FECAL N-ALKANES VARIATION IN LACTATING DAIRY COWS GRAZING A TROPICAL PASTURE (*Cynodon nlemfüensis* VANDERYST VAR. NLEMFÜENSIS)

[VARIACIÓN FECAL EN N-ALCANOS EN VACAS LACTANTES PASTOREANDO EN PRADERA TROPICAL (*Cynodon nlemfüensis* VANDERYST VAR. NLEMFÜENSIS)]

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SUMMARY

The objective of this study was to measure fecal n-alkane concentration variation and herbage intake in grazing lactating dairy cows fed with two sources of fat: conjugated linoleic acid (CLA) or Megalac (Control). Cows were dosed with n-alkanes using a controlled-release capsule. There was no difference in herbage dry matter intake between CLA and Control. In the first period, there were no differences in fecal concentration of both natural and synthetic n-alkanes from the capsules. For the second period, there was a difference in fecal concentration for time of collection for all natural, dosed, and alkanes ratios. In the third period, there was a difference for time of collection for natural n-alkanes and their respective ratios, with higher excretion values recorded in the afternoon. In addition, there was an effect of day of collection for all n-alkanes and ratios, with higher excretion values recorded on days 2 and 4. Overall, our results highlight the importance of strict control of grazing management and sward structure in studies where external markers are used. Monitoring herbage, sward and animal managements might have an influence on n-alkanes excretion, causing a change in the ratio of their fecal concentration. These modifications may result in erroneous estimates of intake.

Key words: Diet Composition; Grazing Animals; Intake; Markers)

RESUMEN

El objetivo de este estudio fue determinar la variación en la concentración fecal de n-alcanos y el consumo de forraje en vacas lactantes en pastoreo y suplementadas con dos fuentes de grasa: Ácido linoleico conjugado (ALC) o Megalac (Control). Los n-alcanos fueron administrados utilizando una capsula de liberación controlada. No hubo diferencias en el consumo de materia seca de forraje entre las vacas suplementadas con ALC o con Control. Durante el primer periodo, no hubo diferencias en la concentraciones fecales de n-alcanos naturales, de los alcanos sintéticos. Durante el segundo periodo hubo un efecto del momento de colecta sobre en la concentración fecal de los n-alcanos naturales, sintéticos y sobre sus relaciones. En el tercer periodo, hubo un efecto del momento de colecta sobre la concentración de n-alcanos naturales y sobre sus respectivas relaciones. Adicionalmente, hubo un efecto del día de la colecta sobre todos los n-alcanos y las relaciones, siendo los valores más altos aquellos registrados en los días 2 y 4. En general, nuestros resultados resaltan la importancia del control estricto del manejo de pastoreo y de la estructura de la pradera en estudios que utilizan marcadores externos. El monitoreo del forraje y su estructura y el manejo animal pueden tener un efecto sobre la excreción de n-alcanos. Estas modificaciones pueden dar lugar a errores en la estimación del consumo.

Palabras clave: composición de la dieta; animales en pastoreo; consumo; marcadores

INTRODUCTION

Grazing animals modify their diet composition by selecting different plant species or plant parts (e.g., leaves and stem), increasing the difficulty in the determination of herbage intake and composition of the consumed herbage, hence interfering with the accuracy and planning of feeding programs. The most common method for estimating herbage intake in grazing ruminants is the calculation of fecal output (FO) using an external marker (e.g., chromium oxide) and herbage dry matter (DM) digestibility using *in vitro* procedures (Mayes and Dove, 2000). The assumptions of this method are that the external marker is fully recovered in the feces and a single herbage DM digestibility value is applicable to all animals involved. Additionally, the discrete infusion of markers has shown variations on diurnal and/or daily excretions on fecal concentration of markers resulting in erroneous estimates of intake. The use of natural to dosed n-alkanes ratio has been proposed as an alternative method in an attempt to overcome these limitations (Mayes et al., 1986) and it has been used to estimate herbage DM intake (Dove and Mayes, 1991; Oliveira et al., 2007; Oliveira et al., 2008). However, some studies with stall fed animals (Dillon, 1989) have shown that the n-alkanes technique may also present some problems such as daily and/or diurnal fecal concentration variations affecting estimates of herbage intake (Aguiar et al., 2013). In spite of that, data on fecal concentration variation in grazing animals on tropical pastures is scarce.

