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N-ALKANES TO ESTIMATE VOLUNTARY FORAGE INTAKE OF CATTLE USING CONTROLLED-RELEASE CAPSULES

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ABSTRACT: N-alkanes have been used as internal markers in digestibility trials with ruminants and non-ruminants for more than 20 years. In this study, two trials were conducted under different feeding regimes to (i) evaluate the release rate of n-alkanes of controlled-release capsules in the rumen of rumen-cannulated steers either grazing or restrained in metabolic stalls and (ii) estimate voluntary forage intake of the same steers in metabolic stalls. Six rumen-cannulated Nelore steers were allocated to individual metabolic stalls and were fed diets with varying forage to concentrate ratios (80:20, 60:40, and 40:60; respectively). Corn silage was the only forage source. In the grazing trial, the same steers were evaluated under three feeding managements (*Brachiaria brizantha* cv. Marandu unsupplemented or supplemented with either 0.3% or 0.6% of live weight of a concentrate). The release rate of the n-alkanes (mg d^{-1}) was measured by multiplying the distance (mm d^{-1}) the capsule plunger travelled after 3, 7, 10, 13, and 17 d of rumen infusion to the n-alkanes concentration of capsule tablets (mg mm^{-1}). There was an effect of day of measurement ($P < 0.05$) on the release rate of animals restrained in metabolic stalls and grazing. However, no effect ($P > 0.05$) of feeding management or feeding management × day of measurement interaction was observed. Values averaged 6.9 and 14.8%, lower than proposed by the manufacturer for the C_{32}, when animals were restrained in metabolic stalls and at grazing, respectively. Similarly, the values of C_{36} were 15.9 and 23.1% lower for those animals in metabolic stalls and grazing, respectively. The average release rate of C_{32} into the rumen was 372 and 341 mg d^{-1} for animals restrained in metabolic stalls and grazing, respectively. There was no difference in the release rate between feeding regimes ($P > 0.05$). The regression of the pooled data indicated an average release rate of 345 mg d^{-1}. Estimated values of voluntary forage intake using the pair of C_{33}:C_{32} n-alkanes using the animals restrained in metabolic stalls was not different from those effectively measured ($P > 0.05$). These findings indicated that n-alkanes capsules can be used to estimate forage intake of stall-fed animals, but concentration of n-alkanes in capsules and the release rate has to be measured before dosing animals to accurately predict intake.
Key words: bovine, fecal recovery, hydrocarbons, markers, dry matter intake

N-ALCANOS PARA ESTIMAR O CONSUMO VOLUNTÁRIO DE FORRAGEM EM BOVINOS USANDO CÁPSULAS DE LIBERAÇÃO CONTROLADA

RESUMO: N-alcanos têm sido usados como marcadores internos em ensaios de digestibilidade com ruminantes e não-ruminantes por mais de 20 anos. Neste estudo dois ensaios foram conduzidos para avaliar: 1) a taxa de liberação de alcanos de cápsulas de liberação controlada inseridas no rúmen de novilhos fistulados em pastejo ou mantidos em gaiolas de metabolismo e; 2) o uso de n-alcanos para estimar o consumo voluntário de forragem dos novilhos mantidos em gaiolas de metabolismo. Seis novilhos Nelore, fistulados no rúmen foram colocados individualmente em gaiolas de metabolismo e alimentados com dietas variando a relação forragem:concentrado (80:20, 60:40 e 40:60, respectivamente). A silagem de milho foi a única fonte de forragem. No ensaio de pastejo, os animais foram avaliados sob três regimes de alimentação (Brachiaria brizantha cv. Marandu sem ou com suplementação com concentrado na proporção de 0,3 ou 0,6% do peso vivo, PV). A taxa de liberação de n-alcanos (mg d^{-1}) foi medida multiplicando-se a distância percorrida pelo êmbolo da cápsula (mm d^{-1}) após 3, 7, 10, 13 e 17

