1   Estimation of feed intake and digestibility in Zebu type Arsi steers fed natural pasture using the

2   n-alkane technique

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32   **Abstract**

33   An experiment was conducted to validate the use of n-alkanes technique to estimate feed intake

34   and digestibility in cattle under the sub-humid tropical conditions. The experiment was conducted

35   using Zebu type Arsi steers fed natural pasture at different levels of dry matter intake (DMI). Eight

36   animals, blocked into four groups based on body weight (BW), were used for the experiment. The

37   animals in each group were randomly assigned to either low intake (0.011 dry matter (DM) of

38   BW) or high intake (*ad libitum* at − 0.05 refusal rate) diet. The animals were housed in individual

39   pens, and each animal was dosed twice daily with paper bung containing 400 mg C_{32} alkane using

40   a balling gun for 15 days. Animals received pasture diets twice a day (at 8:00 and 16:00 h) with

41   half of the daily allocation offered at each feeding. Feed intake, refusal, and total fecal outputs

42   were recorded, weighed and sub sampled for proximate and n-alkane concentrations analysis. The

43   odd-chain n-alkanes comprised the highest percentage during both wet and dry seasons. The

44   alkanes C_{29}, C_{31} and C_{33} were present in concentrations greater than 59 mg/kg DM in the two

45   seasons. The mean fecal recovery rates ranged from 0.49 to 0.79 for low and 0.62 to 0.99 for high

46   intake group, respectively during the wet season, whereas 0.68 to 1.05 for low and 0.61 to 0.9 for

47   high intake group during the dry season, respectively. The DMI predictions using the double n-

48   alkane technique were affected by season (P<0.05). The C_{31}/C_{32} and C_{33}/C_{32} n-alkane pairs

49   accurately estimated the DMI regardless of intake levels during the wet season. During the dry

50   season, the prediction for the low intake level improved after feacal recovery corrections, whereas

51   that for the high intake level were accurate both with or without fecal recovery corrections.

52   Moreover, using C_{35} alkane as internal marker provided an accurate estimate of dry matter

53   digestibility (DMD) during both seasons. The results obtained in this study confirm the accuracy

54   of the *n*-alkane markers to estimate DMI and DMD in cattle consuming different levels of wet and

55 dried pasture. However, accuracy can be reduced for digestibility estimation at higher levels of

56 intake which need further validation.

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58 *Keywords*: cattle; digestibility; estimation; grazing pasture; intake; n-alkane

59

60 *Abbreviations:* ADF, acid detergent fiber expressed inclusive of residual ash; ADL, acid detergent

61 lignin; BW, body weight; CP, crude protein; D, diet; DM, dry matter; DMD, dry matter

62 digestibility; DMI, dry matter intake; EE, ether extract; FID, flame ionization detector; FO, fecal

63 output; GC, gas chromatograph; HI, high intake; ISwt, internal standard weight; IAEA,

64 international atomic energy agency; LI, low intake; N, nitrogen; NAS, natural and agricultural

65 science; NDF, neutral detergent fiber assayed without a heat stable amylase and expressed

66 inclusive of residual ash; OM, organic matter; OARI, Oromia Agricultural Research Institute; S,

67 season; SD, standard deviation; SDW, sample dry weight; SRF, standard response factor

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78 **1. Introduction**

79   Livestock serve as a source of income and food security and also an integral component for

80 most of the agricultural activities in the country (Mengistu et al., 2017). The performance of

81 animals is mainly limited by inadequate nutrition both in terms of quantity and quality. The main

82 feed resources used for livestock production in Ethiopia include natural pasture, crop residues,

83 improved forages and agro-industrial by-products, of which the first two are the most important

84 (Tolera et al., 2012). Recently, the share of natural grazing pasture at the national level as livestock

85 feed resource, has become reduced to about 57% (CSA, 2013). The production performance of

86 grazing ruminants, within their genetic boundaries, depends on the level of nutrient intake (Tolera

87 et al., 2012). Since providing feed for animals can represent up to 65% of total production costs

88 (Arthur et al., 2004) and also to meet nutritional requirements of the animal (Mayes and Dove,

89 2000), accurate measure of feed intake and digestibility is necessary to evaluate production

90 efficiency. However, estimation of feed intake and digestibility are difficult and complicated in

91 grazing conditions due to limitations of available methods of measurement (Keli et al., 2008).

