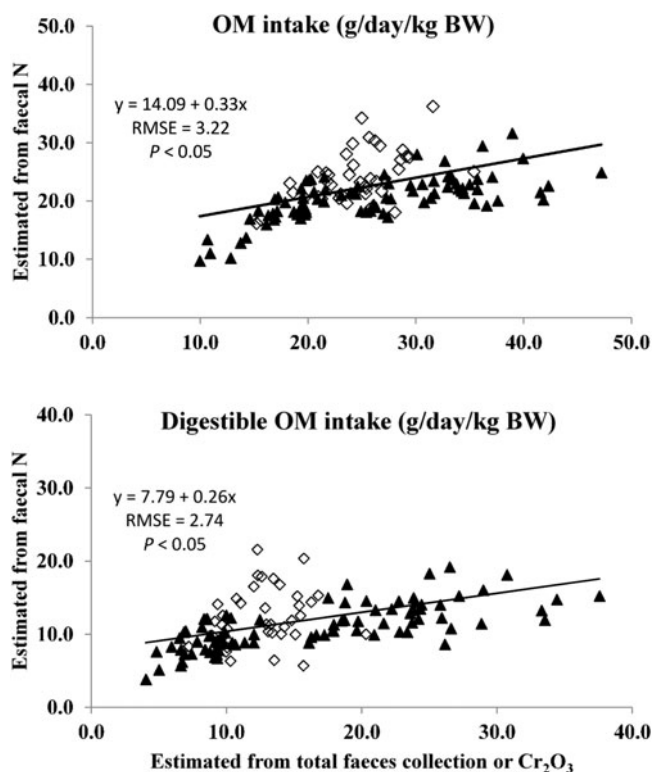


Fig. 2. Relationship between intake values estimated through either technique the conventional (i.e. total faeces collection or Cr_2O_3 marker) or faecal N in trials with free-ranging sheep (\blacktriangle) and cattle (\diamond). The OM intake was estimated from the conventional techniques as: faecal OM (g/day/kg BW)/(1 - *in situ* OM digestibility) and, from faecal N as: $1.10 + (101.2 \times \text{faecal N (g/day/kg BW)})$, which is the equation showed in Fig. 1. The digestible OM intake was calculated as: OM intake - faecal OM. RMSE, root mean square error; $n = 123$. OM, organic matter; BW, body weight.

different ($P < 0.05$) from 0 and 1, respectively. No significant relationship was obtained between the ME requirement and ME supply estimated through either technique (results not shown). On average, the ME supply was similar to the ME requirement (188 ± 65.1 kJ/day/kg BW) when estimated through faecal N (171 ± 51.3 kJ/day/kg BW) and higher ($P < 0.05$) than the ME requirement when estimated through *in situ* technique (226 ± 111.0 kJ/day/kg BW).

Faecal N v. *n*-alkanes

There was no significant relationship between techniques for estimating digestible OM intake whereas the OM intake values estimated using *n*-alkanes were linearly ($P < 0.05$) related to those estimated by faecal N (Fig. 3). However, the intercept of the equation was different from zero, and the slope was different from 1. No significant relationship was obtained between the ME requirement and ME supply estimated through either technique (results not shown). On average, ME supply was similar to ME requirement (158 ± 54.2 kJ/day/kg BW) when estimated through faecal N (184 ± 87.3 kJ/day/kg BW) and higher ($P < 0.05$) than the ME requirement when estimated from *n*-alkanes (184 ± 119.2 kJ/day/kg BW).

Discussion

Wang *et al.* (2009) developed an equation for estimating OM digestibility of forage from the crude protein content in faeces

