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Using n-alkanes to estimate herbage intake and diet composition of cattle fed with natural forages in Madagascar

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Abstract

Accurate estimates of feed intake are important in order to develop accurate ration formulations, , for selecting livestock according to production efficiency, and for reducing environmental impact of livestock. The present study assessed the accuracy of n-alkanes to estimate individual herbage intake and diet botanical composition of cattle fed natural forages, typical to Madagascar. The effect of two different forage allowances and seasons on the herbage intake was also tested. Eight Norwegian red pie sires (232±20 kg BW) were orally dosed twice daily with paper pellets containing 456.61±21.64 mg of C32 alkane as external marker. Animals were housed in individual pens and fed with mixtures of five forages species typically used by farmers in the rainy season (*Aristida multicaulis, Hyparrhenia rufa, Imperata cylindrical, Urochloa brizantha* and *Stylosanthes guyanensis*) and the dry season (*Chrysopogon serrulatus, Cynodon dactylon, Imperata cylindrica, Lolium multiflorum* and *Leersia hexandra Sw*). The sires were randomly assigned to two different forage allowances: (i) *ad libitum* with a refusal of 5% and (ii) 1.1% DM of body weight (BW). The n-alkane pairs C31:C32 and C32:C33 and the ratio C32/Acid Insoluble Ash (AIA) in plants and faeces were used to estimate the herbage intake. The botanical composition of the diet was estimated using the n-alkane profile from C27 to C35 in individual plant species and faeces, by least squares optimization. The n-alkane pairs underestimated (P<0.0001) the herbage intake by 25% in the dry season, for both forage allowances and 26% for animals receiving high forage allowance, regardless of the season. In contrast, the intake estimates based on both n-alkane pairs did not differ from the measured intake for animals receiving low forage allowance during the rainy season. The C32/AIA underestimated the actual herbage intake,by 50%, for cattle consuming high forage allowance for both seasons (*P*<0.0001). The n-alkane faecal recovery (AFR) corrections factors set affected (P<0.01) the

estimated proportions of each plant species, that comprised the diet in both seasons. The application of appropriate AFR permitted to have a better accuracy of diet botanical composition estimates. It is concluded that plant wax n-alkanes are advantageous for estimating both diet botanical composition and herbage intake in cattle. However, for improving the prediction, it is important to measure the actual AFR before the calculation.

Keywords: botanical composition, feed intake, markers, sires, recovery

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; AFR, N-alkane faecal recovery; AIA, acid insoluble ash; DM, dry matter; BW, body weight; NDF, neutral detergent fibre; SAS, statistical analysis system

1. Introduction

The increased global demand for protein and the environmental issues facing livestock systems require enhanced production efficiency. Accurate assessment of individual feed intake and nutrient digestibility are relevant to meet nutritional needs of animals, optimize their production levels, and improve farm profitability (Bezabih et al., 2012). Moreover, accurate estimates of feed intake are important to recommend precise ration formulation, to select animals according to production efficiency, and to reduce the environmental impact of livestock (Bani et al., 2014). Individual feed intake is difficult to estimate in group-housed and grazing animals (Ferreira et al., 2004). Internal markers, naturally present in plants, have been largely used to estimate herbage intake, nutrient digestibility, and diet botanical composition in ruminants. Acid insoluble ash (AIA) is an internal marker to determine digestibility and feed intake in cattle and sheep (Sales and Janssens, 2003). Nevertheless, this technique is limited by analytical inaccuracy and low repeatability. The concurrent use of adjacent natural odd-chain and dosed even-chain n-alkanes is considered as the most accurate technique, mainly for group-housed and grazing animals (Mayes et al., 1986, Keli et al., 2008). The n-alkane technique has been used successfully to estimate herbage intake by dairy cattle (Bani et al., 2014; Richmond et al., 2015), beef cattle (Oliván et al., 2007; Chavez et al., 2011), and sheep (Keli et al., 2008; Amaral et al., 2013). However, there is a lack of information on the use of the n-alkane technique to estimate the individual herbage intake and diet composition of sires fed with natural forages and under conditions typical to Madagascar.

The aim of this study was to evaluate the reliability of the n-alkane technique, compared to the AIA technique to predict the individual herbage intake and diet botanical composition in sires fed natural pastures. The second objective of this study was to determine the effect of the diet botanical composition by season and the forage allowance on the accuracy of the intake estimates.