dias de inserção no rúmen, pela concentração de alcanos dos tabletes das cápsulas (mg mm^{-1}). Houve um efeito de dia ($P < 0,05$) sobre a taxa de liberação para os animais em gaiolas de metabolismo e em pastejo. Contudo, não houve efeito ($P > 0,05$) do regime de alimentação ou da interação regime da alimentação × dia da medida. Os valores foram 6,9 e 14,8% menores do que o proposto pelo fabricante para o C_{32} quando os animais foram mantidos em gaiolas de metabolismo e pastejo, respectivamente. Os valores para o C_{36} foram 15,9 e 23,1% menores para os animais em gaiolas de metabolismo e pastejo, respectivamente. A liberação média do C_{32} dentro do rúmen foi 372 e 341 mg d^{-1} para os animais em gaiolas de metabolismo e pastejo, respectivamente. Não houve diferença na taxa de liberação entre os animais em gaiolas de metabolismo e em pastejo e a regressão linear usando os dois conjuntos de dados resultou em uma taxa de liberação de 345 mg dia^{-1} do C_{32}. Os consumos estimados de forragem usando o par C_{33}:C_{32} de alcanos não diferiu dos consumos observados para os animais em gaiolas de metabolismo. Estes resultados indicam que as cápsulas de liberação controlada podem ser utilizadas para estimar o consumo de forragem em animais estabulados mas a taxa de liberação e a concentração de alcanos nas cápsulas devem ser medidas para gerar predições mais exatas.
Palavras-chave: bovinos, recuperação fecal, hidrocarbonetos, marcadores, consumo de matéria seca

INTRODUCTION

N-alkanes are saturated, aliphatic hydrocarbons with length varying from 21 to 37 carbon atoms. They are part of the cuticular wax of plant leaves and usually are part of the ether extract. The oral administration of n-alkanes has been used in digestibility trials with domestic and wild ruminants as well as monogastrics animals to measure feed digestibility (Mayes et al., 1984; Oliveira et al., 2000), feed intake (Mayes et al., 1986; Dove et al., 1989; Oliveira et al., 2007), and composition of the available herbage or diet (Dove, 1992; Dove & Mayes, 1991; Mayes & Dove, 2000; Dove & Moore, 1995).

N-alkanes can be supplied to animals in different forms. Mayes et al. (1986) fed pellets made of paper strip embedded with synthetic n-alkanes as external markers to estimate feed intake of sheep. Similarly, Dove et al. (1989) fed sheep with n-alkanes (C_{28} and C_{32}) in the form of gelatin capsules of powder cellulose, previously added with a known amount of n-alkane dissolved with n-hexane or n-heptane, to estimate forage intake. Vulich et al. (1991) developed a different method that consisted of mixing n-alkanes with solvents and powder cellulose, resulting in a homogenous suspension that, after being evaporated and dried, was inserted into gelatin capsules. Marais et al. (1996) developed another technique in which particles of *Pennisetum clandestinum* (Hochst.) were impregnated with n-alkanes suspended in a xanthan gum (0.4%) and infused into the rumen of sheep using either dose guns or disposable syringes.

In general, estimates of intake are based on the fecal ratio of odd chain n-alkane, a natural component of feeds, to homologous form of even chain (Mayes et al, 1986). Unfortunately, these techniques require daily infusions, which may cause stress and affect normal eating behavior of animals. Additionally, the discrete infusion of markers may lead to variations on diurnal excretions resulting in erroneous estimates of intake. The use of controlled-release devices of n-alkanes may minimize the measurement errors associated with diurnal and/or daily variations on fecal concentration of markers.

Therefore, the objectives of this study were to measure the release rate of synthetic n-alkanes from controlled-release capsules (MCM Alkanes code 60421 - Captec, NZ), and to evaluate the estimated forage intake of Nelore steers restrained in metabolic stalls.

MATERIAL AND METHODS

Trial 1

Experimental procedures

A trial was conducted with six rumen-cannulated Nelore steers (18 months old and average 380 kg of body live weight; LW) in metabolic stalls in Nova Odessa, SP, Brazil (22º7' S; 48º0' W; and altitude of 528 m). Two animals were randomly assigned to one of three diets that were formulated to meet energy and protein requirements in a maintenance level according to the Cornell Net Carbohydrate and Protein System (CNCPS) model (Fox et al., 2004). Humane animal care and handling procedures were followed.