The main objectives of this study were to measure fecal n-alkane concentration variation and dry matter intake estimations in lactating dairy cows grazing stargrass (*Cynodon nlemfüensis* Vanderyst var. nlemfüensis), supplemented with two sources of fat.

MATERIALS AND METHODS

Animals, treatments, and experimental design

The experiment was designed to test the fecal n-alkanes variation and DM intake in lactating dairy cows fed with two sources of supplemental fat. All procedures regarding treatments, management and feeding animals, concentrate feeds, milk measures and analysis were described by Medeiros et al. (2010) and will not be addressed here. Briefly, the experiment was carried out in Valença, RJ, Brazil (43º42' W, 22º21' S) using thirty lactating Holstein x Gir crossbred cows receiving one of the following two treatments: 150 g/d of Megalac (Dwight & Church, salts of calcium from palm oil = Control) or 150 g/d of CLA-60 (Dwight & Church, salts of calcium of a mixture with 60% of isomers of CLA containing 24% of cis-9, trans-11, 35% of trans-10, cis-12, 15% of cis-8, trans-10, 17% trans-11, trans-13 and 9% of others).

All cows were rotationally grazed on a stargrass (*Cynodon nlemfüensis* Vanderyst var. nlemfüensis) pasture managed according to a 28-day grazing cycle (i.e., 2 days of pasture occupation, 26 days of pasture resting period) on fourteen 0.5 ha-paddocks. The experiment was divided into three 28-day evaluation periods during which estimates of herbage DMI were performed using the double n-alkanes technique (Mayes et al., 1986).

Herbage Sampling and Analysis

Herbage sampling (12 per period) was carried out on paddocks in which animals were grazing. The herbage sampling was performed during the same period in which fecal sample collection was performed to calculate DMI.

Herbage samples from each paddock were collected twice daily (morning and afternoon) using the hand-plucking procedure (Prates, 1974) during the two days of occupation. Samples were harvested during a 30-minute period each day to allow a larger proportion of the paddock area to be covered. Herbage samples were hand-separated into leaf (leaf lamina/blade), stem (leaf sheath + stem), and dead material. Herbage samples were dried at 65 ºC in a forced-draught oven until constant weight. The results were used to calculate the percentage of each morphological component in sward herbage mass.

Concurrently, pre- and post-grazing herbage mass were measured by cutting the herbage at 10 cm from ground level using ten 0.25 m^2 metallic frames randomly positioned in each paddock used by animals at the time of sampling. Similarly, samples were dried in forced-draught oven as described above. Before grazing, four areas were randomly selected in each paddock to measure morphological composition of sward herbage mass above the post-grazing height (30 cm). Samples received the same treatment as herbage samples used to estimate morphological composition of sward herbage mass.

The hand-plucked herbage whole sample and components plus concentrate feed were analyzed for DM (method #934.01), ash (method #942.05), ether extract (EE, method #920.29) and crude protein (CP, method #988.05)) content according to AOAC (1997). Determinations of DM and ash were also performed on fecal samples. Herbage samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent insoluble protein (NDFIP), acid detergent insoluble protein (ADFIP), and sulfuric lignin according to Van Soest et al. (1991). Because the detergent system is not

accurate for animal byproducts (i.e. fish meal), fractions of NDF, ADF, NDFIP, ADFIP, and lignin of the concentrate were estimated based on individual composition of each ingredient and their corresponding proportion in the concentrate mixture. The rations were formulated using the Large Ruminant Nutrition System (LRNS; http://nutritionmodels.tamu.edu/lrns.html). The herbage carbohydrate and protein fractional degradation rates (%/h) were assigned to match those reported by Tedeschi et al. (2002). The chemical composition of the feeds is shown in Table 1. Each animal was individually characterized and data used to compute animal metabolizable energy requirement using the LRNS model. The results of feed analyses were used to estimate metabolizable energy content of feedstuffs used.