92   In the late 1980, the *n*-alkanes of plant cuticular wax were used as markers to indirectly

93 estimate feed intake (Mayes et al., 1986). The n-alkane marker technique uses a combination of

94 internal and external markers to estimate intake (Mayes et al., 1986; Dove and Mayes, 1991) and

95 digestibility (Unal and Garnsworthy, 1999) of the diet. The advantages of the n-alkane method

96 over other approaches include low invasiveness, accuracy and the possibility of taking into account

97 diet-animal interactions (Dove and Mayes, 1991; Mayes and Dove, 2000). In addition, n-alkanes

98 are chemically discrete components which can be easily analyzed by gas chromatography. A

99 crucial point in the analysis of a marker is its recovery rate, the ratio of the excreted concentration

100 of that marker over that of the ingested amount. Dove and Mayes (1996) explained that the error

101 in intake estimation is proportional to the fecal recovery difference between the dosed and natural

102 n-alkanes.

103 Different scholars support the recommendation that the n-alkane method needs diet and species

104 specific trials to increase the accuracy of its predictions since lower fecal recovery rate were

105 observed for tropical forage species compared to temperate species (Ferreira et al., 2009; Bezabih

106 et al., 2012). Similarly, environmental conditions and geographical locations could influence the

107 pattern of the cuticular wax profile of plant species growing in different places (Samuels et al.,

108 2008). Although n-alkane technique as feed intake and digestibility estimation is widely applied

109 in other parts of the world, its validation was done only in the Mid Rift Valley grassland of Ethiopia

110 (Bezabih et al., 2012), and no information is available on its applications in the highlands of

111 Ethiopia, where the pasture composition is distinctly different from the Rift Valley grasslands due

112 to its sub-humid tropical agro-ecology. Therefore, the objectives of this study were to measure the

113 fecal recovery rate of n-alkanes from zebu type Arsi steers fed different levels of pasture forage

114 from the central highlands of Ethiopia and to validate feed intake and digestibility estimation both

115 during wet and dry seasons by using n-alkane technique. The information generated would help to

116 build on the pool of knowledge available for wider application under tropical conditions.

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118 **2. Materials and Methods**

119 *2.1. Study area description*

120 This study was conducted in Kofele district, West Arsi Zone of Oromia Regional state,

121 Ethiopia situated at 7°07'N and 38°48'E at an altitude of 2660 m above sea level during wet

122 (December, 2017) and dry (April, 2018) seasons. The long term average rainfall and temperature

123 per annum of the district are 1800 mm and 19.5 °C, respectively and the district has bi-modal

124 rainfall distribution with the short rain lasting from March to May and the main rainy season

125 extending from June to September/October. The district is predominantly a loam soil type. The

126 area has a high potential for crop-livestock farming system, where cattle and sheep are the most

127 predominant livestock species (CSA, 2015). The natural pasture of the experimental site is

128 dominated by *Pennisetum thunbergii* and *Andropogon amethystinus* and additionally consisted

129 mainly other grasses such as *Ischaemum afrum, Sporobolus pyramidalis, Eragrostis botryodes,*

130 *Poa leptoclada, Helictotrichon elongatum, Brachiaria scalaris,* legumes such as *Trifolium*

131 *cryptopodium, Trifolium mattirolianum, Trifolium rueppellianum, Trifolium simense, Trifolium*

132 *tembense,* sedges which include *Cyperus rigidifolius, Scleria schimperiana, Scleria hispidula* and

133 other herbs like *Centella asiatica, Uebelinia abyssinica, Haplocarpha hastata, Satureja paradoxa*

134 *and Oldenlandia monanthos.*

135 *2.2. Experimental animals and housing*

136　　The experiment was conducted in two rounds, the first being in the main rainy (wet) season

137 and the second during the dry season. Eight Zebus type Arsi steers aged about 48 months were

138 purchased from the local market and used during each of the trial periods. For the wet season trial,

139 the average initial live weight of the animals was 148.1 ± 9.18 and that for the dry season trial was

140 155.4 ± 4.8 kg. Up on arrival at the experimental site, the steers were treated with Albendazole to

141 control internal parasites and fed a diet similar to the subsequent feeding period *ad libitum* with

142 free access to water for 21 days. A temporary experimental shed was constructed at the grazing

