ESTIMATING FORAGE INTAKE OF LACTATING DUAL-PURPOSE COWS USING CHROMIUM OXIDE AND N-ALKANES AS EXTERNAL MARKERS

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ABSTRACT: The n-alkanes have been used to estimate forage dry matter intake, digestibility and the diet composition in grazing animals. The objective this study was to compare chromium oxide and n-alkanes techniques used to estimate forage intake. Twenty lactating dual-purpose cows receiving two sources of fat (treatments: conjugated linoleic acid (CLA) or Megalac (control)) plus 4 kg of concentrate were dosed with n-alkanes and chromium oxide to estimate the intake of stargrass (*Cynodon nlemfüensis* Vanderyst var. nlemfüensis). The *in vitro* dry matter (DM) digestibility of the stargrass and concentrate were used to estimate the nutritive value of the digesta. The n-alkanes between C_{23} and C_{36} were quantified in the digesta and feces. The regression between metabolizable energy requirement (MEr, Mcal d^{-1}) and supply derived from forage DM intake estimated using chromium oxide was ME $Intake_{Cr}$ = 19.1 + 0.62 MEr (R^2 = 0.27) and the same relationship estimated using C_{35}:C_{36} n-alkane ratio was ME $Intake_{C35:C36}$ = 9.3 + 0.77 MEr (R^2 = 0.52). There was a treatment effect on fecal concentration of chromium oxide with daily and period variations. For the n-alkane technique, treatment and period effects and a linear effect of day of collection on the fecal concentrations of C_{35} were found. For C_{36} fecal concentrations, there was a treatment effect and a quadratic effect of collection day. There was no treatment effect on the fecal concentration of the C_{35}:C_{36} ratio, but a period effect and a linear effect of day of collection were found. Estimates of daily intake using the two markers were different, but those obtained using the C_{35}:C_{36} pair of n-alkanes were more precise than those obtained using chromium oxide and *in vitro* digestibility. Management of experimental animals could have influenced the concentration of markers in the feces, determining variations and inconsistencies that partially explain the inaccuracy of the estimates.
Key words: diet composition, digestibility, grazing

ESTIMATIVA DO CONSUMO DE FORRAGEM EM VACAS MESTIÇAS LACTANTES USANDO ÓXIDO CRÔMICO E N-ALCANOS COMO MARCADORES EXTERNOS

RESUMO: Os n-alcanos têm sido utilizados para estimar o consumo de matéria seca, a digestibilidade e a composição da dieta de animais em pastejo. O objetivo desse estudo foi comparar as técnicas de óxido crômico e n-alcanos usadas para estimar o consumo de forragem. Vinte vacas lactantes cruzas Holandês × Gir recebendo duas fontes de gordura (tratamentos: CLA (ácido linoleico conjugado) ou Megalac (controle)) mais 4 kg de concentrado foram dosadas com n-alcanos e óxido crômico para estimar o consumo de estrela-africana (*Cynodon nlemfüensis* Vanderyst var. nlemfüensis). A digestibilidade in vitro da matéria seca da estrela africana e do concentrado foi usada para estimar o valor nutritivo da dieta. Foram quantificados n-alcanos entre C_{23} e C_{36} na dieta e fezes. A regressão entre exigência e o consumo de energia metabolizável (EMr, Mcal d^{-1}) derivada da matéria seca da forragem calculada usando óxido de cromo foi ConsumoCr = 19,1 + 0,62 EMr (R^2 = 0,27) e a mesma relação estimada usando a relação C_{35}:C_{36} de n-alcanos foi ConsumoC_{35}:C_{36} = 9,3 + 0,77 EMr (R^2 = 0,52). Houve efeito de tratamento na concentração fecal de óxido de cromo com variação diária e entre turnos. Para técnica de n-alcanos, houve efeito de tratamento e período e efeito linear de dia de coleta

nas concentrações fecais do C_{35}. Para as concentrações fecais do C_{36}, houve efeito de tratamento e um efeito quadrático de dia de coleta. Não houve efeito de tratamento na concentração fecal da relação C_{35}:C_{36}, mas houve efeito de turno e efeito linear de dia de coleta. As estimativas do consumo de forragem obtidas com os dois marcadores foram diferentes, mas aquela obtida com o par de alcanos C_{35}:C_{36} foi mais precisa que aquela obtida com o óxido crômico e a digestibilidade *in vitro*. O manejo dos animais experimentais pode ter influenciado a concentração dos marcadores nas fezes, determinando variações e inconsistências que explicam parcialmente a falta de acurácia das estimativas.
Palavras-chave: composição da dieta, digestibilidade, pastejo

