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

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15  
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## 17 Abstract

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

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

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

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

49

## 50 **1. Introduction**

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

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

73

## 74 **2. Materials and methods**

### 75 *2.1. Study site*

76 The study was conducted in Madagascar, at the Rural Development and Applied Research  
77 Center (Fifamanor), Antsirabe, during the rainy (February-March 2018) and dry seasons  
78 (September 2018). The center is located at 18°59' South and 46°17' East and at an altitude of  
79 1,644 m above sea level. Temperatures during the experimental period ranged from 14 to 22°C  
80 (average of 18°C) and 10 to 22°C (average of 16°C) during the rainy and dry season, respectively.

### 81 *2.2. Animals and diets*

82 Two experiments were conducted with sixteen Norwegian red pie sires. Average initial BW of  
83 the sires was 237±16 kg and 226±23 kg (mean±SD) during the rainy and dry season experiments,  
84 respectively. Each experiment lasted 13 days, with seven days of adaptation and six days of data  
85 collection. The animals were housed in individual pens and were randomly allocated to two  
86 different forage allowances: (i) *ad libitum* with a refusal of 5%; (ii) 1.1% DM of BW per day. Two  
87 forage-based diets that differ in composition, depending on the season were distributed to the  
88 animals (Table 1). The forage species were harvested at the vegetative stage and at ground level,  
89 every two days. All forages were weighed out, thoroughly mixed by hand, stored in a bag and fed  
90 fresh to animals. The forage species and their composition into the diets were chosen to represent  
91 the average composition of pastures during each season. The botanical composition of the diets of  
92 each season was estimated from surveys applied to farmers in the region and direct observations  
93 of the density, level of coverage and appearance of the plant species in the pasture. The animals  
94 received an accurately measured amount of the experimental diet twice a day at 7:00AM and  
95 3:00PM. The diet offered and refusals, that were collected daily shortly before the first morning

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

### 100 2.3. Sampling and laboratory analysis

101 Representative samples (~250 g) of feed offered and refusals were collected daily. From days  
102 11 to 13, approximately 500 g of faecal samples were collected twice daily by rectal grab sampling,  
103 with 12h intervals, advanced by 4h for the consecutive days. After collection, faecal samples were  
104 refrigerated, thoroughly mixed by hand, and pooled to create one composite sample per animal.  
105 Samples were dried at 60°C in a forced air oven for 72h for dry matter analysis, and ground to pass  
106 a 1-mm mesh prior to analysis. Total ash, crude protein (CP), and crude fibre (CF) were determined  
107 according to AOAC procedure (2005). Total ash was determined by incineration at 550°C during  
108 8h (Method No. 930.05), the CP concentration ( $6.25 \times N$ ) by the Kjeldahl method (Method No.  
109 978.04) and the CF by successive digestion with 1.25% dilute sulfuric acid and 1.25% dilute  
110 sodium hydroxide (Method No. 978.10). Neutral detergent fibre (NDF), acid detergent fibre (ADF)  
111 and acid detergent lignin (ADL) concentrations were determined according to Van Soest and  
112 Robertson (1985). The NDF concentration was determined without a heat stable amylase. Both  
113 NDF and ADF were expressed inclusive of residual ash. AIA concentration of feed and faeces  
114 were determined gravimetrically after boiling the ashes in hydrochloric acid 3N, filtering, washing  
115 the hot hydrolysate, and re-ashing, according to Van Keulen and Young (1977). For n-alkane  
116 analysis, ground samples were pulverized using a bullet mill (MM 2000; 4 min at 80 Hz; Retsch  
117 Technology GmbH, Haan, Germany) before extraction and analysis of n-alkanes as described by  
118 Mayes et al. (1986), using C22 and C34 alkanes as internal standards. The extracted samples were

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

### 121 2.3. Calculations

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

$$124 \text{ Herbage intake (kg DM/day)} = D_j / ((F_j / F_i) * H_i - H_j)$$

125 where  $F_i$ : concentration of natural odd-chain n-alkanes in faeces (C31 or C33, mg/kg DM);  $H_i$ :  
126 concentration of natural odd-chain n-alkane in herbs (C31 or C33, mg/kg DM);  $F_j$ : concentration  
127 of even-chain n-alkane in the faeces (C32, mg/kg DM);  $H_j$ : concentration of even-chain n-alkane  
128 in herbs (C32, mg/kg DM);  $D_j$ : concentration of even-chain n-alkane dosed to animals  
129 (C32, mg/day).