The dietary ingredients and chemical composition of each diet are presented in Table 1. Pelleted soybean hulls and corn silage were mixed with concentrate immediately before feeding. The daily dry matter intake (DMI) was adjusted to be equivalent to 100 g of DM $kg^{-0.75}$ of shrunk LW. The three diets were formulated to contain 80:20, 60:40, and 40:60 of forage to concentrate ratio on a dry matter (DM) basis.

After a week of adaptation, animals were dosed with one n-alkane controlled-release capsule via the rumen cannula with n-dotriacontane (C_{32}) and n-hexatriacontane (C_{36}) (MCM Alkanes - Captec, NZ). The capsule was made of a 16 cm long and 3.8 cm diameter plastic cylinder, and it was closed at one end

Table 1 - Dietary ingredient proportions (forage:concentrate) and chemical composition of diets on dry matter basis.

Ingredients (%)	80:20	60:40	40:60
Corn silage	79.6	58.5	39.4
Ground corn	4.2	9.4	13.5
Ground sorghum	2.8	8.0	13.5
Corn gluten feed	2.1	4.6	6.0
Soybean meal	3.5	3.6	5.4
Cottonseed meal	2.2	3.6	4.0
Urea	1.3	1.0	0.5
Soybean hulls	2.8	9.8	16.2
Mineral mix*	1.5	1.5	1.5
Estimated chemical composition, %			
DM	34.0	40.0	49.0
TDN	71.0	73.0	77.0
CP	14.2	14.8	14.9

*Calcium sulfate (28.1%), limestone (46.8%), sodium chloride (18.7%), manganese sulfate (0.34%), dicalcium phosphate (5.2%), cupper sulfate (0.08%), zinc sulfate (0.12%), cobalt sulfate (0.004%), sodium selenite (0.004%). Dry matter (DM); Total Digestible Nutrient (TDN); Crude Protein (CP).

with a wire spring attached to a plunger. The plunger applied pressure on five tablets containing the n-alkanes in their matrix. Once in the rumen, tablets were maintained in close contact with the rumen liquor at the open end of the plastic cylinder. Capsules were equipped with a transversal wing (18 cm length) located at the closed end of the plastic cylinder to avoid regurgitation. A long string was attached to the wing and to the ruminal cannula to provide easy recovery of capsules. The string allowed capsules to move freely in the rumen.

On days 3, 7, 10, 13 and 17 after dosing, capsules were removed and quickly washed with sprinkled warm water. The distance traveled by the plunger was measured with a caliper and capsules were then reinserted into the rumen. Measurements were taken on four diametrically opposite points alongside the cylinder. Two capsules of the same batch were opened to measure the thickness and weight of the tablets. N-alkanes concentration was determined on two tablets and daily DMI measurements and fecal collections were performed during five consecutive days from day 8 after the administration of the capsules.

N-alkanes extraction

Samples of corn silage, concentrates, and feces were dried in a forced-draught oven at 60ºC for 48 h and then finely ground to pass a 1-mm sieve. Extraction and determination of n-alkanes in samples were made in duplicates as described by Oliveira (2004).

Gas chromatography analysis

Alkanes determinations were made using a capillary column CP-Sil 8CB (Crompack) with 30 m $\times$ 0.25 mm $\times$ 0.1 μm film thickness in a gas chromatography (ThermoQuest-Finnigam - USA) fitted with a flame ionization detector (FID). The injector temperature was 280ºC and the detector's 310ºC. Column temperature was programmed to start at 200ºC raising 6ºC min^{-1} and to stop at 300ºC for 15 min. The carrier gas was Helium with a linear speed of 32 cm s^{-1}, and flame gases were N, H and O_2 at rates of 30, 35 and 430 mL min^{-1}, respectively. The injection mode was "split" with a ratio of 20:1 and 2 μL of a sample was automatically injected.

