Estimation of feed intake and digestibility in Zebu type Arsi steers fed natural pasture using the n-alkane technique

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Highlights

•N-alkane technique was validated under sub-humid tropical conditions.

•For the majority of n-alkanes evaluated, recovery in the feces were incomplete.

•Faecal recovery correction considerably improved feed intake estimation.

Abstract

An experiment was conducted to validate the use of n-alkanes technique to estimate feed intake and digestibility in cattle under the sub-humid tropical conditions. The experiment was conducted using Zebu type Arsi steers fed natural pasture at different levels of dry matter (DM) intake (DMI). Eight steers, blocked into four groups based on body weight (BW), were used for the experiment. The steers in each group were randomly assigned to either low intake (11 g DM/kg BW) or high intake (*ad libitum* at 50 g refusal per kg diet offered) diet. The steers were housed in individual pens, and each steer was dosed twice daily with paper bung containing 400 mg C_{32} alkane using a balling gun for 15 days. Steers received pasture diets twice a day (at 8:00 and 16:00 h) with half of the daily allocation offered at each feeding. Feed intake, refusal, and total fecal outputs were recorded, weighed and sub sampled for proximate and n-alkane concentrations analysis. The odd-chain n-alkanes comprised the highest percentage during both wet and dry seasons. The alkanes C_{29}, C_{31} and C_{33} were present in concentrations greater than 59 mg/kg DM in the two seasons. The mean fecal recovery rates ranged from 0.49 to 0.79 for low and 0.62 to 0.99 for high intake group, respectively during the wet season, whereas 0.68 to 1.05 for low and 0.61 to 0.9 for high intake group during the dry season, respectively. The DMI predictions using the double n-alkane technique were affected by season ($P<0.05$) after fecal recovery correction. The C_{31}/C_{32} and C_{33}/C_{32} pairs accurately estimated the DMI regardless of intake levels during the wet season. During the dry season, the prediction for the low intake level improved after feacal recovery corrections, whereas that for the high intake level was accurate both with and without fecal recovery corrections. Moreover, using C_{35} alkane as internal marker provided an accurate estimate of DM digestibility (DMD) during both seasons for low intake group. The results obtained in this study confirm the accuracy of the *n*-alkane markers to

estimate DMI and DMD in cattle consuming different levels of wet and dried pasture. However, accuracy can be reduced for digestibility estimation at higher levels of intake which need further validation.

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

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; BW, body weight; CP, crude protein; D, diet; DM, dry matter; DMD, dry matter digestibility; DMI, dry matter intake; EE, ether extract; FID, flame ionization detector; FO, fecal output; GC, gas chromatograph; HI, high intake; ISwt, internal standard weight; IAEA, international atomic energy agency; LI, low intake; N, nitrogen; NAS, natural and agricultural science; NDF, neutral detergent fiber; OM, organic matter; OARI, oromia agricultural research institute; S, season; SD, standard deviation; SDW, sample dry weight; SRF, standard response factor

1. Introduction

Livestock serve as a source of income and food security and also an integral component for most of the agricultural activities in the country (Mengistu et al., 2017). The performance of animals is mainly limited by inadequate nutrition both in terms of quantity and quality. The main feed resources used for livestock production in Ethiopia include natural pasture, crop residues, improved forages and agro-industrial by-products, of which the first two are the most important (Tolera et al., 2012). Recently, the share of natural grazing pasture at the national level as livestock feed resource, has become reduced to about 57% (CSA, 2013). The production performance of grazing ruminants, within their genetic boundaries, depends on the level of

nutrient intake (Tolera et al., 2012). Since providing feed for animals can represent up to 65% of total production costs (Arthur et al., 2004) and also to meet nutritional requirements of the animal (Mayes and Dove, 2000), accurate measure of feed intake and digestibility is necessary to evaluate production efficiency. However, estimation of feed intake and digestibility are difficult and complicated in grazing conditions due to limitations of available methods of measurement (Keli et al., 2008).