130 The herbage intake was also estimated from C32/AIA procedure using the formula (Ferreira et al.,  
131 2004):

$$132 \text{ Herbage intake (kg DM/day)} = \text{Faecal output} / (1 - \text{Digestibility})$$

133 Faecal output was calculated from the equation:

$$134 \text{ Faecal output (kg/day)} = D_j / F_j$$

135 where  $D_j$ : concentration of n-alkane dosed to animals (C32, mg/day) and  $F_j$ : concentration of even-  
136 chain n-alkane in faeces (C32, mg/kg DM).

137 Digestibility was calculated as:

$$138 \text{ Digestibility} = 1 - (C_i / C_f)$$

139 where  $C_i$  and  $C_f$  are the concentrations of AIA in diet and faeces, respectively (g/kg DM).



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

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

144 where actual = actual faeces concentration of marker  $i$  corrected for incomplete recovery;

145 calculated = calculated faeces concentration of marker, using the following formula:

146  $\text{calculated} = (xA_i + yB_i + zC_i)/(1 - \text{digestibility})$

147 where  $x$ ,  $y$  and  $z$  are the proportion of plant species A, B and C identified in the diet in each season,

148 respectively;  $A_i$ ,  $B_i$  and  $C_i$  represent the concentrations of marker  $i$  in plants A, B and C,

149 respectively. The diet botanical composition calculations were performed assuming all plant

150 species identified in each season. The n-alkanes from C27 to C35 were used in the calculations

151 because they were found in higher concentrations in the faeces. The n-alkane faecal recovery

152 (AFR) correction factors were taken from published studies, in order to assess the effect of the

153 AFR rates on the accuracy of the diet botanical composition estimates (Table 2).

154

#### 155 *2.4. Statistical analysis*

156 The effect of forage allowance level, the season and their interaction on the accuracy of feed intake

157 was analyzed as a split-split plot factorial analysis of variance, where season was the main plot,

158 forage allowance the split-plot, and method the split-split plot, according to the model:

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

160 Where  $Y_{ijkl}$  is the response variable,  $\mu$  represents the overall mean, R represents the random effect

161 of replication (in this case, animal), S represents the fixed effect of season (main plot), RS is the

162 interaction replication and season, F is the fixed effect of forage allowance (split-plot), SF is the

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

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

178

### 179 **3. Results**

#### 180 *3.1 Marker concentrations in herbage, diet and faeces*

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

186 alkanes (C31 to C33). Odd-chain n-alkanes were 93% of the total concentration of n-alkanes in  
187 the diet of both seasons. For diet, the greatest n-alkane concentration (183 mg/kg DM) occurred  
188 for C31 in dry season while the lowest (6 mg/kg DM) was for C28 in rainy season. In rainy season,  
189 the concentration of C31, C32 and C33 contributed to 34, 3, and 36% of the total n-alkane  
190 concentration, respectively. In dry season, n-alkanes C31, C32 and C33 contributed to 39, 2 and  
191 20% of the total n-alkane concentration, respectively. The greatest n-alkane concentration in faeces  
192 was attributed to C32 in dry season (560 mg/kg DM) while the lowest was for C28 in rainy season  
193 (6 mg/kg DM). The faecal concentration of C31, C32, and C33 in rainy season contributed to 20,  
194 43 and 23% of the total n-alkane concentration. In dry season, the faecal concentration of C31,  
195 C32, and C33 contributed to 25, 41 and 13% of the total n-alkane concentration. The greatest  
196 concentration of acid insoluble ash (54 g/kg DM) occurred in *Hyparrhenia rufa* while the lowest  
197 (10 g/kg DM) was in *Stylosanthes guyanensis*. The concentration of acid insoluble ash averaged  
198 44 g/kg DM and 68 g/kg DM in feed and faeces, respectively.