The area of each n-alkane chromatographic peak was calculated using ChromQuest version 2.53 (Thermo Electron Corporation). The identification of each n-alkane was made via correlation and linear regression analyses between the number of carbons in each chain and the logarithm of the retention time. The areas of each peak under the curve were converted into quantities of n-alkanes based on internal standard reference (n-tetratriacontane) according to McNair & Bonnelli (1968). The calculation of the response factors of each n-alkane was made as suggested by Untz & Tranchant (1982). The concentration of each n-alkane (kg kg^{-1} of DM) of feed, feces, and tablets were calculated. The two tablets that were extracted for calibration purposes had an average of 1.54 g (SD = 63 mg) of C_{32} and 1.39 g (SD = 11.9 mg) of C_{36}.

Calculations

The average thickness of alkane tablets was 10.3 mm (SD = 0.06) with an average of 149.5 and 135 mg mm^{-1} of C_{32} and C_{36}, respectively. Controlled

release capsules had a mean length (distance from the closed end to the plunger) of 86.85 mm (SD = 0.05) (ID). The partial mean distance traveled (DP_{3-17}) was calculated as the difference between the initial capsule length (ID) and the distance traveled by the plunger at every measurement day (DISTA). These values were then divided by the number of days for each measurement event (3, 7, 10, 13 and 17 days after dosing).

The final values of fecal recovery (FRn) of n-alkanes (n) C_{31}, C_{32}, C_{33}, C_{35} e C_{36} were calculated based on actual intake (Int), fecal dry matter output (FP, kg), and n-alkane concentrations (mg kg^{-1} of DM) in the diets (Di) and feces (Fe) according to Equation 1.

$$FRn = [(FP \times Fe)/(Int \times Di)] \times 100 \quad (1)$$

The FDMI was estimated using the pair C_{33} (odd chained, natural in the diet) and C_{32} (synthetic, even chained, from the controlled release capsule) according to Mayes et al. (1986), as shown in Equation 2.

$$FDMI = \{F_o/F_e \times [D_e + (C_c \times C_e)] - (C_c \times C_o)\} / [Fo_o - (F_o/F_e) \times Fo_e] \quad (2)$$

where: FDMI = forage dry matter intake (kg of DM d^{-1}); D_e = even chain n-alkane dosed daily (mg d^{-1}); C_c = concentrate intake (kg of DM d^{-1}); C_e = even chain n-alkane naturally occurring in concentrate (mg kg^{-1} of DM); C_o = odd chain n-alkane naturally occurring in concentrate (mg kg^{-1} of DM); Fo_o = odd chain n-alkane naturally occurring in forage (mg kg^{-1} of DM); F_o = odd chain n-alkane fecal concentration (mg kg^{-1} of DM); F_e = even chain n-alkane fecal concentration (mg kg^{-1} of DM); Fo_e = even chain n-alkane naturally occurring in forage (mg kg^{-1} of DM).

Trial 2

Experimental procedures

Six rumen-fistulated Nelore steers (four of the six ones used in trial 1), averaging 470 kg of LW (SD = 36.8 kg) and 24 months of age, were used to evaluate the release rates of the same n-alkanes used in trial 1, but under grazing conditions. Two animals per treatment were allocated to three 1-ha paddocks of *B. brizantha* cv. Marandu along with other non-fistulated animals to maintain the herbage allowance. Experimental treatments investigated were: (i) only grazing with no concentrate supplementation or supplemented with either (ii) 0.3 % or (iii) 0.6 % of LW of a concentrate supplement. The composition of the supplements used is presented in Table 2. Supplements were prepared in order to feed the same amount of kg of protein kg^{-1} of LW to the non-fistulated animals that were weighing 350 kg. Procedures related to dosing of controlled release capsules and n-alkanes determinations were similar to those described for Trial 1.

Statistical analysis

The effect of trials (metabolic stalls or grazing) on daily distance traveled by the capsule plunger and the release rates of n-alkanes were tested with the PROC MIXED procedure of SAS (2000). Diet and its interactions with evaluation days as well as the effect of days after dosing on capsule release rate were analyzed. Release rate of n-alkanes was estimated as the slope of the linear regression between the distance traveled of the capsule plunger (DISTA) and days after dosing using the procedure PROC REG of SAS (2000). Data points outside of the range -2 to 2 of the studentized residue were considered outliers and removed from the database.