In the late 1980, the *n*-alkanes of plant cuticular wax were used as markers to indirectly estimate feed intake (Mayes et al., 1986). The n-alkane marker technique uses a combination of internal and external markers to estimate intake (Mayes et al., 1986; Dove and Mayes, 1991) and digestibility (Unal and Garnsworthy, 1999) of the diet. The advantages of the n-alkane method over other approaches include low invasiveness, accuracy and the possibility of taking into account diet-animal interactions (Dove and Mayes, 1991; Mayes and Dove, 2000). In addition, n-alkanes are chemically discrete components which can be easily analyzed by gas chromatography. A crucial point in the analysis of a marker is its recovery rate, the ratio of the excreted concentration of that marker over that of the ingested amount. Dove and Mayes (1996) explained that the error in intake estimation is proportional to the fecal recovery difference between the dosed and natural n-alkanes.

Different scholars support the recommendation that the n-alkane method needs diet and species specific trials to increase the accuracy of its predictions since lower fecal recovery rate were observed for tropical forage species compared to temperate species (Ferreira et al., 2009; Bezabih et al., 2012). Similarly, environmental conditions and geographical locations could influence the pattern of the cuticular wax profile of plant species growing in different places (Samuels et al., 2008). Although n-alkane technique as feed intake and digestibility estimation is

widely applied in other parts of the world, its validation was done only in the Mid Rift Valley grassland of Ethiopia (Bezabih et al., 2012), and no information is available on its applications in the highlands of Ethiopia, where the pasture composition is distinctly different from the Rift Valley grasslands due to its sub-humid tropical agro-ecology. Therefore, the objectives of this study were to measure the fecal recovery rate of n-alkanes from zebu type Arsi steers fed different levels of pasture forage from the central highlands of Ethiopia and to validate feed intake and digestibility estimation both during wet and dry seasons by using n-alkane technique. The information generated would help to build on the pool of knowledge available for wider application under tropical conditions.

2. Materials and Methods

2.1. Study area description

This study was conducted in Kofele district, West Arsi Zone of Oromia Regional state, Ethiopia situated at 7°07'N and 38°48'E at an altitude of 2660 m above sea level during wet (December, 2017) and dry (April, 2018) seasons. The long term average rainfall and temperature per annum of the district are 1800 mm and 19.5 °C, respectively and the district has bi-modal rainfall distribution with the short rain lasting from March to May and the main rainy season extending from June to September/October. The district is predominantly a loam soil type. The area has a high potential for crop-livestock farming system, where cattle and sheep are the most predominant livestock species (CSA, 2015). The natural pasture of the experimental site is dominated by *Pennisetum thunbergii* and *Andropogon amethystinus* and additionally consisted mainly other grasses such as *Ischaemum afrum, Sporobolus pyramidalis, Eragrostis botryodes, Poa leptoclada, Helictotrichon elongatum, Brachiaria scalaris,* legumes such as *Trifolium*

cryptopodium, Trifolium mattirolianum, Trifolium rueppellianum, Trifolium simense, Trifolium tembense, sedges which include *Cyperus rigidifolius, Scleria schimperiana, Scleria hispidula* and other herbs like *Centella asiatica, Uebelinia abyssinica, Haplocarpha hastata, Satureja paradoxa and Oldenlandia monanthos.*

2.2. Experimental animals and housing

The experiment was conducted in two rounds, the first was in the main rainy (wet) season and the second during the dry season. Eight Zebus type Arsi steers aged about 48 months were purchased from the local market and used during each of the trial periods. For the wet season trial, the average initial live weight of the steers was 148 ± 9.2 and that for the dry season trial was 155 ± 4.8 kg. Upon arrival at the experimental site, the steers were treated with Albendazole to control internal parasites and fed a diet similar to the subsequent feeding period *ad libitum* with free access to water for 21 days. A temporary experimental shed was constructed at the grazing site to provide protection to the steers from strong cold weather of the highland area and allow individual feeding and observation. The shed was partitioned into 2 m × 1.5 m pens which contained a separate feeding and watering troughs. The steers were handled and maintained throughout the experiment according to the experimental protocol approved by the Animal Ethics Committee for animal research of the University of Pretoria (NAS086/2019).