2. Materials and methods

2.1. Study site

The study was conducted in Madagascar, at the Rural Development and Applied Research Center (Fifamanor), Antsirabe, during the rainy (February-March 2018) and dry seasons (September 2018). The center is located at 18°59' South and 46°17' East and at an altitude of 1,644 m above sea level. Temperatures during the experimental period ranged from 14 to 22°C (average of 18°C) and 10 to 22°C (average of 16°C) during the rainy and dry season, respectively.

2.2. Animals and diets

Two experiments were conducted with sixteen Norwegian red pie sires. Average initial BW of the sires was 237±16 kg and 226±23 kg (mean±SD) during the rainy and dry season experiments, respectively. Each experiment lasted 13 days, with seven days of adaptation and six days of data collection. The animals were housed in individual pens and were randomly allocated to two different forage allowances: (i) *ad libitum* with a refusal of 5%; (ii) 1.1% DM of BW per day. Two forage-based diets that differ in composition, depending on the season were distributed to the animals (Table 1). The forage species were harvested at the vegetative stage and at ground level, every two days. All forages were weighed out, thoroughly mixed by hand, stored in a bag and fed fresh to animals. The forage species and their composition into the diets were chosen to represent the average composition of pastures during each season. The botanical composition of the diets of each season was estimated from surveys applied to farmers in the region and direct observations of the density, level of coverage and appearance of the plant species in the pasture. The animals received an accurately measured amount of the experimental diet twice a day at 7:00AM and 3:00PM. The diet offered and refusals, that were collected daily shortly before the first morning

meal, were registered daily to calculate the observed intake. Drinking water was available *ad libitum* throughout the experimental period. During the experimental period, paper pellets containing 471.9±8.04 mg and 441.3±2.95 mg of n-dotriacontane (C32 alkane) were administrated twice daily to the animals, at 6:30AM and 2:30PM during the rainy and dry season, respectively.

2.3. Sampling and laboratory analysis

Representative samples (~250 g) of feed offered and refusals were collected daily. From days 11 to 13, approximately 500 g of faecal samples were collected twice daily by rectal grab sampling, with 12h intervals, advanced by 4h for the consecutive days. After collection, faecal samples were refrigerated, thoroughly mixed by hand, and pooled to create one composite sample per animal. Samples were dried at 60°C in a forced air oven for 72hfor dry matter analysis, and ground to pass a 1-mm mesh prior to analysis. Total ash, crude protein (CP), and crude fibre (CF) were determined according to AOAC procedure (2005). Total ash was determined by incineration at 550°C during 8h (Method No. 930.05), the CP concentration (6.25×N) by the Kjeldahl method (Method No. 978.04) and the CF by successive digestion with 1.25% dilute sulfuric acid and 1.25% dilute sodium hydroxide (Method No. 978.10). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) concentrations were determined according to Van Soest and Robertson (1985). The NDF concentration was determined without a heat stable amylase. Both NDF and ADF were expressed inclusive of residual ash. AIA concentration of feed and faeces were determined gravimetrically after boiling the ashes in hydrochloric acid 3N, filtering, washing the hot hydrolysate, and re-ashing, according to Van Keulen and Young (1977). For n-alkane analysis, ground samples were pulverized using a bullet mill (MM 2000; 4 min at 80 Hz; Retsch Technology GmbH, Haan, Germany) before extraction and analysis of n-alkanes as described by Mayes et al. (1986), using C22 and C34 alkanes as internal standards. The extracted samples were

analyzed for n-alkanes (C21 to C35) using a gas chromatograph (GC; Carlo Erba HRGC Mega 2 series) fitted to a flame ionizing detector (FID), using helium as the carrier gas.

2.3. Calculations

Herbage intake was estimated from the concentration of n-alkane pairs C31:C32 and C32:C33 in the faeces and herbage (Mayes et al., 1986), as follows:

Herbage intake (kg DM/day)=Dj/((Fj/Fi)*Hi-Hj)

where Fi: concentration of natural odd-chain n-alkanes in faeces (C31 or C33, mg/kg DM); Hi: concentration of natural odd-chain n-alkane in herbs (C31 or C33, mg/kg DM); Fj: concentration of even-chain n-alkane in the faeces (C32, mg/kg DM); Hj: concentration of even-chain n-alkane in herbs (C32, mg/kg DM); Dj: concentration of even-chain n-alkane dosed to animals (C32,mg/day).