INTRODUCTION

Pasture is the main source of nutrients for grazing animals. They modify their diet by selecting different species and/or parts of plants, and this confers complexity to the determination of the diet composition, intake, and digestibility under grazing conditions. The most common methodology for estimating forage intake by grazing ruminants is the calculation of fecal output and forage dry matter (DM) digestibility. Fecal output is usually estimated by chromium oxide as an external marker and forage DM digestibility is measured using *in vitro* methods (Schneider & Flatt, 1975; Le Du & Penning, 1982; Mayes & Dove, 2000). The main constraints of this methodology are the assumption that chromium oxide is completely recovered in the feces and the use of a single forage DM digestibility value for all animals. An alternative methodology has been proposed to overcome these limitations. Currently, the aliphatic saturated hydrocarbons (n-alkanes) in the cuticular wax of plants have been extensively used to estimate forage DM intake (Mayes et al., 1986; Dove & Mayes, 1991; Genro et al., 2000) and digestibility (Mayes & Lamb, 1984; Oliveira et al., 2000). Additionally, this technique allows the study of the botanical and/or morphological composition of plants available in a pasture or ingested by the grazing animals (Dove, 1992; Dove & Moore, 1995; Oliveira et al., 2003).

The objective of the present research was to compare chromium oxide and n-alkane techniques for estimating forage DM intake and digestibility in lactating dual-purpose cows supplemented with or without conjugated linoleic acid (CLA) and grazing a tropical grass pasture.

MATERIAL AND METHODS

As part of a project evaluating the effects of CLA in lactating cows, an experiment was carried out in Valença, RJ, Brazil (43º42' W, 22º21' S). Twenty crossbred Holstein x Gir lactating cows were used, and based on previous milk yield, parity, body condition score (BCS), and body weight (BW) assigned to the treatments. Humane animal care and handling procedures were followed. Treatments comprised daily dosing of animals grazing a *Cynodon nlemfüensis* Vanderyst var. nlemfüensis pasture with 150 g of Megalac (Dwight & Church, salts of calcium of palm oil) (control) or 150 g of CLA-60 (Dwight & Church, salts of calcium of a mixture of isomers of CLA). Each cow received, individually, 2.0 kg (as–fed basis) of concentrate after the morning and afternoon milking for a total of 4.0 kg day^{-1}. The pasture was divided into fourteen 0.5-ha paddocks and rotationally grazed according to a 28-day grazing cycle (14 paddocks and 2-day grazing duration per paddock). Concentrate composition (DM basis) was: ground corn, 58.5%; fish meal, 25%; soybean meal, 5.5%; wheat bran, 5.5%; and a vitamin-mineral mix, 5.5%.

Fat supplements (CLA or Megalac) were mixed into the concentrate used for the first meal, using the second meal in the afternoon to offer any refusal from the morning period as a means of assuring total consumption, although most of the time this was not necessary. Rations were formulated with the Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) and forage composition was assumed to be (DM basis) 35% DM, 75% neutral detergent fiber (NDF), 9.7% lignin, 15% crude protein (CP), 30% N bound to the NDF as a fraction of total N (N-NDF), 8% N bound to the acid detergent fiber (ADF) as a fraction of total N (N-ADF), 1.6% ether extract, and 8% ash (Tedeschi et al., 2002).

Forage samples were hand-plucked (Prates, 1974) in the morning and afternoon during the two days of grazing per paddock. Three animals were followed for 30 minutes during the forage sampling procedure in an attempt to cover the entire area of the paddock and to mimic the pattern of intake and forage selection of the animals. Morphological separation of leaf lamina (leaf), leaf sheath and stem (stem), and senescent or dead material (dead) was carried out in three subsamples. The pre- and post-grazing herbage mass were measured by clipping herbage 10 cm above ground level at ten randomly selected areas (0.25 m^2) per paddock in three paddocks. Forage sampling, 12 hand-plucked samples and 60 for pre and post-grazing herbage mass, were carried out in the paddocks animals in which were grazing when fecal collections were carried out.