2.3. Experimental forages and diets

Natural pasture harvested from enclosed grassland was used for the current study. The harvested pasture diet used for the wet season trial contained five forage species, whereas the one used for the dry season trial contained six forages species (Table 1). For wet season trial, the 'pasture' diets were created by cutting the fresh pasture daily and forming a mixture of the dominant species by excluding all sedges, forbs and other herbs which were found in small

quantities. For the dry season trial, standing hay which was harvested and stored was used for the trial. The species composition of the hay used for the trial was calculated based on pre-determined quadrants for each species composition determination during wet season when forage species were easily identified. The pasture diets were chopped to 3 − 4 cm before feeding to the steers during the whole experimental periods.

Table 1

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

DM=dry matter

2.4. Experimental design and procedure

The experimental design used was a randomized complete block design with 2 × 2 factorial arrangement of the treatments that involves two levels of diet intake (low or high) and season (wet or dry). Steers in each group were randomly assigned to a low intake (11 g DM/kg BW) or a high intake (*ad libitum* at a refusal level of 50 g per kg feed offered) pasture diets and housed in individual pen with individual access to feed and water. The low intake level was considered to be equivalent to maintenance level of intake. The experiment lasted for 15 days, which included 5 days of adaptation and 10 days of feeding and data collection. During the experimental period, each steer was dosed with a paper bung containing 400 mg C_{32} alkane (n-dotriacontane) twice daily (at 6:00 h and 18:00 h) using a balling gun. Steers received pasture diets twice a day (at 8:00 and 16:00 h) with half of the daily allocation offered at each feeding. Feed intake, refusal and total fecal outputs were recorded during the last 5 days of the feeding period. The amounts of feed offered were weighed by using a scale during diet offer time (morning and afternoon) while diet refusals were removed and weighed once before the next day morning feeding time. Water was provided freely and 20 g sodium chloride per steer was given daily together with water each morning. Similar procedures were implemented during both wet and dry season trials.

2.5. Sampling and sample preparation

Samples of pasture (wet season) and hay (dry season) fed to each steer (200 g at each feeding time) were taken and pooled across the trial for each intake group. Diet refusals were collected from days 11 to 14, from each steer which has any and weighed and pooled for the four days and the mixed refusals were sub sampled to create one sample per steer per experiment of 500 g fresh weight. Total feces were collected twice daily (days 13 to 15), weighed and after being homogenized, sub-samples of 200 g fresh weight per collection were taken from each steer to

create one sample per steer per experiment for chemical and n-alkane analysis. Fecal samples were retained in refrigerator at − 20 °C during the collection period, mixed thoroughly after all samples were collected and sub sampled to create one sample per steer per period. Samples of feeds, refusals and feces were partially dried at 60 °C in a forced draft oven for 48 h and ground to pass through a 1 mm mesh size sieve and stored in plastic bags.

2.6. Chemical analysis

Chemical compositions of feeds, refusals and feces samples were analyzed for DM, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) at the animal nutrition laboratory of Hawassa University. Crude protein (CP) and ether extract (EE) were performed at the animal nutrition laboratory of National Veterinary Institute, Debre Zeit, Ethiopia. Samples were analyzed for DM (method 934.01), ash (method 942.05), EE (method 954.02) and N (method 954.01; CP = N × 6.25) by using Kjeldahl method according to AOAC (2006). Organic matter (OM) content was determined as 100 − %ash. Neutral detergent fiber content was determined according to method of Van Soest et al. (1991) without adding heat stable amylase. The ADF and ADL contents were determined according to method of Van Soest and Robertson (1985). Both NDF and ADF were expressed inclusive of residual ash.