The herbage intake was also estimated from C32/AIA procedure using the formula (Ferreira et al., 2004):

Herbage intake (kg DM/day) = Faecal output/ (1 – Digestibility)

Faecal output was calculated from the equation:

Faecal output (kg/day) = Dj/Fj

where Dj: concentration of n-alkane dosed to animals (C32, mg/day) and Fj: concentration of even-chain n-alkane in faeces (C32, mg/kg DM).

Digestibility was calculated as:

Digestibility = 1- (Ci/Cf)

where Ci and Cf are the concentrations of AIA in diet and faeces, respectively (g/kg DM).

The diet botanical composition was estimated using a least-squares optimization procedure where the sum of the squared discrepancies between the actual marker proportions and those predicted to occur in faeces were minimized, as follows:

Minimization = $\sum[(\text{actual-calculated})^2]$ marker i…n

where actual = actual faeces concentration of marker i corrected for incomplete recovery; calculated = calculated faeces concentration of marker, using the following formula:

calculated = (xAi + yBi + zCi)/(1 – digestibility)

where x, y and z are the proportion of plant species A, B and C identified in the diet in each season, respectively; Ai, Bi and Ci represent the concentrations of marker i in plants A, B and C, respectively. The diet botanical composition calculations were performed assuming all plant species identified in each season. The n-alkanes from C27 to C35 were used in the calculations because they were found in higher concentrations in the faeces. The n-alkane faecal recovery (AFR) correction factors were taken from published studies, in order to assess the effect of the AFR rates on the accuracy of the diet botanical composition estimates (Table 2).

2.4. Statistical analysis

The effect of forage allowance level, the season and their interaction on the accuracy of feed intake was analyzed as a split-split plot factorial analysis of variance, where season was the main plot, forage allowance the split-plot, and method the split-split plot, according to the model:

$$Yijkl = \mu + R_i + S_j + RS_{ij} + F_k + SF_{jk} + RSF_{ijk} + M_l + SM_{jl} + FM_{kl} + SFM_{jkl} + \varepsilon_{ijkl}$$

Where Yijkl is the response variable, μ represents the overall mean, R represents the random effect of replication (in this case, animal), S represents the fixed effect of season (main plot), RS is the interaction replication and season, F is the fixed effect of forage allowance (split-plot), SF is the

interaction between season and forage allowance, RSF represents the interaction R*S*F, M represents the fixed effect of method (split-split plot), SM the interaction season and method, FM the interaction forage and method, SFM the triple interaction season, forage allowance and method, and ε_{ijkl} is the residual variation.

The PROC MIXED procedure of the SAS statistical package (version 8.01) was used for the analysis by using the restricted maximum likelihood (REML) method. The Solver routine of the Microsoft Excel program was used without non-negative restrictions for the estimation of diet composition (Keli et al., 2008). The accuracy of the estimation of diet botanical composition was assessed by using the Kulcyznski similarity index, according to Ferreira et al. (2009); as KSI=100*∑2ci/(ai +bi), where ci is the lesser percentage of i component between the known and estimated diet proportion, and (ai +bi) is the sum of the known and estimated proportions of each plant component. The effect of different AFR sets used in the estimation of diet botanical composition was examined by analysis of variance (ANOVA).Regression parameters were estimated by PROC REG procedure of the SAS statistical package. Statistical results were considered to be significant at the 0.05 α level.

3. Results

3.1 Marker concentrations in herbage, diet and faeces

Herbage and diet concentrations of odd-chain n-alkanes were greater than the even-chain n-alkanes (Table 3). For herbage components, the greatest n-alkane concentration (274 mg/kg DM) occurred for C33 in *Stylosanthes guyanensis* while the lowest (3 mg/kg DM) was for C28 in *Urochloa brizantha*. *Lolium multiflorum* has the highest concentrations for short chain n-alkanes (C27 to C30) while *Stylosanthes guyanensis* has the highest concentrations for long chain n-