### 199 3.2. *Herbage intake*

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

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

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

### 219 *3.3. Diet botanical composition*

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

225 The most accurate estimate of diet botanical composition was with AFR1, with higher KSI values  
226 (76 and 86 in the rainy and dry season, respectively).

227

## 228 **4. Discussion**

### 229 *4.1. Marker concentrations in feed and faeces*

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

232 the even-chain n-alkanes is in agreement with the observations from other authors who reported  
233 that over 90% of the n-alkanes measured in plants have an odd number of carbon atoms (Bani et  
234 al., 2014). As expected, the concentration of C32 alkane in most plant species and diet was very  
235 low (<20 mg/kg DM). This was the reason for using C32 alkane as external marker for estimating  
236 herbage intake as its presence in plants is typically negligible (Hu et al., 2014). N-alkanes C29,  
237 C31, and C33 in forage species are also reported as the predominant hydrocarbons of the odd-  
238 chains (Ferreira et al., 2004), indicating their importance for estimating diet composition (Lewis  
239 et al., 2003). They are also suitable to be used as internal markers with the even-chain dosed n-  
240 alkane (C32) for estimating the herbage intake. A value of 50 mg/kg is the minimum threshold  
241 value for using an n-alkane as a marker (Laredo et al., 1991). For these reasons, C31 and C33 were  
242 chosen as the internal marker for estimating intake. The n-alkane profiles in faeces were  
243 characterized by an increase with the carbon chain length (Hu et al., 2014). Faecal concentration  
244 of n-alkanes reflected that of the consumed diet (Keli et al., 2008). The higher concentration of  
245 even chain C32 in faeces was due to the amount of dosed C32 alkane to the animals. The  
246 concentrations of AIA of the forage diet in this study were greater, compared to the threshold of  
247 7.5 g/kg DM for obtaining precise estimates of digestibility (Thonney et al., 1895).

#### 248 4.2. Factors influencing the accuracy of herbage intake estimate

249 According to our results, the herbage intake was underestimated by both pairs of n-alkanes  
250 (C31:C32 and C32:C33) during the dry season, and when the animals were fed *ad libitum*. In order  
251 to obtain accurate estimates of herbage intake, the faecal recoveries of the two n-alkanes of each  
252 pair must be similar (Oliván et al., 2007; Keli et al., 2008), so that the errors associated with the  
253 incomplete recoveries cancel out in the equation (Sun et al., 2008). Underestimation of intake  
254 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

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

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

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

#### 316 *4.3. Accuracy of diet botanical composition estimate*

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



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

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

336

## 337 **5. Conclusions**

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

346

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352

### 353 **References**

354 Ahvenjärvi, S., Nyholm, L., Nousiainen, J., Mäntysaari, E.A., Lidauer M. Polyethylene glycol as  
355 an indigestible marker to estimate fecal output in dairy cows. *J. Dairy Sci.* 101:4245–4258.  
356 <https://doi.org/10.3168/jds.2017-13337>.

357 Amaral, G.A., Genro, T.C., Neto, A.B., Savian, J.V., David, D.B., Azevedo, E.B., Jochims, F.,  
358 Carvalho, P.C., 2013. Use of n-alkane technique to estimate sheep dry matter intake.  
359 Revitalising grasslands to sustain our communities. Conference paper in Proceedings of the  
360 22nd International Grassland Cong. 22: 704-705. <https://doi.org/10.13140/2.1.4676.0969>.