Milking management

Cows were milked at 0500 h and individually fed half of their daily concentrate allowance after milking. At 1130 h, animals were removed from the pasture and housed in a sheltered barn until the afternoon milking at 0230 h. After milking, cows were fed the second half of their daily concentrate allowance and at 0400 h they were returned to the pasture.

Herbage intake and n-alkane analysis

Cows were dosed with a MCM Alkanes code 60421 – Captec, NZ controlled-release capsule containing 8 g of n-hexatriacontane (C_{36}) and 8 g of n-dotriacontane (C_{32}) to estimate herbage DMI, in each of the 28-day periods of measurement. The release rate used for the C_{32} marker was 345 mg/d as previously determined by Oliveira et al. (2008). Eight days after dosing the n-alkane capsules, fecal samples were directly taken from the rectum of the animals immediately after the morning (0500 h) and afternoon (0230 h) milking for five consecutive days.

For the first and second periods of evaluation, samples from each cow from five days of collection were pooled for each collection time of the day (morning and afternoon). For the third period, for each animal, samples were analyzed separately for each different collection time and day. All fecal samples were first stored at -20 ºC and then dried at 65 ºC in a forced-draught oven until constant weight. Dried fecal samples were ground to pass a 1 mm mesh sieve and stored in plastic containers for subsequent n-alkane analyses.

Table 1. Chemical composition and LRNS estimated metabolizable energy for herbage and concentrate

Sample	n[1]	CP	EE	NDF	NDFIP	ADF	ADFIP	LIGN	ASH	ME
Period 1	12									
Herbage		14.8	2.3	64.8	46.8	30.9	14.9	4.6	9.6	1.95
Leaf		19.4	2.9	66.3	55.6	29.7	12.0	4.8	9.3	2.02
Stem		11.3	0.9	71.3	45.4	34.7	22.5	6.3	9.5	1.64
Concentrate		24.7	5.6	11*	6.6*	3.9*	2.5*	3.3*	16.1	2.99
Period 2	12									
Herbage		14.2	1.7	71.1	42.8	34.3	17.4	2.8	9.6	1.91
Leaf		17.0	2.4	75.1	72.1	32.9	15.8	2.3	8.1	2.11
Stem		11.8	1.3	81.8	65.8	40.7	15.7	4.5	10.1	1.66
Concentrate		25.5	5.6	11*	6.6*	3.9*	2.5*	3.3*	16.1	2.79
Period 3	12									
Herbage		14.3	1.9	66.9	41.2	27.5	12.4	5.2	9.9	1.96
Leaf		16.9	2.7	67.4	60.8	28.7	13.0	3.4	9.0	2.26
Stem		11.8	1.3	72.0	41.8	36.1	13.4	5.0	10.1	1.84
Concentrate		25.5	5.6	11*	6.6*	3.9*	2.5*	3.3*	16.1	2.79

* Estimated using individual ingredient composition and their percentage of inclusion in the concentrate. Where: CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; NDFIP = insoluble protein bound to the NDF (%CP); ADF = acid detergent fiber; ADFIP = insoluble protein bound to the ADF (% CP); LIGN = lignin; ASH = ash; ME = metabolizable energy (Mcal/kg DM estimated by LRNS); (DM basis, %).
[1] Sample of three paddocks.

The n-alkane concentrations in herbage and fecal samples, tablets of controlled-release capsules, and concentrate feeds used were determined in duplicates using the method described by Dillon and Stakelum (1990) and modified by Oliveira (2004). Basically, modifications corresponded to: 1) n-heptane was used as solvent; 2) during the saponification procedure a bath-water was used instead of a dry-block heater and; 3) plastic 5 mL syringes were used to replace the disposable columns (Oliveira and Tedeschi, 2010). The n-alkane determinations and quantifications were made as described by Oliveira et al. (2008). Two tablets from two controlled-release capsules were extracted for calibration purposes and presented an average of 1540 mg (±63 mg) of C_{32} and 1390 mg (±12 mg) of C_{36}. Herbage DMI was computed as proposed by Mayes et al. (1986) using the C_{33}:C_{32} and C_{31}:C_{32} n-alkane ratios.