alkanes (C31 to C33). Odd-chain n-alkanes were 93% of the total concentration of n-alkanes in the diet of both seasons. For diet, the greatest n-alkane concentration (183 mg/kg DM) occurred for C31 in dry season while the lowest (6 mg/kg DM) was for C28 in rainy season. In rainy season, the concentration of C31, C32 and C33 contributed to 34, 3, and 36% of the total n-alkane concentration, respectively. In dry season, n-alkanes C31, C32 and C33 contributed to 39, 2 and 20% of the total n-alkane concentration, respectively The greatest n-alkane concentration in faeces was attributed to C32 in dry season (560 mg/kg DM) while the lowest was for C28 in rainy season (6 mg/kg DM). The faecal concentration of C31, C32, and C33 in rainy season contributed to 20, 43 and 23% of the total n-alkane concentration. In dry season, the faecal concentration of C31, C32, and C33 contributed to 25, 41 and 13% of the total n-alkane concentration. The greatest concentration of acid insoluble ash (54 g/kg DM) occurred in *Hyparrhenia rufa* while the lowest (10 g/kg DM) was in *Stylosanthes guyanensis.* The concentration of acid insoluble ash averaged 44 g/kg DM and 68 g/kg DM in feed and faeces, respectively.

3.2. Herbage intake

Intake estimated by n-alkane pairs during the rainy season did not differ from the measured intake (Table 4). In contrast, in dry season, the intake estimates using n-alkane pairs were both significantly lower by 25% ($P<0.01$) compared with the actual intake. The intake estimates based on both n-alkane pairs did not differ from the measured intake for animals receiving low intake. In contrast, the intake estimates obtained for both n-alkane pairs were 26% lower ($P<0.001$) for animals receiving high forage allowance, for both seasons. The C32/AIA underestimated the herbage intake by 29 and 44% for the rainy and dry seasons, respectively, regardless of the forage allowances. The intake estimated by C32/AIA was 50% lower compared to the actual intake for animals consuming high forage allowance, regardless of the seasons. The intake estimates based

on C32/AIA did not differ from the measured intake for animals receiving low forage allowance, for both seasons.

Estimate of intake using the ratio C31:C32 (R^2=0.61, P<0.001) was more reliable than the ratio C32:C33 (R^2=0.41, P<0.01, Figure 1). The intercept was different from 0 (P=0.0465 and 0.0348 for C31:C32 and C32:C33, respectively). According to the seasons, the intake estimates had greater R^2 during the rainy season (data non-published, R^2=0.79 and 0.78 for C31:C32 and C32:C33, respectively) compared to the dry season (data non-published, R^2=0.63 and 0.07 for C31:C32 and C32:C33, respectively). Moreover, the discrepancies between the observed and estimated intakes were significantly greater during the dry season (25%, P<0.0001), compared to the rainy season (9 and 3%, P>0.05, for C31:C32 and C32:C33, respectively).

3.3. Diet botanical composition

In general, the AFR set used in the calculations of diet botanical composition affected (P<0.01) the estimated proportions of each plant species, that comprised the diet in both seasons (Table 5). The application of AFR corrections factors, previously determined in controlled studies resulted in the accuracy of the estimates, with KSI values ranging from 47 to 76 in the rainy season, and from 60 to 86 in the dry season.

The most accurate estimate of diet botanical composition was with AFR1, with higher KSI values (76 and 86 in the rainy and dry season, respectively).

4. Discussion

4.1. Marker concentrations in feed and faeces

The n-alkane profiles of diets were different among the seasons, as different forage species were mixed for each diet. The fact that odd-chain n-alkane concentrations are higher, compared to

the even-chain n-alkanes is in agreement with the observations from other authors who reported that over 90% of the n-alkanes measured in plants have an odd number of carbon atoms (Bani et al., 2014). As expected, the concentration of C32 alkane in most plant species and diet was very low (<20 mg/kg DM). This was the reason for using C32 alkane as external marker for estimating herbage intake as its presence in plants is typically negligible (Hu et al., 2014). N-alkanes C29, C31, and C33 in forage species are also reported as the predominant hydrocarbons of the odd-chains (Ferreira et al., 2004), indicating their importance for estimating diet composition (Lewis et al., 2003). They are also suitable to beused as internal markers with the even-chain dosed n-alkane (C32) for estimating the herbage intake. A value of 50 mg/kg is the minimum threshold value for using an n-alkane as a marker (Laredo et al., 1991). For these reasons, C31 and C33 were chosen as the internal marker for estimating intake. The n-alkane profiles in faeces were characterized by an increase with the carbon chain length (Hu et al., 2014). Faecal concentration of n-alkanes reflected that of the consumed diet (Keli et al., 2008). The higher concentration of even chain C32 in faeces was due to the amount of dosed C32 alkane to the animals. The concentrations of AIA of the forage diet in this study were greater, compared to the threshold of 7.5 g/kg DM for obtaining precise estimates of digestibility (Thonney et al., 1895).