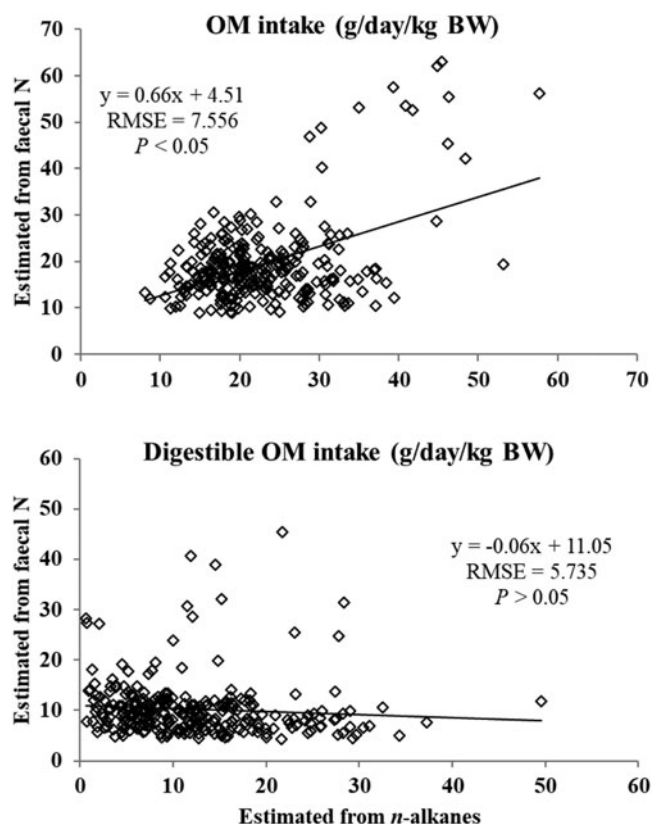


Fig. 3. Relationship between intake values estimated through either technique *n*-alkanes or faecal N in trials with free-ranging cattle. The OM intake was estimated from *n*-alkanes as: $((\text{faecal } C_{33} \text{ (mg/g OM)})/(\text{faecal } C_{32} \text{ (mg/g OM)} - \text{herbage } C_{32} \text{ (mg/g OM)}) \times \text{dosed } C_{32} \text{ (mg/day)})/ \text{herbage } C_{33} \text{ (mg/g OM)}/\text{BW}$ and, from faecal N as: $1.10 + (101.2 \times \text{faecal N (g/day/kg BW)})$, which is the equation showed in Fig. 1. The digestible OM intake was calculated as: OM intake - faecal OM. RMSE, root mean square error; $n = 272$. OM, organic matter; BW, body weight.

of sheep. However, the general objective of the present study was more to test whether nutrient intake by free-ranging sheep and cattle could be estimated through faecal N excretion than to estimate herbage attributes. There is no standard approach for measuring herbage or nutrient intake by grazing ruminants, so faecal N excretion was compared with other conventional techniques commonly used to estimate these variables. Previous studies have reported a high correlation between OM intake and faecal N excretion (Peripolli *et al.*, 2011), and linear equations have been proposed to estimate OM intake based on the amount of N excreted in the faeces of sheep fed with a specific type of forage, or forage supplemented with concentrate feedstuffs (Azevedo *et al.*, 2014; David *et al.*, 2014; Kozloski *et al.*, 2014). The results from indoor trials in the present study corroborated this relationship even when diet was a mixture of forage species taken from a natural grassland and generated a linear equation with a relatively low RMSE (i.e., 2.06 g/day/kg BW to a general mean of 13.26 g/day/kg BW). Moreover, despite the differences between sheep and cattle in chewing activity and digestive responses (De Boever *et al.*, 1990; Aguerre *et al.*, 2013) the effect of animal species was not significant in the present study, so it was assumed that the same equation could be used for both sheep and cattle. The parameters of this linear equation were expressed as a proportion of BW and thus it was not compared with other similar equations in the literature, which were developed using absolute values of OM intake and faecal N, or crude protein, excretion.

The validity of the equation was then tested with data taken from two groups of free-range trials where the *in situ* technique (Group 1) or *n*-alkanes (Group 2) techniques were used to estimate the nutrient intake, and regardless of group, the estimates obtained based on faecal N did not closely fit those obtained with either conventional technique. In some cases, a linear relationship was observed between techniques, but in all linear relationships, the intercept differed from zero and the slope was lower than 1. In general, for all variables in this group, increased values obtained by the *in situ* technique were paired with lower values estimated through faecal N.

The *n*-alkanes technique has been proposed to be more accurate than other conventional techniques based on the use of external markers (Dove and Mayes, 1996), mainly because it overcomes the limitations of the *in vitro* (*in situ* in the present study) technique when estimating *in vivo* digestibility (Peyraud, 1997). However, in the present study, the *n*-alkanes yielded broadly variable digestible OM intake values, some of which were probably unrealistic estimates. This discrepancy was probably the consequence of an incomplete and variable faecal recovery rate of *n*-alkanes (Ohajuruka and Palmquist, 1991; Morais *et al.*, 2011; Keli *et al.*, 2013; Kozloski *et al.*, 2014), which might have introduced bias in the estimation of herbage digestibility. Moreover, the chemical composition of the herbage was measured in samples collected by the hand-clipping procedure, where the ingestion behaviour of each animal was monitored by an observer during some hours of a day in each experimental period. Independently of whether the herbage samples were pooled by pasture plot or not, it is unlikely the herbage attributes used for the calculations in both groups have accounted for the variability in grazing selectivity along a day and throughout the days, which is expected to be significant by free-ranging animals (Orr *et al.*, 2012; Bonnet *et al.*, 2015; Provenza *et al.*, 2015).