143 site to provide protection to the animals from strong cold weather of the highland area and allow

144 individual feeding and observation. The shed was partitioned into 2 m x 1.5 m pens which

145 contained a separate feeding and watering troughs. The steers were handled and maintained

146 throughout the experiment according to the experimental protocol approved by the Animal Ethics

147 Committee for animal research of the University of Pretoria (NAS086/2019).

148 *2.3. Experimental forages and diets*

149 Natural pasture harvested from enclosed grassland was used for the current study. The

150 harvested pasture diet used for the wet season trial contained five forage species, whereas the one

151 used for the dry season trial contained six forages species (Table 1). For wet season trial, the

152 'pasture' diets were created by cutting the fresh pasture daily and forming a mixture of the

153 dominant species by excluding all sedges, forbs and other herbs which were found in small

154 quantities. For the dry season trial, standing hay which was harvested and stored was used for the

155 trial. The species composition of the hay used for the trial was calculated based on pre-determined

156 quadrants for each species composition determination during wet season when forage species were

157 easily identified. The pasture diets were chopped to $3 - 4$ cm before feeding to the animals during

158 the whole experimental periods.

159 *2.4. Experimental design and procedure*

160 The experimental design used was a randomized complete block design with factorial

161 arrangement of the treatments. Animals were blocked into four groups of two animals based on

162 BW predicted from heart girth measurement. Animals in each group were randomly assigned to a

163 low intake (11 g DM/kg BW) or a high intake (*ad libitum* – at a refusal level of 50 g per kg feed

164 offered) pasture diets and housed in individual pen with individual access to feed and water. The

165 low intake level was considered to be equivalent to maintenance level of intake. The experiment

166 lasted for 15 days, which included 5 days of adaptation and 10 days of feeding and data collection.

167 During the experimental period, each animal was dosed with a paper bung containing 400 mg C_{32}

168 alkane (n-dotriacontane) twice daily (at 6:00 h and 18:00 h) using a balling gun. Animals received

169   pasture diets twice a day (at 8:00 and 16:00 h) with half of the daily allocation offered at each

170   feeding. Feed intake, refusal and total fecal outputs were recorded during the last 5 days of the

171   feeding period. The amounts of feed offered were weighed by using a scale during diet offer time

172   (morning and afternoon) while diet refusals were removed and weighed once before the next day

173   morning feeding time. Water was provided freely and 20 g sodium chloride per animal was given

174   daily together with water each morning. Similar procedures were implemented during both wet

175   and dry season trials.

176   *2.5. Sampling and sample preparation*

177   Samples of pasture (wet season) and standing hay (dry season) fed to each animal (200 g at

178   each feeding time) were taken and pooled across the trial for each intake group. Diet refusals were

179   collected from days 11 to 14, from each animal which has any and weighed and pooled for the

180   four days and the mixed refusals were sub sampled to create one sample per animal per experiment

181   of 500 g fresh weight. Total feces were collected twice daily (days 13 to 15), weighed and after

182   being homogenized, sub-samples of 200 g fresh weight per collection were taken from each animal

183   to create one sample per animal per experiment for chemical and n-alkane analysis. Feces samples

184   were retained in refrigerator at − 20 °C during the collection period, mixed thoroughly after all

185   samples were collected and sub sampled to create one sample per animal per period. Feeds, refusals

186   and feces samples were partially dried at 60 °C in a forced draft oven for 48 h and ground to pass

187   through a 1 mm mesh size sieve and stored in plastic bags.

188   *2.6. Chemical analysis*

189   Chemical compositions of feeds, refusals and feces samples were analyzed for DM, ash, neutral

190   detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) at the animal

191   nutrition laboratory of Hawassa University. Crude protein (CP) and ether extract (EE) were

192   performed at the animal nutrition laboratory of National Veterinary Institute, Debre Zeit, Ethiopia.