4.2. Factors influencing the accuracy of herbage intake estimate

According to our results, the herbage intake was underestimated by both pairs of n-alkanes (C31:C32 and C32:C33) during the dry season, and when the animals were fed *ad libitum*. In order to obtain accurate estimates of herbage intake, the faecal recoveries of the two n-alkanes of each pair must be similar (Oliván et al., 2007; Keli et al., 2008), so that the errors associated with the incomplete recoveries cancel out in the equation (Sun et al., 2008). Underestimation of intake would be the result of a negative discrepancy of equal proportion between the AFR of the natural

255 (C31 or C33) and dosed n-alkanes (C32) of each pair (Oliván et al., 2007). Faecal recovery of
256 markers is not predictable but requires experiments with total faecal sampling. N-alkanes that are
257 not excreted in faeces are absorbed, taken up by the liver and metabolized mainly to phospholipids
258 and carbon dioxide (Hargrove et al., 2004). It is recommended that the application of the n-alkane
259 ratio technique should be preceded by the calculation of the actual AFR to accurately estimate the
260 herbage intake. Furthermore, the representative sampling of forage and faeces are also important
261 to assure reliable estimates. The sampling of forage must closely and accurately represent the
262 ingested diet. Representative sampling of faeces is also important to prevent the diurnal variation
263 in the ratio between faecal alkane concentrations. In this study, the faecal sampling regime was
264 done twice daily, with 12h intervals but advanced by 4h in consecutive days, in order to obtain
265 representative samples that would cover the excretion pattern variability of markers during the
266 days of collection. N-alkanes C31, C32 and C33 were generally proposed to estimate intake
267 because they had the lowest discrepancy in faecal recovery (Mayes et al., 1986; Dove et al 2002;
268 Oliván et al., 2007). As stated in the results, the estimates of intake using the C31:C32 pair
269 (R^2=0.61) were more reliable than the C32:C33 pair (R^2=0.41). Nevertheless, there is no significant
270 difference on the discrepancies between the observed and estimated intake for C31:C32 and
271 C32:C33 pairs. These results are in agreement with those reported by Berry et al. (2000) with
272 C31:C32 (R^2=0.60) and C32:C33 (R^2=0.52). Bani et al. (2014) found similar results that on
273 average, C31:C32 pair better predicted intake (R^2=0.71) compared to the C32:C33 pair (R^2=0.60),
274 with dairy cattle receiving a mixed foragediet. Oliván et al (2007) found R^2 of 0.61 and 0.18, with
275 an underestimation about 25% and 19% for estimating intake in cattle fed with Lucerne hay with
276 C31:C32 and C32:C33, respectively. Halfa (2012) obtained R^2 of 0.63 and 0.61 with C31:C32 and
277 C32:C33, respectively. Ferreira et al. (2005) reported that characteristics of the diet, as then-alkane

concentrations may influence the recovery of dosed and naturally occurring n-alkanes. Nevertheless, as stated above, the profiles of n-alkanes in each diet were different among seasons, which may impact the AFR and the intake estimates. This assumption has to be confirmed because in this study, the AFR was not measured. Even the animals in the present study were adapted to the diet and the external marker C32 during 10 days before the faecal sampling, the excretion pattern of dosed and natural n-alkanes in faeces could differ and have possible effect on the intake estimates. While the excretion pattern of external markers could be influenced by the timing and amount of dosing, the faecal excretion of internal markers would be the result of the characteristics of the diet, feeding time and the difference in feed intake behavior of the animals. According to Dove and Mayes (1991), natural odd-chain alkanes are associated with the particle phase of digesta and dosed even-chain n-alkanes are linked with the liquid phase of digesta. Therefore, natural odd-chain alkanes pass slowly along the digestive tract, and can be recovered in faeces in a lower proportion, compared to the dosed even-chain n-alkanes. Ahvenjarvi et al. (2018) illustrated nicely the diurnal pattern of a liquid marker in the faeces when dosed twice per day at 12h intervals. According to the level of intake, the animals receiving an *ad libitum* diet showed a significantly greater discrepancy of 25% between the observed and estimated intake ($P<0.0001$), compared to those with a restricted diet allowance of 1.1% BW. The effect of intake level on AFR of natural and dosed n-alkanes, and its consequences on the accuracy of intake estimates needs to be investigated further. Several studies stipulated that feeding level and characteristics of the diet might affect AFR of naturally occurring and dosed n-alkanes that could have consequences for the accuracy of intake estimates (Unal and Garnsworthy, 1999; Oliván et al., 2007; Bani et al., 2014). Bani et al. (2014) specified that when intake increases, also the individual variability in feeding behavior and rumen passage rate becomes more important. Unal and Garnsworthy (1999)