To verify if equations generated from the metabolic stall and grazing trial data could be pooled, intercept and slope values were compared according to the method described by Neter & Wasserman (1996) using a 95% confidence interval. Additionally, the comparison of feed intake estimates using n-al-

Table 2 - Ingredient proportions and chemical composition of concentrate, concentrate - intake and mean forage mass.

Ingredients	0.3% LW	0.6% LW
Citrus pulp	15.3	52.4
Corn gluten feed	75.6	42.4
Urea	4.9	2.9
Mineral mix*	4.2	2.3
Estimated chemical composition, %		
DM	88.1	88.4
CP	40.8	23.8
NDF	41.7	34.9
Concentrate intake (kg of DM)	1.4	2.8
Forage Mass (kg ha^{-1})	5998	5998

*Same as trial 1; Neutral Detergent Fiber (NDF).

kanes and the actual measured values was made by fitting a linear regression between those two variables using PROC REG of SAS (2000). The mean bias associated with the technique was tested using Student-*t* test for paired values.

RESULTS AND DISCUSSION

N-alkanes release rate of steers fed under metabolic stall or grazing conditions

The data of daily distance traveled by the capsule plunger and the computed release rate of n-alkanes (Tables 3 and 4, respectively) were analyzed individually within trials. Data were collected up to 17 days after inclusion of the capsules into the rumen for both trials because adequate measurements of tablets thickness were not possible thereafter. Measurements of fecal concentrations taken before day 13 were used to estimate intake (7 days for rumen stabilization after capsule dosing and 5 consecutive days of fecal collections).

Data of one animal in trial 1 between day 10 and 13 were considered outliers and removed from the regression analysis. There was no diet effect or diet × day of measurement interaction ($P > 0.05$) on either the distance traveled by the plunger or the daily release rate of n-alkanes. Under grazing conditions, there was no effect of diet or diet × day of measure-

Table 3 - Plunger traveled distance and n-alkane releasing rates during days 3 and 17 after capsule infusion in the rumen of animals in the metabolic stall trial.

Diets	3 days	7 days	10 days	13 days	17 days
			Distance		
			mm		
20:80	7.27	17.50	25.69	32.53	50.40
40:60	8.00	17.34	23.55	31.90	50.40
60:40	7.67	18.06	25.79	32.79	47.80
Means	7.65	17.63	25.89	33.28	49.53
CV (%)	6.4	3.2	6.9	6.6	2.7
			n-alkane releasing rates		
			mm day^{1}		
20:80	2.42	2.50	2.57	2.50	2.96
40:60	2.66	2.48	2.62	2.65	2.96
60:40	2.56	2.58	2.58	2.52	2.81
Means	2.55	2.52	2.59	2.56	2.91
CV (%)	6.3	3.2	6.9	6.6	2.7

Table 4 - Plunger traveled distance and n-alkane releasing rates of capsules infused in the rumen of grazing animals.

Diets	3 days	7 days	10 days	13 days	17 days
			Distance		
			mm		
Brachiaria	8.35	17.10	24.50	31.08	47.40
Brach. + 0.3% LW	7.28	16.44	23.53	30.19	49.75
Brach. + 0.6% LW	7.74	15.82	23.52	30.11	49.05
Mean	8.02	16.76	24.12	30.86	49.08
CV (%)	11.7	7.1	4.5	4.4	1.9
			n-alkane releasing rates		
			mm day^{1}		
Brachiaria	3.01	2.57	2.53	2.48	2.85
Brach. + 0.3% LW	2.42	2.35	2.35	2.32	2.92
Brach. + 0.6% LW	2.58	2.26	2.35	2.31	2.88
Mean	2.67	2.39	2.41	2.37	2.89
CV (%)	11.6	7.1	4.6	4.2	1.7

ment interaction ($P > 0.05$) on the distance traveled by the plunger. N-alkanes release rates were not affected either by diet or diet × day of measurement interaction ($P > 0.05$).