The hand-plucked forage samples, leaf and stem components, and concentrate were analyzed for DM, ash and CP according to AOAC (1997). The DM and ash of the fecal samples were also analyzed. Forage samples were also analyzed for NDF, ADF, NDF-N, ADF-N, and lignin according to Van Soest et al. (1991). Due to the fact that the detergent system produces unrealistic values of fiber for fish meal, the fractions of NDF, ADF, N-NDF, N-ADF, and lignin of the concentrate were estimated based on individual composition of each ingredient and their corresponding proportion of inclusion in the concentrate mix. The chemical composition of the feeds is shown in Table 1. Characterization of the ration, animal, and environment were used in the CNCPS to estimate the biological values of the used feeds. Other information, as the characterization of each animal for live weight (means, CLA= 485, CV= 7.7%; MEG= 463, CV= 10.9%), age (84 months), milk production (means, CLA= 15.4 kg, CV= 17.9%; MEG= 14.5 kg, CV= 17.7%) and composition (CLA, protein= 3.1%, CV= 9.2%, fat= 2.8%, CV= 19.2%; MEG, protein= 2.9%, CV= 8.2%, fat= 3.3%, CV= 11.6%), days in milk (56 days) and body condition score (CLA= 3.8, CV= 20.0%; MEG= 3.6, CV= 15.7%), plus the data of the environmental conditions (previous temperature= 23.5ºC, current temperature= 24.5ºC; humidity= 75%; wind speed= 4.2 km/h; coat condition= some mud on lower body), were also entered to estimate the metabolizable energy requirements (Mcal/day, means, CLA= 30.1, CV= 9.6%; MEG= 29.1, CV= 8.5%), rations and animal performance. The metabolizable energy of the feeds estimated by the CNCPS was used.

Cows were milked at 05:00 a.m. After milking, cows were fed individually, half of the daily concentrate allowance and returned to pasture. At 11:30 a.m., animals were retrieved from pasture and housed in a sheltered barn until the afternoon milking at 02:30 p.m. After the afternoon milking, cows were fed the second half of the concentrate and at 04:00 p.m. returned to pasture until the next day. Milk samples were taken three times per week to estimate milk composition.

Animals were dosed with a controlled-release capsule containing 8 g of n-dotriacontane (C_{32}) and 8 g of n-hexatriacontane (C_{36}) to estimate forage DM intake. The release rate of the marker C_{36} was 312 mg day^{-1} as reported by Oliveira (2003). Chromium oxide (70.96%) was dosed twice a day and started two days after the n-alkanes capsules were dosed. Chromium oxide was administered in the morning and in the afternoon, always after milking, in 5 g of shredded paper during 12 d. After dosing, animals were observed for a short period of time to ensure that there was no regurgitation. Additionally, two crossbred steers weighing 300 kg, using harness and bags for total fecal collection, were similarly dosed with the two markers to measure fecal recovery during three days. The bags were emptied twice a day.

Eight days after dosing the n-alkanes capsules, fecal samples were taken from rectum immediately after morning (06:30 a.m.) and afternoon (04:30 p.m.) milking during five consecutive days. Samples from different periods (morning and afternoon) and from different collection days for each animal were stored at –20ºC. Fecal samples were dried at 60ºC in a forced–draught oven up to constant weight. Dried samples were ground to pass a 1-mm mesh sieve and stored in plastic containers for subsequent chemical analysis.

Fecal samples were analyzed for chromium oxide according to Kimura & Miller (1952) and the digestibility of the forage and concentrate samples was determined by an *in vitro* procedure (Tilley & Terry, 1963 as modified by Goering & Van Soest, 1970), discounting 11.9 percentage units to account for fecal metabolic matter (Van Soest, 1994). The n-alkane concentration in forage and fecal samples, tablets of the controlled-release capsules, and concentrate was determined in duplicate using the methodology described by Oliveira (2004).

To obtain the fecal output from forage (PFF) necessary to estimate forage intake, the fecal production associated with concentrate (PFC) was discounted from the total fecal output with the chromium oxide (TFP) using Equation 1.