Alternatively, when the nutritional variables were estimated through faecal N, any herbage attribute was required and, independently of using total collection or external marker, faeces excretion was individually measured or estimated over three to five collection days under normal grazing conditions. Thus, this technique has some advantages over the conventional approaches as it does not include errors associated with herbage sampling and it accounts for the individual variability in diet selectivity. Moreover, the digestible OM intake estimated through faecal N is probably more representative of the total *in vivo* digestion processes than that obtained through the *in situ* technique. However, the largest source of faecal N in ruminants is of metabolic origin, most of them represented by rumen microbial cell wall residues, whereas a lower proportion is undigestible N from the feed (Van Soest, 1994). Thus, although the faecal excretion of both is expected to increase at increased forage intake, it is probable that the absolute faecal N excretion, even as the proportion of BW, will be affected by forage type and/or chemical composition. Azevedo *et al.* (2014), for example, in a study with sheep fed Italian grass, obtained linear equations between OM intake and faecal crude protein excretion which were not similar at the increased phenological stage of the forage. This might limit the reliability of using a general equation, as that obtained in the present study, for all ranging systems.

Despite the above differences, standard values are not available and, thus, it is not possible to draw conclusions about the validity of one technique or another in estimating the nutrient intake by free-ranging animals based on simple comparisons. Thus, all techniques were evaluated in the present study based on their

accuracy in estimating the ME supply relative to the ME requirement. When individual paired values were compared, any technique was suitable for estimating ME supply, which would be expected when the individual variability on the energy required for activity was not accounted for in calculations. Moreover, whereas BW change was calculated over measurement intervals varying from 28 up to 90 days, energy supply was calculated from samples and data taken over a short period of time in each experimental period (i.e. 3–5 days). Despite these discrepancies, when averaging all individual values within groups, the ME supply estimated through faecal N in both groups was similar to the requirement, whereas both the *n*-alkanes and *in situ* techniques over-estimated the ME supply to sheep and/or cattle.

Conclusion

The OM intake by free-ranging sheep and cattle can be estimated from the amount of N excreted in faeces with advantages over the conventional *in situ* or *n*-alkanes techniques: knowledge about herbage attributes is not required and it accounts for individual variability on selectivity and digestion processes. However, the reliability of the linear equation obtained in the present study on estimating herbage intake in other ranging systems needs to be evaluated.

Financial support. The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for scholarship support.

Conflicts of interest. None.

Ethical standards. All procedures were conducted in accordance with the guidelines in the Code of Practice for the Care and Use of Animal for Experimental Purposes of the Brazilian College of Animal Experimentation.

References

- Aguerre M, Cajaville C, Kozloski GV and Repetto JL (2013) Intake and digestive responses by ruminants fed fresh temperate pasture supplemented with increased levels of sorghum grain: A comparison between cattle and sheep. *Animal Feed Science and Technology* **186**, 12–19.
- AOAC (1997) *Official Methods of Analysis*. 16th ed. 3rd revision. Gaithersburg, MD, USA: Association of Official Analytical Chemists, Inc.
- Azevedo EB, Poli CHEC, David DB, Amaral GA, Fonseca L, Carvalho PCF, Fischer V and Morris ST (2014) Use of faecal components as markers to estimate intake and digestibility of grazing sheep. *Livestock Science* **165**, 42–50.
- Bilencá D and Miñarro F (2004) *Identificación de Áreas Valiosas de Pastizal (AVPs) en las Pampas y Campos de Argentina, Uruguay y sur de Brasil*. Buenos Aires, Argentina: Fundación Vida Silvestre Argentina.
- Bonnet OJF, Meuret M, Tischler MR, Cezimbra IM, Azambuja JCR and Carvalho PCF (2015) Continuous bite monitoring: a method to assess the foraging dynamics of herbivores in natural grazing conditions. *Animal Production Science* **55**, 339–349.
- Cannas A, Tedeschi LO, Fox DG, Pell AN and Van Soest PJ (2004) A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. *Journal of Animal Science* **82**, 149–169.
- Cezimbra IM (2015) *Emissão de metano por bovinos sob níveis de oferta de forragem em pastagem nativa do Bioma Pampa*. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
- Czarnocki J, Sibbald IR and Evans EV (1961) The determination of chromic oxide in samples of feed and excreta by acid digestion and spectrophotometry. *Canadian Journal of Animal Science* **4**, 167–179.
- David DB, Poli CHEC, Savian JV, Amaral GA, Azevedo EB, Carvalho PCF and McManus CM (2014) Faecal index to estimate intake and digestibility in grazing sheep. *Journal of Agricultural Science, Cambridge* **152**, 667–674.