Trial 1 (metabolic stalls) The linear regression presented in Equation 3 was highly significant ($P < 0.0001$) with an $r^2 = 0.99$ and MSE of 0.85. The coefficient of variation (4.2%) was similar to that reported by Dove et al. (2002) with sheep.

$$DISTA = 0.19 + 2.49 \times DAY \quad (3)$$

where DISTA is the distance traveled by the plunger, mm.

The product of the regression coefficient (2.49 mm d^{-1}) by the marker concentration (mg mm^{-1}) indicated a mean release rate of 372 mg d^{-1} of C_{32} and 336 mg day^{-1} of C_{36}. These values were 7 and 16% lower than those proposed by the manufacturer (400 mg d^{-1}). This is in agreement with Ferreira et al. (2004), who reported values that were 14% less than the manufacturer's release rate for the same n-alkanes. The feed intake simulation with the labeled release rate of C_{32} provided by the manufacturer indicated values 9.2% (± 1.5%) lower than those determined using the actual release rate values. Ferreira et al. (2004) also reported a difference of 12.2% for feed intake estimated using the manufacturer's release rate or the calculated release rate with C_{32} as an external marker supplied by the same type of capsule.

Trial 2 (grazing condition): The linear regression represented by Equation 4 was highly significant ($P < 0.0001$) with a r^2 of 0.99, MSE of 0.53, and a coefficient of variation of 2.7%, indicating that days after dosage explained almost all variations in the n-alkane release rate. The quantities released were 341 and 308 mg day^{-1} for C_{32} and $C_{36,}$ respectively.

$$DISTA = 0.69 + 2.28 \times DAY \quad (4)$$

where DISTA is the distance traveled by the plunger, mm.

The coefficient of variation in metabolic stalls (trial 1) was higher than that observed in the grazing trial. That could be explained by a constant feed intake in grazing conditions, even with the supplemented animals, since animals in the metabolic stall trial were fed twice daily. Other possible explanations are the physical structure, rumen motility, rumen volume, and dilution rate of liquid and solid fractions.

Since coefficients in equations for trial 1 and 2 were not different ($P > 0.05$), data were pooled in one single data set. The combined equation for trials 1 and 2 ($DISTA = 0.76 + 2.31 \times DAY$; $r^2 = 0.99$, CV= 3.7%, and MSE = 0.69, $P < 0.0001$) estimated a release rate of 345 and 312 mg day^{-1} of C_{32} and C_{36}, respectively.

The estimate end point of capsules (release rate equals zero) was 20 and 22 days for the metabolic stall and grazing trials, respectively. These values are within the useful period of 17 to 23 days suggested by the manufacturer.

The results indicated no difference among diets (grazing and metabolic trials) on the capsule release rate. The daily release of marker was constant after dosing, indicating that n-alkanes could be used with diets of varying ratios of forage to concentrate.

Under grazing conditions, the diet chemical composition may vary due to selective grazing (leaves vs stem, live vs dead material, or immature vs mature plant tissues). This chemical modification can alter the dynamics of rumen fermentation (e.g. degradation and passage rates). Because natural n-alkanes are more associated with the solid phase and synthetic n-alkanes with the liquid phase (Mayes et al., 1988), these factors could interfere with forage intake predictions. Additionally, the physical structure of the diet could vary according to the herbage allowance and selective grazing by individual animals, which could result in changes in the leaf:stem ratio of the ingested herbage, affecting the predictions of forage intake. However, in the present study, the coefficient of variation and the error of the regression were low (2.7% and 0.53 mm, respectively), suggesting that these factors were not a problem. The range of diet types used in this experiment is similar to those used in practical conditions for both feedlot and grazing production systems, with or without supplementation, indicating that n-alkanes could be successfully used under field conditions.

Similar to our findings, Ferreira et al. (2004) reported a constant release rate of n-alkane capsules used to predict forage intake in two breeds of cattle. They determined a concentration of 141.7 mg of C_{32} mm^{-1}, which is only 5.5% less than value estimated in this experiment (149.5 mg of C_{32} mm^{-1}).