Table 1 - Chemical composition and Cornell Net Carbohydrate and Protein System (CNCPS) estimated metabolizable energy for forage and concentrate

Sample	n	CP	EE	NDF	NDF-N	ADF	ADF-N	LIGN	ASH	ME
Forage	12♣	13.3	1.7	61.4	5.4	28.0	1.6	3.2	9.1	2.11
Concentrate	1	25.5	5.6	11*	6.6*	3.9*	2.5*	3.3*	16.1	2.86

*Estimated using individual ingredient composition and their percentage of inclusion in the concentrate. Where: n= number of samples; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; NDF-N = N bound to the NDF as a fraction of total N; ADF = acid detergent fiber; ADF-N = N bound to the ADF as a fraction of total N; LIGN = lignin; ASH = ash; ME = metabolizable energy (Mcal kg^{-1} DM); (DM basis, %). ♣Sample of three paddocks.

PFF= TFP - [CMSc - (CMSc × DIVC)] [1]

where CMSc is the concentrate DM intake and DIVC is the *in vitro* digestibility of the concentrate.

The forage DM intake measured with chromium oxide was estimated according to Le Du & Penning (1982) and with n-alkanes as proposed by Mayes et al. (1986).

The experimental design was completely randomized and the statistical analyses were performed using the SAS software (SAS Institute Inc., 2000). The fecal C_{35}, C_{36} and C_{35}:C_{36} n-alkane ratios and the chromium oxide variations between the morning and afternoon periods during the five days of collection, as well as their interactions were analyzed using the PROC MIXED procedure. A contrast analysis between days of collection was conducted with the HELMERT option, in which the mean of the current period (morning or afternoon) of every day was compared to the subsequent ones. The relationship between estimated metabolizable energy intake (MEI) and metabolizable energy (ME) requirement was analyzed through linear regression by the PROC REG procedure and a studentized residue analysis was used for identifying outliers. For studentized residues outside the -2 to 2 range, the observation was considered an outlier and excluded from the analysis. The command TEST of PROC REG was used to verify if the slope was different from unity. The difference in the estimated forage MEI between the two methods was detected using a pair wise comparison with the *t*-test analysis. The dry matter intake (DMI) was analyzed with the PROC GLM procedure using the command LSMEANS at 5% of probability.

RESULTS AND DISCUSSION

The mean values of pre- and post-grazing herbage mass were 1800 (CV = 1%) and 1310 (CV = 22%) kg of DM ha^{-1}, respectively. The odd-chain n-alkanes profile between C_{23} and C_{35} was quantified for the whole sample, for the leaf and stem components of the hand-plucked forage and concentrate (Table 2). Concentrations were higher for odd- than even-chained alkanes (data not shown). The n-alkanes C_{33}, C_{31}, and C_{29} presented the highest concentrations, in this order, except that C_{31} for the leaf component was slightly lower than C_{29}, in agreement with results for other tropical forages (Oliveira et al., 1997; Genro et al., 2001). Forage intake estimated using the chromium oxide and the C_{35}:C_{36} n-alkane ratio, the measured daily individual consumption of concentrate, and the mean intake of estimated metabolizable energy (Mcal day^{-1}) are shown in Table 3.

Because the two treatments (CLA and control) had the same intake of concentrate and there was no difference in body weight and body condition score (Oliveira, 2003), the intake of metabolizable energy was assumed to be the total of the maintenance and lactation requirements in a steady-state condition. Thus, the regression between the ME requirement and the intake of ME from concentrate and the estimated forage intake was used to evaluate if markers were suitable to estimate forage intake. If the intercept equals zero and the slope equals one, estimates of energy intake are considered to properly balance the ME requirement. The linear regression (R^2 = 0.27) using forage intake estimates based on the chromium oxide technique is shown in equation (2):

$$ME Intake_{Cr} = 19.1 + 0.62\ MEr\ (RMSE = 2.71) \quad [2]$$

where the intercept was different from zero (P = 0.02) and the slope different from zero (P = 0.03) but similar to one (P = 0.17).

Because the intercept was different from zero, the equality of the slope to one cannot be considered as an accurate measurement. The paired comparisons using *t*-test indicated that the difference between ME intake and ME requirement was not equal to zero (P = 0.0001), suggesting that chromium oxide was not a reliable marker to estimate fecal output in this study.