Statistical analysis

For the first and second periods of study, the variations on fecal concentrations of C_{31}, C_{32}, C_{33}, C_{35} and C_{31}:C_{32} and C_{33}:C_{32} n-alkane ratios in relation to time of sample collection (morning and afternoon) were analyzed considering as sources of variation treatments and time of collection as well as their interactions. For the third period of measurement, analysis of variation was performed as described, but also separately for days of collection (day 1 until day 5). In this case, sources of variation corresponded to treatments, time of collection and days of collection, including their interactions. All analyses were performed using PROC MIXED of SAS (SAS Inst. Inc., 2000) and when the interaction was not significant, it was excluded from the model. The DMI was analyzed and means estimated using LSMEANS. Comparisons, when appropriate, were performed using the F test and 5% probability.

RESULTS

The effects of CLA on the milk yield, composition, fatty acid profile, blood parameters, and reproduction were reported by Medeiros et al. (2010) and will not be addressed. The average pre-grazing herbage mass for the first, second and third periods of study were 3810 (±230), 4290 (±600) and 1800 (±170) kg/ha, respectively. The corresponding post-grazing herbage mass were 1730 (±340), 1606 (±128) and 1310 (±290) kg/ha. The proportion of leaf, stem, and dead material in the pre-grazing herbage mass was 47.9% (±3.1), 51.5% (±3.1), 0.6% (±0.06) for the first period, 32.8% (±7.2), 52.4% (±11.4), 14.9% (±4.8) for the second period and 50.8% (±14.0), 44.1% (±9.3), 5.0% (±6.7) for the third period, respectively.

n-Alkane profile

The n-alkane profile for the whole herbage, its morphological components, and the concentrate are shown in Table 2 (data for even-chained alkanes are not shown). The n-alkanes with carbon chain length varying from C_{22} to C_{35} were quantified. The greatest concentrations were recorded for C_{33}, C_{31} and C_{29} n-alkanes.

Table 2. N-alkane content of the dietary feeds offered to the animals used to estimate the herbage intake

Period/Sample	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	C_{32}	C_{33}	C_{35}
Period 1								
[1]HP Herbage	6	15	31	39	79	10	114	25
Leaf	12	25	58	90	122	7	133	15
Stem	43	83	118	113	180	29	256	76
Concentrate	2	3	3	3	1	nd	nd	nd
Period 2								
[1]HP Herbage	17	25	37	51	107	10	171	37
Leaf	2	6	21	49	108	5	141	18
Stem	5	10	13	24	82	3	157	39
Concentrate	50	79	54	26	10	8	6	3
Period 3								
[1]HP Herbage	35	55	68	71	106	20	137	27
Leaf	55	88	99	128	127	37	168	24
Stem	7	12	17	31	88	5	163	36
Concentrate	50	79	54	26	10	8	6	3

[nd] n-alkane not detected; [1] HP herbage sample by the hand-plucked method; n-alkane content (mg/kg of DM) was determined in duplicate.

Intake estimates of herbage

Grazing period 1

The average DM herbage intake (CLA= 11.2 ±1.9, Megalac= 11.1 ±2.6 kg/d, p>0.05) was not different between treatments. In the investigation of the concentration of individual and/or n-alkanes ratio in fecal samples, there was no effect of treatment neither interaction between treatment and time of collection on fecal concentrations of individual C_{31}, C_{32}, C_{33}, C_{35} alkanes as well as C_{31}:C_{32} and C_{33}:C_{32} ratios. There was an effect of time of collection (morning and afternoon) on the individual concentration of C_{31}, C_{33} and C_{35} alkanes in the herbage in which the greatest values of fecal concentrations were recorded during the afternoon period (Table 3).

Grazing period 2

There was no treatment effect on the herbage DMI (CLA= 9.0 ±2.1, Megalac= 8.2 ±2.1, kg/day, p>0.05). There was no effect of treatment on the individual C_{31}, C_{32}, C_{33}, C_{35} fecal concentrations, but there was an effect of treatment on C_{31}:C_{32} and C_{33}:C_{32} ratios. There was an effect of time of collection on all individual n-alkanes and n-alkane ratios (Table 3).