Dove et al. (2002) compared a controlled-release device (CRD) of C_{28} and C_{32} with daily administrations of paper pellets impregnated with C_{36} in sheep and reported that CRD had better stability of release rates than the impregnated paper pellets. They also found no evidence of a curvilinear pattern of alkanes release rates using a CRD over time, which is in agreement with the findings of our experiment.

Genro (1999) determined the n-alkane release rate using fecal concentrations during days 7 and 15 after the administration of capsules (YC, Captec - NZ) into the rumen of rumen-cannulated steers fed Brachiaria hay (*Brachiaria* sp.) plus concentrate

(0.5%BW) in metabolic stalls. The author reported a variation of the release rate of both n-alkanes (C_{32} and C_{36}). Furthermore, fecal concentration of alkanes decreased from day 12 to 14 after administration of the capsules. However, Genro (1999) did not mention the natural occurrence of C_{32} and/or C_{36} in the hay, which could have influenced the alkane profile of the fecal samples.

Some factors could have influenced the release rate of n-alkanes in this study, such as: (i) the tension of the wire spring in the capsules, since it can be very difficult to maintain the initial tension as the solubilization of the tablets proceeds, (ii) the tablet material which is impregnated with markers, and (iii) interactions that may occur in the ruminal environment like pH and volatile fatty acid (VFA) production and concentration.

Prediction of dry matter intake

N-alkanes with chain length from 22 to 36 atoms of carbon were identified in the corn silage and in all three concentrates offered to animals. The most abundant alkanes in the corn silage were in a descending order: C_{31}, C_{29}, C_{27} and C_{33}. This is in agreement with the information that odd chained alkanes are predominant in forage samples (Dove & Mayes, 1991; Oliveira et al., 2000). The N-alkanes concentration in concentrate feeds where lower than that in corn silage, and the most abundant were C_{27}, C_{29} and C_{31} (C_{27} and C_{29} are not shown). The n-alkanes with chain length of C_{31} to C_{36} carbons are shown in Table 5, except C_{34} that was used as an internal standard.

A linear relation was observed ($P < 0.05$) between estimated (mean of 5.0 kg of DM d^{-1}, CV= 28.0%) and observed (mean of 4.9 kg of DM d^{-1}, CV= 28.5%) values of intake. The coefficient of determination (r^2) was 66% and the coefficient of variation was 18.7%, resulting in the following equation: ObsIntake = 0.86 + 0.81 × EstIntC_{33}:C_{32}. The fecal concentrations of C_{33} in two animals were 64 and 22% higher than the mean excretion by the other animals (38.3 ± 2.2%). Figure 1 depicts the relationship of these measurements. A paired *t*-test was performed on the estimated and observed intake values to evaluate the suitability and overall trend of the technique. The mean difference (0.1 kg) was not significant ($P > 0.05$).

The slope of the regression was not different from unity ($P > 0.05$), indicating a high reliability of the technique. This suggests that variations in the estimated values of intake were the same as those in the observed values, but the number of animals may be a limiting factor in this analysis. Future work should consider using animals to repeat measurements over longer periods of time.

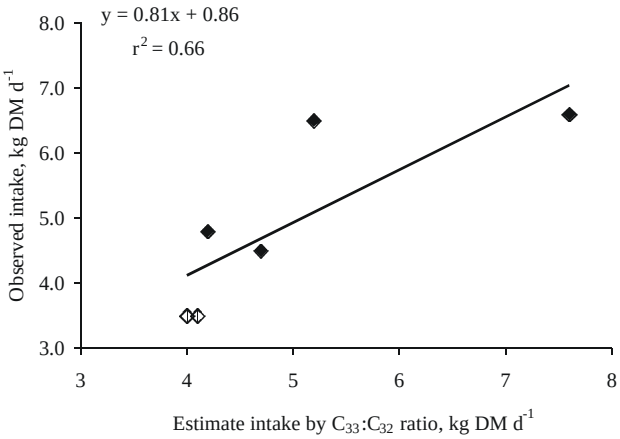

Figure 1 - Relationship between observed and estimated intake with the n-alkane pair C_{33}:C_{32}.