- De Boever JL, Andries JI, De Brabander DL, Cottyn BG and Buysse FX** (1990) Chewing activity of ruminants as a measure of physical structure — A review of factors affecting it. *Animal Feed Science and Technology* **27**, 281–291.
- De-Stefani Aguiar A, Forbes TDA, Rouquette FM, Tedeschi LO and Randel RD** (2013) Evaluating the statistical variation in estimating forage dry matter intake of grazing Brahman bulls using n-alkanes. *Journal of Agricultural Science, Cambridge* **151**, 129–140.
- Dermarquilly C, Chenost M, Aubry J, Chevalier D and Chenost MJ** (1969) Étude de la digestion des fourrages dans le rumen par la méthode des sachets de nylon; liaisons avec la valeur alimentaire. *Annales de Zootechnie* **18**, 419–430.
- Dove H and Mayes RW** (1996) Plant wax components: a new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition* **126**, 13–26.
- Dove H and Mayes RW** (2006) Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. *National Protocols* **1**, 1680–1697.
- Faria BM** (2015) *Emissão de metano e comportamento ingestivo de bovinos de corte em pastagem natural com diferentes níveis de intensificação*. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
- Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, Pell AN and Overton TR** (2004) The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology* **112**, 29–78.
- Keli A, Olmos G, Veja A and Guada JA** (2013) Net transfer of nutrients to the duodenum and disappearance of n-alkanes in the reticulo-rumen and the hindgut of sheep fed grass/legume combinations. *British Journal of Nutrition* **109**, 1765–1778.
- Kozloski GV, Oliveira L, Poli CHEC, Azevedo EB, David DB, Ribeiro Filho HMN and Collet SGJ** (2014) Faecal nitrogen excretion as an approach to estimate forage intake of wethers. *Journal of Animal Physiology and Animal Nutrition* **98**, 659–666.
- Kuinchtner BC** (2016) *Manejo de pastagem natural em pastoreio rotativo utilizando duas somas térmicas como intervalo de descanso*. PhD thesis, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.
- Lancaster RJ** (1949) Estimation of digestibility of grazed pasture from faeces nitrogen. *Nature* **163**, 330–331.
- Mayes RW and Lamb CS** (1984) The possible use of n-alkanes in herbage as indigestible faecal markers. *Proceedings of the Nutrition Society* **43**, 39A.
- Morais JAS, Berchielli TT, Veja A, Queiroz MFS, Keli A, Reis RA, Bertipaglia LMA and Souza SF** (2011) The validity of n-alkanes to estimate intake and digestibility in Nelore beef cattle fed a tropical grass (*Brachiaria brizantha* cv. Marandu). *Livestock Science* **135**, 184–192.
- Ohajuruka OA and Palmquist DL** (1991) Evaluation of n-alkanes as digesta markers in dairy cows. *Journal of Animal Science* **69**, 1726–1732.
- Orr RJ, Tozer KN, Griffith BA, Champion RA, Cook JE and Rutter SM** (2012) Foraging paths through vegetation patches for beef cattle in semi-natural pastures. *Applied Animal Behaviour Science* **141**, 1–8.
- Peripolli V, Prates ER, Barcellos JOJ and Neto JB** (2011) Fecal nitrogen to estimate intake and digestibility in grazing ruminants. *Animal Feed Science and Technology* **163**, 170–176.
- Peyraud JL** (1997) Techniques for measuring herbage intake of grazing ruminants: a review. In Spörndly E, Burstedt E and Murphy M (eds). *Managing High Yielding Dairy Cows at Pasture*. Uppsala, Sweden: SLU, pp. 3–23.
- Provenza FD, Gregorini P and Carvalho PCF** (2015) Synthesis: foraging decisions link plants, herbivores and human beings. *Animal Production Science* **55**, 411–425.
- Saccol AGF** (2015) *Sheep meat production in different feeding systems*. PhD thesis, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.
- SAS** (2002) *SAS User's Guide Statistics*, Version 9.1. Cary, NC, USA: SAS Institute Inc.
- Tedeschi LO, Cannas A and Fox DG** (2010) A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: the development and evaluation of the Small Ruminant Nutrition System. *Small Ruminant Research* **89**, 174–184.
- Van Soest PJ** (1994) *Nutritional Ecology of the Ruminant*, 2nd Edn. New York, USA: Cornell University Press.
- Wang CJ, Tas BM, Glindemann T, Rave G, Schmidt L, Weißbach F and Susenbeth A** (2009) Fecal crude protein content as an estimate for the digestibility of forage in grazing sheep. *Animal Feed Science and Technology* **149**, 199–208.