Grazing period 3

There was no treatment effect on the herbage DMI (CLA= 11.8 ±2.4, Megalac= 12.6 ±2.4, kg/day, p>0.05). The least-square means for treatments, time of collection, days of collection, and their interactions on individual n-alkanes and fecal concentration ratios of n-alkanes are listed in Table 4. There was an effect of day of collection on C_{31} fecal concentration, which also varied with time of collection with greater concentration found in the afternoon. The fecal concentration of C_{32} comprises both the C_{32} coming from pasture and from the slow-release capsule. The fecal concentration of C_{33} varied with time of collection (morning= 323.1 vs. afternoon= 367.6 mg, p<0.01) and an effect of days of collection was observed with the greatest concentration occurring on day two (467.4 mg/kg of DM). Fecal concentration of C_{35} varied with time of collection (morning= 67.1 vs. afternoon= 76.8 mg, p<0.01), and during days of collection (p<0.01). The fecal concentration ratios of C_{31}:C_{32} and C_{33}:C_{32} varied with time and days of collection (p<0.01).

Table 3. Least square means of individual and fecal ratios concentration for the n-alkanes (mg/kg DM) of C_{31}, C_{32}, C_{33}, C_{35}, and C_{31}:C_{32} and C_{33}:C_{32} ratios on the first and second periods

Alkane/ Ratio	P1[1]		p[3]			P2[2]		p[3]		
	M[4]	A[5]	Tr[6]	TC[7]	TrXTC[8]	M[4]	A[5]	Tr[6]	TC[7]	TrXTC[8]
C_{31}	217.8	240.8	ns	*	ns	253.7	275.7	ns	**	ns
C_{32}	89.9	93.3	ns	ns	ns	123.5	137.1	ns	**	ns
C_{33}	236.4	261.9	ns	**	ns	420.8	453.0	ns	**	ns
C_{35}	42.4	47.5	ns	**	ns	92.3	99.2	ns	**	ns
C_{31}:C_{32}	2.43	2.64	ns	ns	ns	2.14	2.32	**	**	ns
C_{33}:C_{32}	2.64	2,88	ns	ns	ns	3.56	3.84	**	**	ns

*(p<0.05); **(p<0.01); [ns](non significant); P1= grazing period 1; P2= grazing period 2; p= probability; M= morning; A= afternoon; Tr= treatment; TC= time of collection; Tr x TC= interaction between treatment and time of collection; mg/kg DM.

Table 4. Least square means of individual and fecal ratios concentration for the n-alkanes (mg/kg DM) between collection time and days of collection on the grazing period 3

Marker	Treatment		Collection Time		Day of Collection				
	CLA	Meg	M	A	1	2	3	4	5
C_{31}	258.3[a]	249.2[a]	237.4[b]	270.1[a]	250.8[b]	344.1[a]	222.4[c]	231.5[c]	219.8[c]
C_{32}	117.0[a]	116.5[a]	114.0[a]	119.5[a]	129.8[b]	160.1[a]	100.6[c]	99.4[c]	93.8[c]
C_{33}	349.9[a]	340.8[a]	323.1[b]	367.6[a]	296.4[d]	467.4[a]	319.2[bc]	336.9[b]	306.9[cd]
C_{35}	73.4[a]	70.5[a]	67.1[b]	76.8[a]	57.6[d]	92.0[a]	67.4[c]	75.4[b]	67.4[c]
C_{31}:C_{32}	2.29[a]	2.18[a]	2.15[b]	2.33[a]	1.99[b]	2.23[a]	2.25[a]	2.35[a]	2.37[a]
C_{33}:C_{32}	3.14[a]	3.01[a]	2.96[b]	3.19[a]	2.35[d]	3.04[c]	3.24[ab]	3.43[a]	3.32[a]

CLA= conjugated linoleic acid; Meg= megalac; M= morning; A= afternoon; Different small letters between treatments within each marker, within each collection time and days of collection are significantly different (p<0.01).