The linear regression (R^2 = 0.52) between the ME consumption and ME requirements using the forage intake estimated with the C_{35}:C_{36} n-alkane ratio is shown in equation (3):

Table 2 - Odd-chain n-alkane concentration of whole, leaf blade, and sheath and stem of hand-plucked forage and concentrate.

Sample*	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}
	mg kg^{-1} DM						
Forage	35	55	68	71	106	137	27
Leaf	55	88	99	128	127	168	24
Stem	7	12	17	31	88	163	36
Concentrate	50	79	54	26	10	6	3

* Samples were analyzed in duplicate.

Table 3 - Estimated metabolizable energy requirements, measured intake of concentrate (Ci), and estimated dry matter intake and metabolizable energy intake of forage[1]

Treatment	MEr	Ci	FCr	$FC_{35}:C_{36}$	MECr	ME $C_{35}:C_{36}$
	Mcal d^{-1}	---------- kg d^{-1} ----------			---------- Mcal d^{-1} ----------	
CLA						
Mean[2]	30.1	3.6	12.7	10.2	37.1	32.5
CV (%)	9.6	3.5	14.1	14.5	9.2	8.8
Megalac						
Mean[2]	29.1	3.5	14.3	10.0	38.6	31.4
CV (%)	8.5	7.9	14.6	14.1	6.6	9.2

[1]Values estimated for intake of DM (kg d^{-1}) and ME (Mcal d^{-1}) using chromium oxide (FCr and MECr, respectively) and n-alkanes ($FC_{35}:C_{36}$ and ME $C_{35}:C_{36}$, respectively). [2]Mean of ten animals per treatment.

$$MEIntake_{C35:C36} = 9.3 + 0.77\ MEr\ (RMSE = 2.04) \quad [3]$$

in which the intercept is not different from zero (P = 0.09) and the slope different from zero (P = 0.0005) but similar to one (P = 0.21)

These findings indicate that the changes in energy requirement resulted in changes of intake of metabolizable energy, suggesting that the $C_{35}:C_{36}$ n-alkane ratio was a more reliable marker than Cr_2O_3 to estimate forage intake, even though 48% of the variation in energy intake was not accounted for by variations in energy requirements.

Although the fecal recoveries for chromium oxide (89.1%) and C_{36} (96%) n-alkane have been measured and used in the calculations of forage intake estimates, variation in excretion for the different markers was investigated among periods and days of collection. In Table 4, the fecal concentrations are presented for the morning and afternoon periods during five consecutive days of fecal collection.

There was no interaction between day of collection and period of the day for both markers. There was a treatment effect on fecal concentration of chromium oxide in the afternoon period on the fifth day of collection (CLA= 976 vs. MEG= 712 mg, P = 0.004). Unlike the findings of this experiment, Ohajuruka & Palmquist (1991), measuring intake in lactating dairy cows with external markers, found no effect of added fat on the concentration and fecal recovery of chromium oxide or concentration of C_{31} and C_{32} n-alkanes. Pereira et al. (1983) used chromium oxide in cattle receiving different levels of soybean oil plus formaldehyde in the diet. The authors found an effect of soybean oil addition on DM digestibility using chromium, but not on fecal recovery of the marker. In this study chromium oxide concentrations varied among days (P = 0.02, Figure 1) within and between periods (morning mg Cr = 1132.7 + 106.5 × day – 26.7 × day^2, afternoon mg Cr = 929.9 + 106.5 × day – 26.7 × day^2, P = 0.0001). These are similar findings in relation to those of Lima et al. (1980), who studied fecal excretion of chromium in grazing cattle and reported largest concentration values during the morning period (morning = 1158 vs. afternoon = 955 mg, P = 0.01). Kameoka et al. (1956) mentioned the possibility of chromium oxide accumulating in some parts of the digestive tract, with later excretion of larger amounts in subsequent times of the day, and that the variation of the amount of daily excreted feces, could also contribute to alter fecal concentration of the marker regardless of animals being kept indoors or grazing.