described a tendency of AFR C32 and C33 by dairy cattle to be lower when animals received a restricted daily diet of 20 kg of silage compared with diets of 30 kg of silage. The n-alkane technique was accurate for estimating intake in cattle because the SEM is low (0.27), with a respective CV of 20 and 25% for C31:C32 and C32:C33, regardless of the seasons and forage allowances. In grazing conditions, the intake does not depend only on diet quality but also on forage distribution and availability. The consideration of two different levels of diet intake during this study is important and interesting. The lower level allowance is generally observed under tropical rangeland, where forage resources may be scarce.

Unlike the n-alkane technique, the use of C32/AIA technique involved separate calculations of faecal output and digestibility, when estimating the herbage intake. This approach allows cumulated errors in the estimated intake from the faecal output and digestibility estimates (Ferreira et al., 2004). For this reason, the herbage intake estimated by C32/AIA was not reliable, unless it is used for low forage allowance. Moreover, analytical errors could be the reason for failure when using AIA procedure in digestibility and intake studies, particularly in feed with low content of AIA or feeds contaminated with soil (Sales and Janssens, 2003; Ferreira et al., 2004).

4.3. Accuracy of diet botanical composition estimate

The discriminatory information carried by the n-alkanes is suitable for use as diet composition markers. Since the differences of n-alkane concentrations patterns between forage species are high, hence the estimates of diet composition are accurate. In this study, the forage species showed differences in n-alkane concentrations. In addition, differences in n-alkane profiles were observed between plant species. Each plant species had a unique profile of markers that could be discerned from the others. These differences between plant species that comprised the diet, in terms of concentrations and profiles of n-alkanes have influenced the good estimates of the diet

botanical composition. Since the calculation of the diet botanical composition used n-alkane marker concentrations recovered in faeces, it is important to apply a suitable AFR correction factor prior to diet composition estimation (Ferreira et al., 2009). In the present study, since total collection of feces was not done, some data on AFR rates from published studies were used. The accuracy of diet composition estimates using AFR illustrated the importance of using appropriate recovery correction factors prior to application of these markers. Indeed, AFR used mean recovery data with similar experiments conditions of this present study, in terms of animal species (dairy cattle) and diets (fresh grass).

The estimation of diet botanical composition allows a more detailed qualitative evaluation of what animals consume and how this contributes to a balanced nutrient supply. Furthermore, such knowledge of the plant–animal interactions is important for sustainable ecosystem management and optimization of both animal and land productivity.

5. Conclusions

The results from the present study confirm the potential of n-alkane technique to estimate both herbage intake and diet botanical composition in cattle. The C31:C32 alkane pair better estimated the herbage intake compared to the C32:C33. The combination of n-alkanes allowed a satisfactory prediction of the botanical composition of feed consumed in cattle. For accurate estimation of herbage intake and diet botanical composition, the actual faecal recovery of n-alkanes should be measured and used in any given situation. Since animal characteristics, diet type, composition and allowance have been shown to affect faecal recovery rates of n-alkanes, accuracy of intake estimations was variable across the two seasons and the two forage allowances.

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Table 1

Botanical and chemical composition of the experimental diet

	Rainy Season		Dry Season	
	Mean	S.E.M.	Mean	S.E.M.
Botanical composition				
Aristida multicaulis	0.05	-		
Chrysopogon serrulatus			0.05	-
Cynodon dactylon			0.27	-
Hyparrhenia rufa	0.51	-		
Imperata cylindrica	0.08	-	0.08	-
Leersia hexandra Sw.			0.10	-
Lolium multiflorum			0.50	-
Stylosanthes guyanensis	0.11	-		
Urochloa brizantha	0.25	-		
Chemical composition				
Dry matter (g/kg)	429	9	417	7
Total ash (g/kg)	77	3	112	4
Crude protein (g/kg)	78	3	80	2
Crude fibre (g/kg)	385	9	241	8
Neutral detergent fibre (g/kg)	711	20	480	20
Acid detergent fibre (g/kg)	455	9	306	11
Acid detergent lignin (g/kg)	64	7	47	0