DISCUSSION

The results of n-alkane profile of our study are in agreement with the results from other studies with tropical pasture species (Oliveira et al., 1997; Delgado et al., 2000; Genro et al., 2001). There was a variation in the concentration of C_{31}, C_{33}, and C_{35} in the whole herbage samples as well as in the leaf and stem fractions between periods of evaluation. Similar results were reported by Laredo et al. (1991) for *Pennisetum glaucum* and *Sorghum* sp, Oliveira et al. (1997) for *Pennisetum purpureum* cv Napier, and Genro et al. (2001) for *Brachiaria brizantha* cv Marandu, *Panicum maximum* cv Mombaça and *Pennisetum purpureum* cv Cameroon.

The first period of study indicated the basic assumption of no variation in the n-alkanes ratios, using the pair C_{33}:C_{32} to estimate DMI as suggested by Mayes et al. (1986). The effects of time and day of collection on fecal concentration of individual n-alkanes and on the n-alkane ratios were probably caused by the management of animals. They were retrieved from paddocks at 1130 h to a sheltered place, returning to pasture after the afternoon milking, around 0230 h. Therefore, grazing was more intense during the afternoon period and at night than during the morning period, certainly resulting in variations in rumen fill. A larger rumen fill after the more intense grazing period might have caused larger excretions and greater fecal concentrations of n-alkanes in the afternoon period of the following day once concentrate amounts were constant and only forage DMI varied. Therefore, differences in rates of passage of both solid and liquid phases of the digesta throughout the day might have resulted in larger diurnal variation in fecal concentration of the markers. Moreover, changes in sward structure (e.g. leaf to stem ratio) may have resulted in consumption of different plant parts, altering fecal concentration of the markers used. Dillon (1989) worked with lactating cows and found diurnal variations for the pairs C_{31}:C_{32}, C_{33}:C_{32} and C_{35}:C_{36} of n-alkanes, pointing out that feeding management might have contributed to those variations.

The changes in fecal concentration of alkanes with day of collection were likely a consequence of variations in morphological composition of the pasture. As grazing progressed, the effect on fecal concentration of the marker could be related to the depletion of leaf in sward herbage mass, a fact reinforced by the smaller proportion of the leaf component in the following paddocks of the grazing sequence (Figure 1).

In the second period, the difference between treatments was due to differences in rumen metabolism of fatty acids, which might have influenced fiber digestion by altering passage rate and modifying fecal concentration of the markers. According to Mayes et al. (1986), smaller fecal concentrations of natural C_{27} and C_{29} alkanes were found when animals were receiving a synthetic source of C_{28} and C_{32} n-alkanes with stearic and palmitic acids mix. On the other hand, Ohajuruka and Palmquist (1991) measured the intake of lactating dairy cows using external markers and found no effect of added fat on the concentration and fecal recovery of C_{31} and C_{32} n-alkanes.

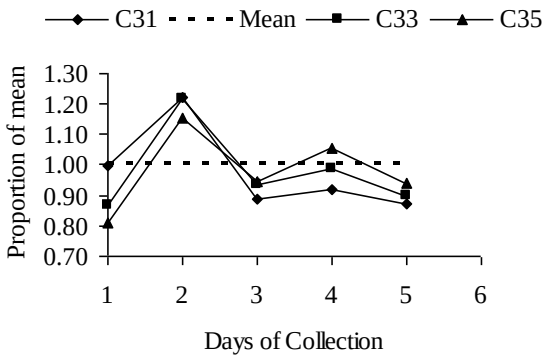

Figure 1 - Mean variation in the fecal concentrations for C_{31}, C_{33} and C_{35} (mg/kg of DM), expressed as proportions of the mean concentration for each cow over five days.

Although it was not in the scope of this study, monitoring and maintenance of swards under control is important because as grazing occurs, changes in sward structure take place throughout the pasture growing season and could affect intake and performance of animals by altering the amount and composition of the herbage consumed. Vulich et al. (1993) suggested that pasture should be sampled frequently because of variations in n-alkanes concentration between days and weeks.

Fecal concentration of C_{33} changed between morning and afternoon, but because this n-alkane had the same concentration in both leaf and stem fractions, fecal concentration was maintained by the increase in intake of the stem component as grazing progressed (Table 4).