193   Samples were analyzed for DM (method 934.01), ash (method 942.05), EE (method 954.02) and

194   N (method 954.01; CP = N x 6.25) by using Kjeldahl method according to AOAC (2006). Organic

195   matter (OM) content was determined as $100 - \%ash$. NDF content was determined according to

196   method of Van Soest et al. (1991) whereas ADF and ADL contents were determined according to

197   method of Van Soest and Robertson (1985). Heat stable amylase was not added and both NDF and

198   ADF were expressed inclusive of residual ash.

199   N-alkane extraction and analysis was conducted at isotope nutrition laboratory of James Hutton

200   Institute, UK. N-alkane for feed and feces samples were extracted and analyzed in duplicate by

201   gas chromatography (GC) according to the method of Dove and Mayes (2006) using n-

202   tetratriacontane (C_{34}) as an internal standard with a minor modification. For GC analysis, the

203   derivatised extract was injected (1 μl) into a Trace (Thermo Finnegan) gas chromatograph fitted

204   with a splitless injector (running in splitless mode at 275 °C, with a splitless time of 5 min) and

205   flame ionization detector (FID), using helium (flow rate of 1 ml/min) as the carrier gas. The GC

206   column was a non-polar bonded-phase capillary type Rtx-5 MS (Restek) (30 m × 0.25 mm i.d. ×

207   0.25 μm film thickness). The temperature used for the GC column oven was 170 °C for 5 min; 30

208   °C/min to 210 °C; held at 210 °C for 1 min; 5.3 °C/min to 320 °C; held at 320°C for 12 min.

209   *2.7. Calculations*

210   The concentration of n-alkane was calculated according to the following formula:

211    $$\text{Alkane}_i \text{ (mg/kg DM)} = \frac{[10 \times \text{area \% alkane}_i \times C_{34} \text{ IS wt (mg)}]}{\text{SDW} \times \text{SRF}_i}$$
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213   Where C_{34} IS wt is internal standard solution (g) $\times$ C_{34} concentration in standard solution (mg/g);

214   SDW is sample dry weight and SRF_i is the standard response factor for alkane$_i$, calculated as area

215   % alkane$_i$ in the mixed standard divided by weight % alkane$_i$ in the mixed standard.

216   Fecal recovery of each alkane was calculated as the proportion of ingested compound, which

217   was recovered in feces as follow:

218    $R_i = \dfrac{(FO \times F_i)}{(DMI \times H_i)}$,

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221   Where R_i is the fecal recovery rate of alkane$_i$, FO is the daily fecal output (kg DM), F_i is the

222   concentration of alkane$_i$ in feces (mg/kg DM), DMI is the daily dry matter intake (kg), and H_i is

223   the concentration of alkane$_i$ in the diet consumed (mg/kg DM).

224   Feed intake was estimated by using the double n-alkane method according to Mayes et al.

225   (1986) using the following formula:

226   Daily diet intake (kg DM) $= \dfrac{((F_i/F_j) \times D_j)}{(H_i\text{-}F_i/F_j\times H_j)}$,

227

228

229   Where F_i represents the feacal and H_i the herbage odd-chain alkane$_i$ concentrations (mg/kg DM),

230   F_j resembles the fecal and H_j the herbage even-chain alkane$_j$ concentrations (mg/kg DM), and D_j

231   equals the daily dose of even-chain alkane$_j$. Intake estimate were generated using both C_{31}/C_{32} and

232   C_{33}/C_{32} alkane pairs.

233   Apparent dry matter digestibility (DMD) estimates were calculated by using natural C_{35} alkane

234   as an internal marker according to the following formula:

235   DMD (%) $= \dfrac{(FC_{35} - DC_{35})}{FC_{35}}$

236

237   Where FC_{35} is the fecal C_{35} concentration (corrected for incomplete recovery) and DC_{35} is the

238   dietary C_{35} concentration.

239   *2.8. Statistical analysis*

240   The effect of feeding level, season and their interaction on the n-alkane fecal recoveries, feed

241   intake and dry matter digestibility estimates were assessed by analysis of variance using General

242   Linear Model (GLM) Procedure of Statistical Analysis System (SAS, version 9.0). Block effect

243 was initially included in the model as its effect was not different; it was removed from the final

244 analysis as indicated in the following model:

245     $Y_{ijk} = \mu + D_i + S_j + D*S_{ij} + e_{ijk}$

246 Where, Y_{ijk} is the dependent variable, μ is the overall mean; D_i is the fixed effect of diet (i =1 − 2),

247 S_k the random effect of season (k = 1 − 2), $D*S_{ij}$ the interaction between diet and season and e_{ijk} is

248 the error term. Multiple comparisons among means were determined by Tukey test at 5%

249 probability. To compare the accuracy of measured and estimated feed intake and dry matter

250 digestibility, paired *t*-test were performed.