361 AOAC, 2005. Official method of Analysis. 18th Edition, Association of Official Analytical  
362 Chemists, Washington DC, USA.

363 Bani, P., Cappelli, F.C., Minuti, A., Ficuciello, V., Lopreiato, V., Garnsworthy, P.C., Trevisi, E.,  
364 2014. Estimation of dry matter intake by n-alkanes in dairy cows fed TMR: effect of dosing  
365 technique and faecal collection time. *Anim. Prod. Sci.* 54, 1747-1751.  
366 <http://dx.doi.org/10.1071/AN14342>.

367 Berry, N.R., Scheeder, M.R., Sutter, F., Kröber, T.F., Kreuzer, M., 2000. The accuracy of intake  
368 estimation based on the use of alkane controlled-release capsules and faeces grab sampling in  
369 cows. *Ann. Zootech.* 49, 3-13. <https://doi.org/10.1051/animres:2000104>.

- 370 Bezabih, M., Pellikaan, W.F., Tolera, A., Hendriks, W.H., 2012. Estimation of feed intake and  
371 digestibility in cattle consuming low-quality tropical roughage diets using molasses-based n-  
372 alkane boluses. *Anim. Feed Sci. Technol.* 177, 61–171.  
373 <http://dx.doi.org/10.1016/j.anifeedsci.2012.08.014>.
- 374 Chavez, S.J., Huntington, G.B., Burns, J.S., 2011. Use of plant hydrocarbons as markers to  
375 estimate voluntary intake and digestibility in beef steers. *Livest. Sci.* 139, 245–251.  
376 <https://doi.org/10.1016/j.livsci.2011.01.012>
- 377 Dillon, P.G., 1993. The use of n-alkanes to determine herbage intake, botanical composition of  
378 available or consumed herbage and in studies of digesta kinetics with dairy cows. PhD  
379 Dissertation. Dublin, National University of Ireland. 272 p.
- 380 Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substance in studies of  
381 nutrition herbivores: a review. *Aust. J. Agric. Res.* 42: 913-952.  
382 <https://doi:10.1071/AR9910913>.
- 383 Dove, H., Scharch, C., Oliván, M., Mayes, R.W., 2002. Using n-alkanes and known supplement  
384 intake to estimate roughage intake in sheep. *Anim. Prod. Aust.* 24, 57–60.
- 385 Ferreira, L.M., Oliván, M., Rodrigues, M.A., Osoro, K., Dove, H., Dias-da-Silva, A., 2004.  
386 Estimation of feed intake by cattle using controlled-release capsules containing n-alkanes or  
387 chromium sesquioxide. *J. Agric. Sci. Camb.* 142, 225–234.  
388 <https://doi.org/10.1017/S0021859604004320>.
- 389 Ferreira, L.M., Oliván, M., Garcia, U., Rodrigues, M.A., Osoro, K., 2005. Validation of the alkane  
390 technique to estimate diet selection of goats grazing heather-gorse vegetation communities. *J.*  
391 *Sci. Food Agric.* 85, 1636–1646. <https://doi.org/10.1002/jsfa.2162>.