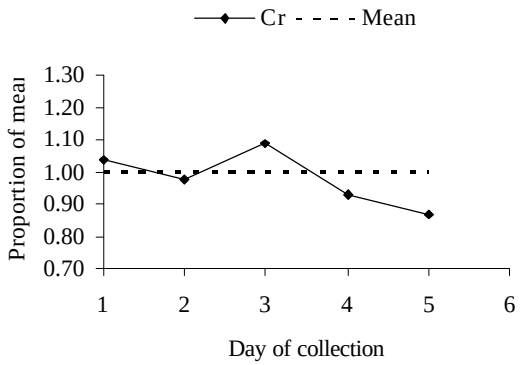

Figure 1 - Mean variation in the chromium concentrations in feces expressed as proportions of the mean concentration for each cow over five days.

Similar to the findings regarding chromium oxide, there were effects of treatment (CLA = 62 vs. MEG = 55 mg, P = 0.01), period (morning = 54 vs. afternoon= 63 mg, P = 0.01); day of collection (linear; CLA Morning, mg C_{35}= 48.05 + 3.4 × day; CLA afternoon, mg C_{35}= 36.2 + 3.4×day; MEG morning, mg C_{35}= 40.7 + 3.4 × day; MEG afternoon, mg C_{35}= 48.9 + 3.4 × day; P = 0.0001) on the fecal concentrations of the natural C_{35} n-alkane of the diet.

Excretion of the C_{36} n-alkane was affected by treatment on the morning of the fourth day of collection (P = 0.01), and there was a quadratic effect

Table 4 - Fecal concentrations of chromium, C_{35}, C_{36} and C_{35}:C_{36} n-alkane ratio for each period (morning and afternoon) in five consecutive days.

Mk/Tt.	M1	M2	M3	M4	M5	A1	A2	A3	A4	A5
	---------- mg kg^{1} DM ----------									
Chr										
CLA	1222	1268*	1367*	1091	995	1052	1102*	1069*	935	976a
MEG	1142	1252*	1183*	1070	983	976	963	943	829	712b
C_{35}										
CLA	55*	81*	63	68*	70a	64*	100*	74*	85*	77a
MEG	55*	86*	64	70*	57b	58*	102*	68*	79*	63b
C_{36}										
CLA	76	106*	69	71*b	66	72*	109*	75	83*	67
MEG	71	107*	74	80*a	62	67*	114*	70	68*	60
C_{35}:C_{36}										
CLA	0.76*	0.80*	0.95	0.96	1.10	0.90*	0.96*	1.01*	1.12	1.19
MEG	0.82*	0.84*	0.90	0.89	0.95	0.93*	0.94*	1.00*	1.15	1.01

Mk/Tt = Marker/treatment; Cr = chromium; CLA = conjugated linoleic acid; MEG = megalac; M1 = morning, day 1; M2; M3; M4; M5 = morning, day 5, A1 = afternoon, day 1; A2; A3; A4; A5 = afternoon, day 5 (mg kg^{-1} DM); Different small letters between treatments within each marker, for each fecal sampling time are different ($P < 0.01$); * Indicates difference between the fecal sampling time mean and the next one ($P < 0.01$) within each marker, for example M2 in comparison to M3.

($P = 0.01$) of day of collection (mg C_{36}= 40.02 + 20.5 × day – 3.02 × day^2). Fecal concentration ratio for the C_{35}:C_{36} alkane pair did not vary with treatments (P = 0.49), but there was a period of the day (morning= 0.89 vs. afternoon= 1.02 mg, $P = 0.01$) and a linear effect of collection day (afternoon mg C_{35}:C_{36}= 0.85 + 0.058 × Day; morning mg C_{35}:C_{36}= 0.72 + 0.058 × day, $P = 0.01$). Figures 2 and 3 show the variation of concentration of individual C_{35} and C_{36} n-alkanes and the C_{35}:C_{36} ratio in the feces throughout the day as proportions of the mean value, respectively.

It is possible that the difference between treatments is due to differences in saturation, which might have influenced the fiber digestion, altering the passage rate, and consequently modified the fecal concentrations of the markers. Mayes et al. (1986) found smaller fecal concentrations for the natural C_{27} and C_{29} n-alkanes coming from the diet when animals received synthetic C_{28} and C_{32} n-alkanes in pellets with a stearic and palmitic acids mix.