251

252 **3. Results**

253 *3.1. Proportions and chemical composition of the experimental diets*

254   Chemical composition and diet proportions used during wet and dry season experiments are

255 presented in Table 1. The proportions of *Pennisetum thunbergii* was 0.65 and 0.51 from the total

256 pasture used during wet and dry season experiments, respectively. Similarly, *Andropogon*

257 *amethystinus* was comprised 0.26 and 0.37 from the total pasture used during wet and dry season

258 experiments, in that order. Crude protein concentrations were 68.8 and 37.6 g/kg DM for wet and

259 dry season diets, respectively whereas the NDF concentrations were 697.1 and 712.4 g/kg DM in

260 that order for wet and dry season diets.

261 *3.2. N-alkane concentrations and fecal recovery*

262   The n-alkane profiles of the diets used during the experiments are shown in Table 2. The

263 concentrations of C_{22} and C_{34} are not presented as they were added to samples at the beginning of

264 the analysis as internal standards for gas chromatography (GC) analysis. The proportions of odd-

265 chain n-alkanes comprised 0.93 and 0.92 from the total alkane content in the diet during wet and

266 dry season, respectively. N-alkane such as C_{29}, C_{31} and C_{33} presented with greater than 59 mg/kg

267 DM concentrations in the two diets and they also made up 0.86 and 0.83 of the total odd-chain

268 proportion for wet and dry season diets in that order. Apart from these three alkanes, other alkanes

269 were presented in low concentration in the two diets. Total n-alkane concentrations for carbon

270 chains lengths C_{23} to C_{35} were 298.6 mg/kg DM during the wet season diet (fresh forage), and

271 301.5 mg/kg DM during the dry season diet (dried hay), excluding C_{34} as it was used as internal

272 standard in the alkane analyses.

273 The mean fecal recoveries of n-alkanes for animals provided different levels of diets during

274 wet and dry seasons are shown in Table 3. From combined analysis data, there were no interactions

275 between the level of diet provision and season on fecal recovery as carbon chain length increases

276 ($C_{27} - C_{35}$) except for C_{29}, but interactions were observed (P<0.05) for lower carbon chain lengths.

277 Similarly, interactions were observed (P=0.02) between the level of diet provision and season on

278 fecal recovery for odd to adjacent even-chain alkane pairs (C_{31}/C_{32} and C_{33}/C_{32}). Fecal recovery

279 for higher carbon chain lengths ($C_{27} - C_{35}$ except C_{29}) were not affected for the level of diet

280 provision (low or high), but higher (P<0.05) fecal recovery were observed for the dry season

281 compared to the wet season except for C_{32}. The mean fecal recovery rates ranged from 0.49 to 0.79

282 and 0.62 to 0.99 during the wet season and from 0.68 to 1.05 and 0.61 to 0.9 during the dry season,

283 for low intake and high intake groups, respectively. Full recoveries were achieved from the present

284 study for C_{31} (1.02) and C_{33} (1.05) for low intake groups during the dry season. The average ratio

285 between the fecal recovery of dosed even-chain and adjacent odd-chain alkanes were 0.84 and

286 0.82 for C_{31}/C_{32} and 0.88 and 0.84 for C_{33}/C_{32}, respectively for the low intake and high intake

287 groups, during the wet season trial.

288 *3.3. Estimate of diet intake and digestibility using n-alkane technique*

289   Table 4 shows mean measured and estimated DMI by using C_{31} and C_{33} as internal markers

290   according to C_{31}/C_{32} and C_{33}/C_{32} pairs used for the calculations. There was no interaction between

291   level of diet provision and season on measured and estimated DMI, whereas the level of diet

292   provision was affected (P<0.01) for all groups as intentionally done from the beginning (low and

293   high intake provision). Season effect was different for the measured DMI (P=0.01) and estimated

294   DMI (P<0.05) after fecal recovery correction, but no differences were observed before fecal

295   recovery correction. Assuming similar fecal recovery between adjacent odd-and-even chain alkane

296   pair during the wet season, the C_{31}/C_{32} and C_{33}/C_{32} pairs underestimated DMI by 0.16 and 0.12 for

297   low intake group and 0.18 and 0.16 for high intake group, respectively. On the contrary, the two

298   alkane pairs accurately predicted DMI for both low and high intake groups with only 0 to 0.014

299   overestimations than the measured DMI after fecal recovery correction.