Fluctuations in the absolute values of the fecal concentrations of markers may occur as a consequence of differences in intake and digestion of the diet. This last one is the best option for our case, because C_{33} naturally occurs in the diets and the concentrations of C_{32} were more homogeneous than the C_{33} (means = 153 mg ± 3.3 vs. 44 mg ± 10.0, respectively). The affinity of synthetic C_{32} n-alkane with the liquid phase of the rumen and the association of the dietary C_{33} n-alkane with the solid phase could also contribute to these differences (Mayes et al., 1988). In our experiment corn silage and several concentrate feeds were used. This may have resulted in different passage rates of digesta, altering the kinetic relation of the marker released by the capsule and that from the feed, which in turn could have resulted in poor estimates of intake. Although the mean intake estimates were good, these limitations have to be taken into account when predicting DMI of grazing animals. There is also a possibility of an analytical bias, especially because of the low concentration of n-alkanes in the diets, including C_{33} that was used as an internal marker in the calculations (Laredo et al., 1991; Dove & Mayes, 1991). Other important consideration that might have influenced feed intake estimates are the fecal recovery (Table 5).

The n-alkane technique works with the underlying assumption that similar fecal recoveries of homologous n-alkanes used as internal (odd chain from dietary sources) and external (dosed even chain) markers are achieved. There was a high discrepancy in the fecal recovery of both markers used in the calculations for three animals, likely causing the differences between estimated and observed intake values.

There were some values of fecal recovery higher than 100%. These values probably occurred because of the low concentrations of natural n-alkanes, as C_{35}, increasing the analytical error or even by an

Table 5 - N-alkanes concentration of feed ingredients, feces (mg kg^{-1} DM) and fecal recoveries.

Ingredients	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}
Corn silage	45	12	25	6	5
80:20[a]	5	4	ND	ND	2
60:40[a]	11	8	5	2	2
40:60[a]	5	3	2	ND	1
Soybean hulls	20	7	5	ND	2
Mean	87	153	44	11	112
CV (%)	23	2	23	28	10
Animals (n = 6)	n-alkane fecal recoveries (%)				
Mean*	100	100	95	115	89
CV*	19	10	17	25	12

[a]Concentrate of different treatments. [ND] n-alkane not detected. [*]Means and coefficient of variations of fecal concentrations.

incomplete extraction of alkanes in feed samples. There are some reports of fecal recoveries higher than 100% (Berry et al., 2000, with C_{32} = 117%, C_{33} = 105% and C_{36} = 118%; Gedir & Hudson, 2000, with C_{36} = 113%; O'Keefe & McMeniman, 1998, with C_{35} = 177%). O'Keefe & McMeniman (1998) suggested that the low concentrations of n-alkanes in the diet may lead to anomalous and inaccurate fecal recoveries. The daily variations of intake and fecal production may also contribute to misinterpretation. When feces are grouped, so are the "errors" associated with such variations. Therefore, it may be noticed that for one animal, for example, fecal recovery was over 100% for the pair C_{33} and C_{32} used in the estimates of intake. However, they were practically the same (C_{32} = 114 and C_{33} = 116%), indicating that even with values of recovery over 100%, the bias was common and similar to both alkanes used as markers, a key feature to estimates of intake using alkanes. In other animals there was no much difference, and the mean fecal recovery was 100 and 95% for C_{32} and C_{33}, respectively.

CONCLUSIONS

N-alkanes can be used to provide reliable estimates of herbage intake by animals fed diets with variable ratios of forage to concentrate feeds. Release rate values were constant, particularly during the first 13 days after dosing, an important feature in estimating intake. However, actual release rates and n-alkanes tablets concentration have to be checked because values provided by the manufacturer may be different. This indicates the need for a pre-experiment or an additional trial to test the release rate of capsules.

ACKNOWLEDGEMENTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). The first author is grateful to Dr. Hugh Dove, CSIRO, Australia, for his advices on the n-alkanes technique.

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Received September 09, 2006
Accepted November 14, 2007