- 392 Ferreira, L.M., Celaya, R., Garcia, U., Rodrigues, M.A., Osoro, K., 2009. Differences between  
393 domestic herbivores species in alkane faecal recoveries and the accuracy of subsequent  
394 estimates of diet composition. *Anim. Feed Sci. Technol.* 151, 128–142.  
395 <https://doi.org/10.1016/j.anifeedsci.2008.11.003>.
- 396 Halfa, A.N., 2012. Examining mechanisms contributing to the biological variation of residual feed  
397 intake in growing heifers and bulls and in mid-gestation females. PhD Dissertation. College  
398 Station, TX, USA. Texas A&M University.
- 399 Hargrove, J.L., Greenspan, P., Hartle, D.K., 2004. Nutritional significance and metabolism of very  
400 long chain fatty alcohols and acids from dietary waxes. *Exp.biol. med.* 239 (3), 215-226.  
401 <https://doi.org/10.1177/153537020422900301>.
- 402 Hu, H., Liu, Y., Li, Y., Lu, D., Gao, M., 2014. Use of the n-alkanes to estimate intake, apparent  
403 digestibility and diet composition in sheep grazing on *Stipa breviflora* desert steppe. *J. Integr.*  
404 *Agric.* 13 (5): 1065-1072. [https://doi.org/10.1016/S2095-3119\(13\)60502-X](https://doi.org/10.1016/S2095-3119(13)60502-X).
- 405 Keli, A., Andueza, D., De Vega, A., Guada, J.A., 2008. Validation of the n-alkane and NIRS  
406 techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne:  
407 ryegrass diets. *Livest. Sci.* 119 (1-3): 42–54. <https://doi.org/10.1016/j.livsci.2008.02.011>.
- 408 Laredo, M.A., Simpson, G.D., Minson, D.J. and Orpin, C.G., 1991. The potential for using n-  
409 alkanes in tropical forages as a marker for determination of dry matter intake by grazing  
410 ruminants. *J. Agric. Sci. Camb.* 117 (3): 355-361.  
411 <https://doi.org/10.1017/S0021859600067101>.
- 412 Lewis, R.M., Magadlela, A.M., Jessop, N.S., Emmans, G.C., 2003. The ability of the n-alkane  
413 technique to estimate intake and diet choice of sheep. *Anim. Sci.* 77 (2): 319–327.  
414 <https://doi.org/10.1017/S1357729800059051>.

- 415 Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as  
416 markers for the determination of herbage intake. *J. Agric. Sci. Camb.* 107, 161–170.  
417 <https://doi.org/10.1017/S0021859600066910>.
- 418 Morais, J. A., T. T. Berchielli, A. de Vega, M. F. Queiroz, A. Keli, R. A. Reis, L. M. Bertipaglia,  
419 and S. F. Souza. 2011. The validity of n-alkanes to estimate intake and digestibility in Nellore  
420 beef cattle fed a tropical grass (*Brachiaria brizantha* cv. *Marandu*). *Livest. Sci.* 135, 184–192.  
421 <https://doi.org/10.1016/j.livsci.2010.07.004>.
- 422 Oliván, M., Ferreira, L.M., Celaya, R., Osoro, K., 2007. Accuracy of the n-alkane technique for  
423 intake estimates in beef cattle using different sampling procedures and feeding levels. *Livest.*  
424 *Sci.* 106, 28–40. <https://doi.org/10.1016/j.livsci.2006.06.015>.
- 425 Richmond, A.S., Wylie, A.R., Laidlaw, A.S., Lively, F.O., 2015. An evaluation of contrasting C32  
426 alkane dosing and faecal sampling regimes to estimate herbage dry matter intake by beef cattle.  
427 *J. Agric. Sci. Camb.* 153, 353-360. <https://doi.org/10.1017/S0021859614000410>.
- 428 Sales, J., Janssens, G.P., 2003. Acid-insoluble ash as a marker in digestibility studies: a review. *J.*  
429 *Anim. Feed Sci.* 12, 2003, 383–401. <https://doi.org/10.22358/jafs/67718/2003>.
- 430 Sun, Z.W., Zhou, D.W., Ferreira, L.M., Zhong, Q.Z., Lou, Y.J., 2008. Diet composition, herbage  
431 intake and digestibility in Inner Mongolian cashmere goats grazing on native *Leymus chinensis*  
432 plant communities. *Livest. Sci.* 116, 146-155. <https://doi.org/10.1016/j.livsci.2007.09.018>.
- 433 Thonney, M.L., Palhof, B.A., DeCarlo, M.R., Ross, D.A., Firth, N.L., Quaas, R.L., Perosio, D.J.,  
434 Duhaime, D.J., Rollins, S.R., Nour, A.Y.M., 1985. Sources of variation of dry matter  
435 digestibility measured by the acid insoluble ash marker. *J. Dairy Sci.* 68(3): 661-668.  
436 [https://doi.org/10.3168/jds.S0022-0302\(85\)80872-9](https://doi.org/10.3168/jds.S0022-0302(85)80872-9).