300   During the dry season, the C_{31}/C_{33} and C_{33}/C_{32} pairs overestimated DMI by 0.22 and 0.26,

301   respectively, for the low intake group by assuming similar fecal recovery. But, after fecal recovery

302   correction, the two alkane pairs accurately predicted the DMI with only 0.06 over estimation from

303   the measured DMI. On the other hand, during the dry season, the two alkane pairs (C_{31}/C_{33} and

304   C_{33}/C_{32}) accurately predicted the DMI both before and after fecal recovery correction for high

305   intake group which did not differ from the mean measured DMI. The coefficient deviation of the

306   estimated DMI from the measured DMI before and after fecal recovery correction followed

307   consistent trends during both seasons for the two alkane pairs.

308   Estimated and measured DMD by using C_{35} alkane as internal marker is shown in Table 5.

309   There was no interaction between levels of diet provision and season on the measured DMD. On

310   the other hand, interaction between level of diet provision and season was observed (P=0.04) for

311   the estimated DMD. Dry matter digestibility coefficient was higher (P=0.001) for the wet season

312   compared to the dry season. Dry matter digestibility was accurately estimated by using C_{35} alkane

313   as internal marker after fecal recovery correction which was not different from the measured DMD.

314   On the contrary, DMD were different ($P<0.05$) for measured and estimated during both seasons

315   for high intake group.

316

317   **4.   Discussion**

318   *4.1. N-alkane concentration and alkane fecal recovery*

319   In most herbage species, large odd chain molecules account for the bulk of n-alkanes present

320   in the plant cuticular wax and over 0.9 of *n*-alkanes have odd-numbers of carbon atoms, with C_{29},

321   C_{31} and C_{33} alkanes being dominant in most pasture species (Mayes et al., 1995; Peiretti et al.,

322   2006). Our findings agrees with the previous results in which odd-chain alkanes accounted for

323   over 0.92 (Table 2) and the three odd-chain alkanes (C_{29}, C_{31} and C_{33}) were predominant

324   hydrocarbons as reported by Dove and Mayes (1991).

325   The incomplete fecal recovery in n-alkanes in the current study was consistent with the

326   previous findings (Dove and Mayes, 1996; Elwert et al., 2004; Bezabih et al., 2012). The increases

327   in fecal recovery rate with increasing carbon chain length (Table 3) were in agreement with

328   previous results (Dove et al., 2002; Dove and Mayes, 2005; Bezabih et al., 2012) for both seasons

329   except for few alkanes during the wet season trial. The higher fecal recovery of some alkanes in

330   high intake group compared to low intake group during the wet season might be due to higher feed

331   intake in the former group (Table 4). The greater fecal recovery of n-alkanes in the dry season than

332   the wet season is related to the lower digestibility of the dried hay compared to fresh pasture. This

333   is consistent with the findings of Ferreira et al. (2005) who demonstrated a negative relationship

334   between fecal recovery and digestibility. Full recoveries for C_{31} and C_{33} for low intake group

335   during the dry season might be due to higher concentrations of these alkanes in the diet (Table 2)

336   as compared to other alkanes as Keli et al. (2008) observed full recovery for alkanes found in

337   highest concentrations in the diet.

338   4.2. *Estimate of diet intake and digestibility using the n-alkane technique*

339   From the present study, feed intakes were constantly underestimated during the wet season and

340   inversely overestimated during the dry season for the low intake group when similar fecal recovery

341   was assumed (Table 4), probably because of the greater differences in fecal recovery between C_{31}

342   and C_{33} compared to C_{32} alkane. The present result was in line with the finding of Bezabih et al.

343   (2012) who observed underestimated actual DMI because of differences in fecal recovery between

344   adjacent alkane pairs. From the current study, during both seasons, estimates of intake were much

345   more accurate after fecal recovery correction for adjacent n-alkanes which confirms the previous

346   observation (Keli et al., 2008; Bezabih et al., 2012). Different scholars used the C_{33}/C_{32} alkane pair

347   (Mayes et al., 1986; Vulich et al., 1991; Dove and Mayes, 1996) and C_{31}/C_{32} (Ordakowski et al.,

348   2001; Peiretti et al., 2006) based on the relative abundance of n-alkanes in the diet which dictates

349   the type of n-alkane used for diet estimation accurately. But our results did not confirm the

350   previous findings as C_{33}/C_{32} alkane pair estimated better diet intake during both seasons even

351   though lower concentration of C_{33} alkane in the diet was observed compared to C_{31} alkane.