S.E.M. standard error of mean

Table 2

Faecal recovery rates of n-alkanes from published data, used to assess the effect on the accuracy of the diet composition estimates

	AFR1 Dillon (1993)	AFR2 Bezabih et al., 2012	AFR3 Morais et al., 2011	AFR4 Oliván et al., 2007
Animal	dairy cattle	zebu	zebu	beef cattle
Diet	fresh herbage	tropical roughages	fresh herbage	hay
Faecal recovery of n-alkanes				
C27	0.68	0.61	0.42	0.85
C28	na	0.67	0.94	0.93
C29	0.77	0.72	0.56	0.93
C30	na	0.74	0.82	0.85
C31	0.81	0.72	0.71	0.75
C33	0.85	0.70	0.75	0.78
C35	0.89	0.73	0.77	0.96

AFR. N-alkane faecal recovery rate; na. not available

Table 3

Concentrations of n-alkanes (mg/kg DM) and acid insoluble ash (AIA; g/kg DM) in herbage, feed and faeces

	n-alkanes (mg/kg DM)								AIA
	C27	C28	C29	C30	C31	C32	C33	C35	(g/kg DM)
Herbage components									
Aristida m.	7.21	4.04	13.89	6.11	71.45	11.48	73.90	24.40	37.84
Chrysopogon s.	14.23	4.31	30.57	6.68	58.37	10.25	53.98	21.52	52.50
Cynodon d.	14.36	5.97	37.51	8.40	76.28	12.04	84.46	32.60	50.66
Hyparrhenia r.	17.54	5.09	42.49	8.03	155.07	10.29	165.02	51.28	54.13
Imperata c.	17.51	9.45	58.63	18.81	220.71	18.47	186.11	46.46	29.45
Leersiah.	13.60	4.21	29.55	5.91	56.01	9.12	52.52	21.54	52.80
Lolium m.	31.60	9.98	176.35	14.60	249.87	9.65	78.98	6.21	20.98
Stylosanthes g.	5.12	4.01	26.38	6.74	259.34	20.67	273.64	3.83	9.50
Urochloa b.	12.95	3.23	34.38	5.18	85.46	10.15	91.12	30.81	26.90
Diet									
Dry season	23.37	7.39	117.09	12.30	183.44	11.52	94.54	21.66	56.81
Rainy season	14.24	5.91	33.42	8.91	119.91	10.98	126.62	33.63	30.55
Low intake	20.01	7.35	75.32	11.23	152.36	11.43	112.90	28.53	44.74
High intake	17.60	5.95	75.19	9.97	150.99	11.08	108.26	26.76	42.62
Faeces									
Dry season	34.52	11.39	199.22	21.19	338.62	559.93	174.80	37.77	81.66
Rainy season	18.15	6.22	57.16	13.44	246.86	532.31	278.04	74.28	53.65
Low intake	21.79	7.73	100.97	15.63	255.00	566.87	217.81	56.60	69.47
High intake	30.88	9.87	155.41	19.00	330.48	525.37	235.02	55.45	65.83

AIA. Acid insoluble ash

Table 4

Effect of season and forage allowance on herbage intake estimates

Herbage intake	Season			Forage allowance		
kg DM/day	Dry	Rainy	S.E.M	Low	High	S.E.M
Observed	4.08a	4.48a	0.27	2.89	5.67a	0.27
C31:C32	3.04b	4.07a		2.88	4.23b	
C32:C33	3.08b	4.35a		3.21	4.23b	
C32/AIA	2.30b	3.17b		2.63	2.84c	

S.E.M. standard error of mean

For a given season or forage allowance, values in the same column with different letters are significantly different ($P<0.05$).

P-values were for dry season ($P<0.01$); rainy season ($P<0.01$); low intake level ($P=0.867$); high intake level ($P<0.001$).

Table 5

Effect of using different sets of n-alkane faecal recovery (AFR) on the estimates of diet composition of cattle and Kulcyznski similarity index (KSI) between the known and estimated diet composition.