- 437 Unal, Y., Garnsworthy, P.C., 1999. Estimation of intake and digestibility of forage-based diets in  
438 group-fed dairy cows using alkanes as markers. *J. Agric. Sci.* 133, 419–425.  
439 <https://doi.org/10.1017/S0021859699007145>.
- 440 Van den Pol-Van Dasselaar, A., Valk, H., de Visser, M., 2006. Recovery of n-alkanes in manure  
441 of dairy cows fed fresh grass supplemented with maize silage. Sustainable grassland  
442 productivity, proceedings of the 21st general meeting of the European Grassland Federation'.  
443 (Eds J Lloveras, A González-Rodríguez, O Vázquez-Yañez, J Piñeiro, O Santamaría, L Olea,  
444 MJ Poblaciones) pp. 451–453. (Badajoz, Spain).
- 445 Van Keulen, J., Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in  
446 ruminant digestibility studies. *J. Anim. Sci.* 44, 282-287.  
447 <https://doi.org/10.2527/jas1977.442282x>.
- 448 Van Soest, P.J., Robertson, J.B., 1985. Analysis of forages and fibrous foods: A laboratory manual  
449 for Animal Science 613. Department of Animal Science, Cornell University, Ithaca, New York,  
450 USA.
- 451

452 Table 1

453 Botanical and chemical composition of the experimental diet

	Rainy Season		Dry Season	
	Mean	S.E.M.	Mean	S.E.M.
Botanical composition				
<i>Aristida multicaulis</i>	0.05	-		
<i>Chrysopogon serrulatus</i>			0.05	-
<i>Cynodon dactylon</i>			0.27	-
<i>Hyparrhenia rufa</i>	0.51	-		
<i>Imperata cylindrica</i>	0.08	-	0.08	-
<i>Leersia hexandra Sw.</i>			0.10	-
<i>Lolium multiflorum</i>			0.50	-
<i>Stylosanthes guyanensis</i>	0.11	-		
<i>Urochloa brizantha</i>	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

454 S.E.M. standard error of mean

455 Table 2

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

	AFR1	AFR2	AFR3	AFR4
	Dillon (1993)	Bezabih et al., 2012	Morais et al., 2011	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

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



459 Table 3

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

	n-alkanes (mg/kg DM)								AIA (g/kg DM)
	C27	C28	C29	C30	C31	C32	C33	C35	
Herbage components									
<i>Aristida m.</i>	7.21	4.04	13.89	6.11	71.45	11.48	73.90	24.40	37.84
<i>Chrysopogon s.</i>	14.23	4.31	30.57	6.68	58.37	10.25	53.98	21.52	52.50
<i>Cynodon d.</i>	14.36	5.97	37.51	8.40	76.28	12.04	84.46	32.60	50.66
<i>Hyparrhenia r.</i>	17.54	5.09	42.49	8.03	155.07	10.29	165.02	51.28	54.13
<i>Imperata c.</i>	17.51	9.45	58.63	18.81	220.71	18.47	186.11	46.46	29.45
<i>Leersiah.</i>	13.60	4.21	29.55	5.91	56.01	9.12	52.52	21.54	52.80
<i>Lolium m.</i>	31.60	9.98	176.35	14.60	249.87	9.65	78.98	6.21	20.98
<i>Stylosanthes g.</i>	5.12	4.01	26.38	6.74	259.34	20.67	273.64	3.83	9.50
<i>Urochloa b.</i>	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

462 AIA. Acid insoluble ash

463 Table 4

464 Effect of season and forage allowance on herbage intake estimates

Herbage intake kg DM/day	Season			Forage allowance		
	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	