352   The result on the use of natural C_{35} alkane as internal marker for DMD in the current study

353   agrees with the earlier finding by Bezabih et al. (2012) who observed accurate estimate of DM and

354   OM digestibility by using C_{35} alkane as internal marker. The present results from low intake group

355   supports the previous finding that the natural C_{35} alkane used as internal marker to conduct

356   digestibility in grazing animals without having to dose synthetic n-alkane. But for high intake

357 group (fed *ad libitum*) during both seasons, the estimated DMD was different from the measured

358 as the level of diet provision increases.

359

360 **5. Conclusions**

361 This experiment validated the accuracy of the n-alkane technique to estimate DMI and DMD

362 in Zebu type Arsi breed cattle fed on wet and dried hay pasture. Regardless of the level of diet

363 provision and season, incomplete fecal recoveries were observed for the majority of n-alkanes

364 evaluated. Dry matter intake was accurately estimated after fecal recovery correction during the

365 wet season. The C_{33}/C_{32} pair accurately estimated the DMI regardless of fecal recovery correction

366 during the dry season. Dry matter digestibility was accurately estimated by using C_{35} alkane as

367 internal marker after fecal recovery correction for restriction feeding. Overall, the C_{31}/C_{32} and

368 C_{33}/C_{32} pairs as n-alkane technique provided a good estimate of DMI and DMD in cattle

369 consuming different amounts of wet and dried pasture after fecal recovery correction. However,

370 accuracy can be reduced for digestibility estimation at higher levels of intake which need further

371 validation.

372

373 **Declarations of interest**

374 The authors certify that there is no conflict of interest related with the development of this

375 original manuscript.

376

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382

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471 Table 1

472 Forage species proportions and chemical compositions of diets used during wet and dry season

473 trials

Ingredients/chemical composition	Wet season diet	Dry season diet
Forage species proportions		
Pennisetum thunbergii	0.65	0.51
Andropogon amethystinus	0.26	0.37
Ischaemum afrum	0.02	0.01
Trifolium cryptopodium	0.01	0.03
Centella asiatica	0.06	0.05
Uebelinia abyssinica	-	0.03
Chemical composition (g/kg DM)		
Organic matter	903.0	898.0
Crude protein	68.8	37.6
Ether extract	6.4	5.2
Neutral detergent fiber	697.1	712.4
Acid detergent fiber	374.0	409.5
Acid detergent lignin	58.1	68.0

474 DM=dry matter

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481 Table 2

482 Concentration of n-alkanes for composite natural pasture diets used during wet and dry season

483 trials

Alkanes	Concentration (mg/kg DM)	
	Wet season diet	Dry season diet
C_{23}	2.2	2.4
C_{24}	1.3	1.8
C_{25}	4.6	5.7
C_{26}	2.0	3.5
C_{27}	20.7	24.7
C_{28}	3.8	5.9
C_{29}	61.7	59.6
C_{30}	4.9	6.4
C_{31}	100.8	92.9
C_{32}	7.6	7.9
C_{33}	77.2	76.2
C_{35}	9.6	12.0
Total odd chain	279.0	276.0
Total	298.6	301.5

484 DM=dry matter

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493 Table 3

494 Mean (SD) feces recovery of n-alkanes coefficient and the ratio of dosed and adjacent odd-chain

495 alkanes in Zebu type Arsi steer fed natural pasture at different levels of feed provision