Fecal concentration of C_{35} might represent an increase in consumption of stems relative to leaves because the leaf to stem ratio of sward herbage mass decreased during grazing and this n-alkane appeared in larger concentrations in the stem relative to the leaf fraction (Table 4).

Although fecal concentration of C_{33} varied over time, variation in the ratios of C_{31} and C_{33} relative to C_{32} must have been caused by variation in fecal concentration of the C_{32} n-alkane (Figures 2 and 3), since the dose was the same each day, being associated with a decreasing intake of C_{32} from pasture because this n-alkane was found in higher concentrations on leaves relative to stems. This finding is in agreement with those of Dove et al. (1991). Those authors reported variation in individual fecal concentrations of C_{29} n-alkane from the diet, C_{28} and C_{32} from the dosed slow-release capsule and C_{36} fed via paper pellet.

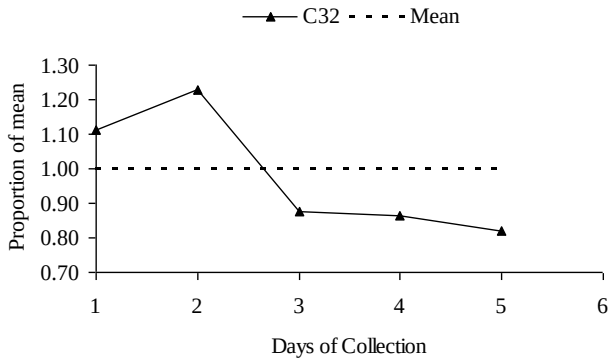

Figure 2 - Mean variation in the fecal concentrations of the C_{32} (mg/kg of DM), expressed as proportions of the mean concentration for each cow over five days.

Dove et al. (1992) found an interaction between herbage intake and feeding level on fecal concentration of the n-alkanes used in their study. It is possible that in this study the changes in sward herbage mass between the two grazing days (occupation days) of each sampling paddock produced a similar effect characterized by high feeding level on the first day and low feeding level on second day, influencing the fecal concentration of n-alkanes. Stakelum and Dillon (1990) used lactating dairy cows to test different patterns (hours) and feeding levels and found a marked daily variation in fecal concentration ratio of the pairs C_{31}:C_{32}, C_{33}:C_{32} and C_{35}:C_{36}. These diurnal and/or daily systematic variations in fecal concentrations of n-alkanes can also be explained by changes in grazing patterns caused by changes in sward structure and selective grazing.

As pointed out by Dove and Mayes (1991) and Mayes and Dove (2000), variations in fecal concentration of individual n-alkanes may not interfere with estimates of herbage intake provided that variation in fecal concentration ratio of the pair of n-alkanes used in the estimation process remains stable.

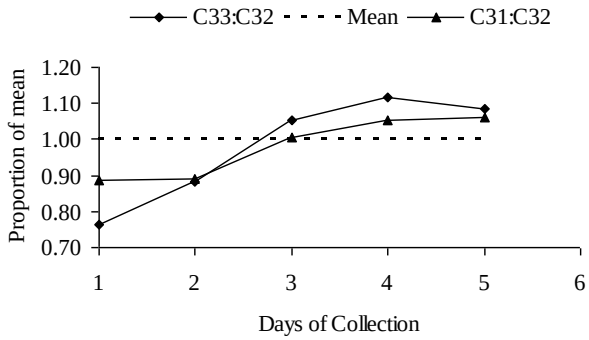

Figure 3 - Mean variation of the ratio in the feces of C_{31} and C_{33} odd- chain (diet) n-alkane concentration to C_{32} even-chain (dosed) n-alkane concentration (mg/kg of DM) expressed as proportions of the mean concentration for each cow over five days.

CONCLUSIONS

Overall, our results highlight the importance of strict control of grazing management and sward structure in studies where external markers are used. We concluded that monitoring herbage and sward and animal managements might have an influence on n-alkanes excretion, causing a change in the ratio of their fecal concentration. These modifications may result in erroneous estimates of intake.

Conflict of interest

The authors declare that they have no conflict of interest.

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