465 S.E.M. standard error of mean

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

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

470 Table 5

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

Seasons	Treatment	<i>Herbage species</i>					KSI
		<i>Aristida m.</i>	<i>Urochloa</i> <i>b.</i>	<i>Hyparrhenia</i> <i>r.</i>	<i>Imperata</i> <i>c.</i>	<i>Stylosanthes</i> <i>g.</i>	
	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.	***	***	***	**	***	
		<i>Chrysopogon</i> <i>s.</i>	<i>Cynodon</i> <i>d.</i>	<i>Imperata c.</i>	<i>Leersia h.</i>	<i>Lolium m.</i>	
	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.	***	***	***	**	**	

473 S.E.M. standard error of mean

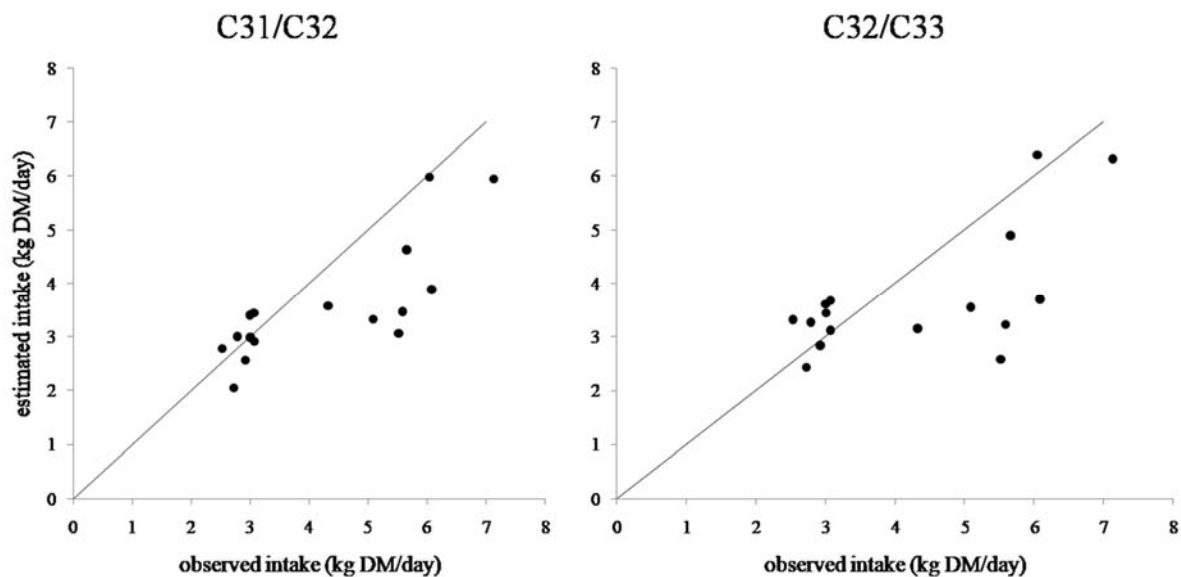
474 AFR is the mean n-alkane faecal recovery data obtained from published studies: AFR1 from Dillon  
475 (1993) as reported by Van den Pol-Van Dasselaar et al. (2006); AFR2 from Bezabih et al. (2012);  
476 AFR3 from Morais et al. (2011); AFR4 from Oliván et al. (2007).

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

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

480 \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

481 Fig 1. Relationship between observed and predicted intake for C31:C32 and C32:C33  
482 C31:C32 [ $Y = (0.56 * X) + 1.17$ ;  $R^2 = 0.61$ ;  $RMSE=0.71$  ;  $CV=20\%$  ;  $P<0.001$ ]; C32:C33 [ $Y =$   
483  $(0.48 * X) + 1.65$  ;  $R^2=0.41$  ;  $RMSE=0.93$  ;  $CV=25\%$  ;  $P<0.01$ ]



484