Alkane	Wet season		Dry season		P value		
	LI	HI	LI	HI	D	S	D*S
C_{23}	0.53 (0.03)	0.62 (0.05)	0.68 (0.07)	0.61 (0.07)	0.63	0.03	0.01
C_{24}	0.49 (0.04)	0.73 (0.02)	0.76 (0.1)	0.65 (0.05)	0.06	<0.01	<0.001
C_{25}	0.57 (0.04)	0.63 (0.06)	0.80 (0.06)	0.68 (0.09)	0.50	<0.01	0.02
C_{26}	0.61 (0.03)	0.71 (0.06)	0.84 (0.07)	0.72 (0.08)	0.87	<0.01	<0.01
C_{27}	0.77 (0.05)	0.77 (0.05)	0.90 (0.05)	0.79 (0.11)	0.13	0.05	0.13
C_{28}	0.65 (0.09)	0.72 (0.05)	0.93 (0.04)	0.84 (0.11)	0.81	<0.001	0.06
C_{29}	0.50 (0.04)	0.79 (0.08)	0.99 (0.05)	0.85 (0.12)	0.10	<0.001	<0.001
C_{30}	0.77 (0.05)	0.80 (0.05)	0.97 (0.05)	0.89 (0.12)	0.52	<0.01	0.19
C_{31}	0.66 (0.06)	0.67 (0.03)	1.02 (0.05)	0.88 (0.12)	0.09	<0.001	0.07
C_{32}	0.79 (0.02)	0.83 (0.09)	0.84 (0.05)	0.88 (0.09)	0.36	0.20	1.00
C_{33}	0.70 (0.03)	0.69 (0.05)	1.05 (0.05)	0.90 (0.13)	0.06	<0.001	0.08
C_{35}	0.73 (0.03)	0.70 (0.05)	0.99 (0.07)	0.90 (0.13)	0.17	<0.001	0.53
C_{31}/C_{32}	0.84 (0.06)	0.82 (0.07)	1.22 (0.06)	1.0 (0.09)	<0.01	<0.001	0.02
C_{33}/C_{32}	0.88 (0.04)	0.84 (0.07)	1.25 (0.06)	1.03 (0.1)	<0.01	<0.001	0.02

496 D=diet (low and high intake); HI=high intake (provided *ad libitum* – 5% refusals); LI=low intake

497 (provided 1.1% DM of BW); S=season (wet and dry); SD= standard deviation.

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500 Table 4

501 Mean measured and estimated DMI (kg/day (SD)) during wet and dry seasons by using two

502 different odd-to-even chain alkanes in Zebu type Arsi steer fed natural pasture

Parameter	Wet Season		Dry season		P value		
	LI	HI	LI	HI	D	S	D*S
Measured	1.87 (0.1)^a	2.78 (0.3)^a	1.58 (0.1)^b	2.29 (0.3)	<0.001	<0.01	0.43
Estimated intake assuming similar fecal recoveries							
C_{31}/C_{32}	1.57 (0.1)^b	2.27 (0.4)^b	1.93 (0.03)^a	2.29 (0.4)	<0.01	0.17	0.25
C_{33}/C_{32}	1.65 (0.1)^b	2.34 (0.4)^b	1.99 (0.03)^a	2.35 (0.4)	<0.01	0.21	0.24
Estimated intake corrected for mean fecal recovery for each group							
C_{31}/C_{32}	1.88 (0.2)^a	2.82 (0.5)^a	1.59 (0.03)^b	2.29 (0.4)	<0.001	0.03	0.49
C_{33}/C_{32}	1.87 (0.2)^a	2.82 (0.5)^a	1.59 (0.02)^b	2.29 (0.3)	<0.001	0.02	0.42

503 D=diet (low and high intake); HI=high intake (provided *ad libitum* – 5% refusals); LI=low intake

504 (provided 1.1% DM of BW); S=season (wet and dry); SD= standard deviation.

505 [a, b]Means with the same superscripts within a column are not different at P>0.05.

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513   Table 5

514   Mean measured and predicted DMD (coefficient (SD)) by using C_{35} as internal marker for Zebu

515   type Arsi steer fed natural pasture after mean fecal recovery correction

Parameter	Wet season		Dry season		*P* value		
	LI	HI	LI	HI	D	S	D*S
Measured	0.58 (0.03)	0.57 (0.02)[a]	0.43 (0.04)	0.50 (0.08)[b]	0.23	<0.001	0.11
Estimated	0.59 (0.05)	0.55 (0.03)[b]	0.43 (0.03)	0.51 (0.08)[a]	0.42	<0.01	0.04

516   D=diet (low and high intake); HI=high intake (provided *ad libitum* – 5% refusals); LI=low intake

517   (provided 1.1% DM of BW); S=season (wet and dry); SD= standard deviation.

518   [a, b]Means with the same superscripts within a column are not different at P>0.05.