Seasons	Treatment	*Herbage species*					KSI
		Aristida m.	*Urochloa b.*	*Hyparrhenia r.*	*Imperata c.*	*Stylosanthes g.*	
	Known	0.05	0.25	0.51	0.08	0.11	
	AFR1	0.15b	0.09b	0.65a	0	0.11b	76
	AFR2	0.15b	0b	0.74a	0	0.11b	67
Rainy	AFR3	0c	0.62a	0.27b	0	0.10b	62
	AFR4	0.51a	0b	0.31b	0	0.18a	47
	S.E.M	0.04	0.04	0.05	0.01	0.01	
	Sign.	***	***	***	**	***	
		Chrysopogon s.	*Cynodon d.*	*Imperata c.*	*Leersia h.*	*Lolium m.*	
	Known	0.05	0.27	0.08	0.10	0.50	
	AFR1	0	0.29a	0.12c	0b	0.58a	86
	AFR2	0	0.26a	0.19b	0b	0.54a	84
Dry	AFR3	0	0.24a	0d	0.24a	0.52b	84
	AFR4	0.01	0.01b	0.39a	0.01b	0.60a	60
	S.E.M	0	0.05	0.02	0.04	0.05	
	Sign.	***	***	***	**	**	

S.E.M. standard error of mean

AFR is the mean n-alkane faecal recovery data obtained from published studies: AFR1 from Dillon (1993) as reported by Van den Pol-Van Dasselaar et al. (2006); AFR2 from Bezabih et al. (2012); AFR3 from Morais et al. (2011); AFR4 from Oliván et al. (2007).

KSI is the Kulcyznski similarity index (KSI) between the known and estimated diet composition.

For a given n-alkane faecal recovery within each plant species. values in the same column with different letters are significantly different ($P<0.05$).

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.
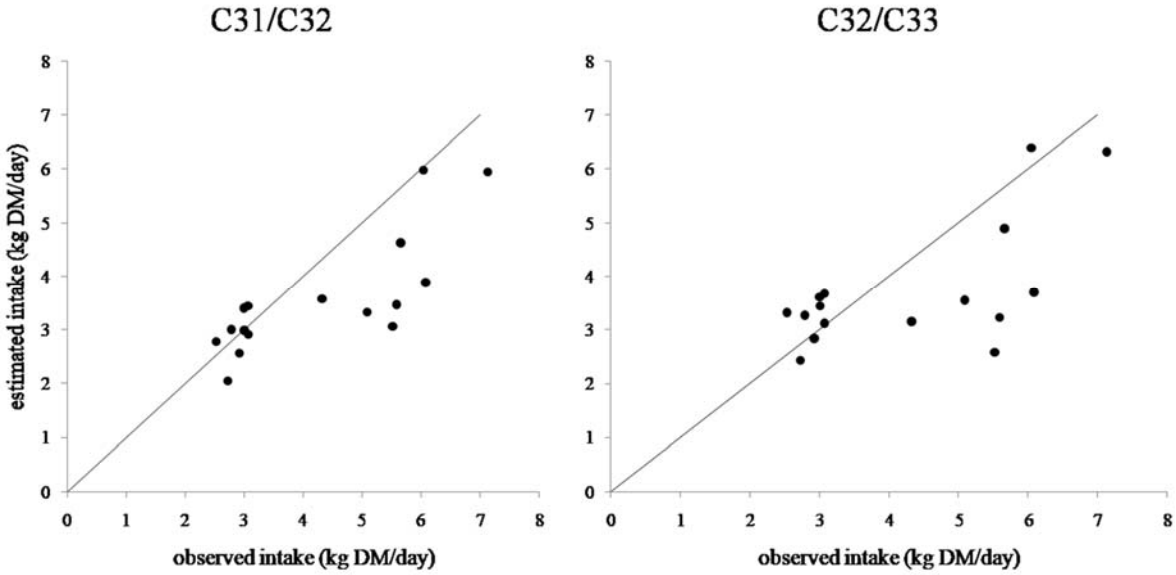
Fig 1. Relationship between observed and predicted intake for C31:C32 and C32:C33 C31:C32 [Y = (0.56*X) + 1.17; R^2 = 0.61; RMSE=0.71 ; CV=20% ; P<0.001]; C32:C33 [Y = (0.48*X) + 1.65 ; R^2=0.41 ; RMSE=0.93 ; CV=25% ; P<0.01]