The period of the day effect on the fecal concentrations of C_{35}, C_{35}:C_{36} ratio, and chromium oxide concentration might have been caused by experimental handling. Grazing was more intense in the afternoon and at night, resulting in a larger ruminal pool at this time, which could have caused larger excretions and smaller fecal concentrations in the afternoon of the following day. So, differences in the rates of flow of both liquid and solid phases of digesta throughout the day could have resulted in the diurnal variation in fecal concentration of the markers. Dillon (1989)

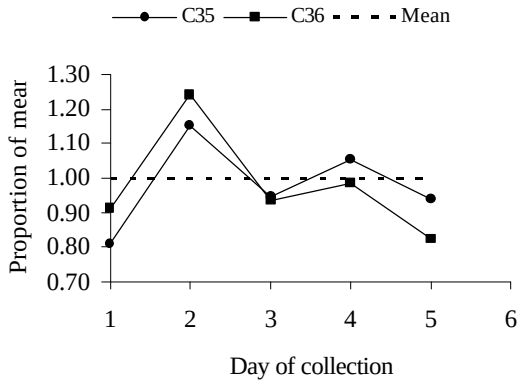

Figure 2 - Mean variation in the feces concentrations of C_{35} and C_{36} n-alkane concentration expressed as proportions of the mean concentration for each cow over five days.

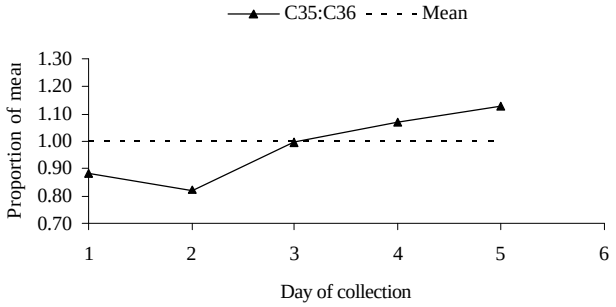

Figure 3 - Mean variation of the ratio in the feces of C_{35} odd-chain (diet) concentration to C_{36} even-chain (dosed) n-alkane concentration expressed as proportions of the mean concentration for each cow over five days.

worked with lactating cows and found day-to-day variation in fecal concentration of chromium oxide and diurnal and daily systematic variations in fecal concentration of the C_{35}:C_{36} ratio. The author concluded that feeding management may have contributed to these variations. The increasing linear effect on C_{35} fecal concentration during the days of collection might represent an increase in consumption of stem fraction of the herbage in relation to leaf since the leaf:stem ratio decreased during the duration of grazing. Moreover, n-alkanes appear in larger concentrations in the stem fraction. The linear increase in consumption of the C_{35} and the quadratic excretion of the C_{36} n-alkanes resulted in the linear increase in fecal concentration of the C_{35}:C_{36} ratio.

Individual variations of the n-alkanes did not interfere with forage intake estimation, but the variation in the fecal concentration ratio of the pair of n-alkanes used in the estimation process is very important (Dove & Mayes, 1991; Mayes & Dove, 2000).

Malossini et al. (1996) worked with lactating cows and compared the estimates of forage intake obtained with chromium oxide and with the C_{31}:C_{32} n-alkane ratio. The authors reported that the methods differed from each other, but there was a positive correlation between them (r = 0.62).

Further investigation is needed for the comparison of these two techniques used to measure forage intake, with special attention on (a) the incomplete fecal recovery of markers, (b) the rectal sampling procedure commonly used to obtain fecal samples may not provide representative estimates of fecal concentration of the markers given the variability related to day and time of the day the collection was carried out, and (c) the forage digestibility values might have been influenced in many ways and may not be accurate.

CONCLUSIONS

The estimates of forage intake obtained with the pair of n-alkanes, the natural C_{35} of the diet and synthetic C_{36} orally supplied by controlled-release capsules, and those obtained with the chromium oxide and *in vitro* digestibility procedure differed. The estimate of forage intake obtained with C_{35}:C_{36} n-alkane ratio was more precise than those obtained with chromium oxide and *in vitro* digestibility. The fecal concentrations of the indicators varied among periods of the day and days of sampling during fecal collection, indicating that handling of animals during the experiment may have influenced fecal concentration of n-alkanes and that rectal collection twice a day failed to provide reliable estimates of daily concentration of both markers in the feces.

ACKNOWLEDGMENTS

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support and to Church & Dwight for the donation of CLA-60 and Megalac.

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Received December 19, 2005
Accepted February 09, 2007