N-alkane extraction and analysis was conducted at isotope nutrition laboratory of James Hutton Institute, UK. N-alkane for feed and feces samples were extracted and analyzed in duplicate by gas chromatography (GC) according to the method of Dove and Mayes (2006) using n-tetratriacontane (C_{34}) as an internal standard with a minor modification. For GC analysis, the derivatised extract was injected (1 μl) into a Trace (Thermo Finnegan) gas chromatograph fitted with a splitless injector (running in splitless mode at 275 °C, with a splitless time of 5 min) and flame ionization detector (FID), using helium (flow rate of 1 ml/min) as the carrier gas. The GC

column was a non-polar bonded-phase capillary type Rtx-5 MS (Restek) (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The temperature used for the GC column oven was 170 °C for 5 min; 30 °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.

2.7. Calculations

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

$$\text{Alkane}_i\ (\text{mg/kg DM}) = \frac{[10 \times \text{area \% alkane}_i \times C_{34}\ \text{IS wt (mg)}]}{\text{SDW} \times \text{SRF}_i}$$

Where C_{34} ISwt is internal standard solution (g) × C_{34} concentration in standard solution (mg/g); SDW is sample dry weight and SRF_i is the standard response factor for $alkane_i$, calculated as area % $alkane_i$ in the mixed standard divided by weight % $alkane_i$ in the mixed standard.

Fecal recovery of each alkane was calculated as the proportion of ingested compound, which was recovered in feces as follow:

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

Where R_i is the fecal recovery rate of $alkane_i$, FO is the daily fecal output (kg DM), F_i is the concentration of $alkane_i$ in feces (mg/kg DM), DMI is the daily dry matter intake (kg), and H_i is the concentration of $alkane_i$ in the diet consumed (mg/kg DM).

Feed intake was estimated by using the double n-alkane method according to Mayes et al. (1986) using the following formula:

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

Where F_i represents the fecal and H_i the herbage odd-chain $alkane_i$ concentrations (mg/kg DM), F_j resembles the fecal and H_j the herbage even-chain $alkane_j$ concentrations (mg/kg DM), and D_j equals the daily dose of even-chain $alkane_j$. Intake estimate were generated using both C_{31}/C_{32} and C_{33}/C_{32} alkane pairs.

Apparent dry matter digestibility (DMD) estimates were calculated using natural C_{35} alkane as an internal marker according to the following formula:

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

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

2.8. Statistical analysis

The effect of feeding level, season and their interaction on the n-alkane fecal recoveries, feed intake and dry matter digestibility estimates were assessed by analysis of variance using General Linear Model (GLM) Procedure of Statistical Analysis System (SAS, version 9.0). Block effect was initially included in the model as its effect was not different; it was removed from the final analysis as indicated in the following model:

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

Where, Y_{ijk} is the dependent variable, μ is the overall mean; D_i is the fixed effect of diet ($i = 1 - 2$), S_j the random effect of season ($j = 1 - 2$), $D*S_{ij}$ the interaction between diet and season and e_{ijk} is the error term. Multiple comparisons among means were determined by Tukey test at 5% probability. To compare the accuracy of measured and estimated feed intake and dry matter digestibility, paired *t*-tests were performed.

3. Results

3.1. Proportions and chemical composition of the experimental diets

Chemical composition and diet proportions used during wet and dry season experiments are presented in Table 1. The proportions of *Pennisetum thunbergii* was 0.65 and 0.51 from the total pasture used during wet and dry season experiments, respectively. Similarly, *Andropogon*

amethystinus was comprised 0.26 and 0.37 from the total pasture used during wet and dry season experiments respectively. Crude protein concentrations were 68.8 and 37.6 g/kg DM for wet and dry season diets respectively, whereas the NDF concentrations were 697.1 and 712.4 g/kg DM respectively, for wet and dry season diets.

Table 2

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

DM=dry matter

3.2. N-alkane concentrations and fecal recovery

The n-alkane profiles of the diets used during the experiments are shown in Table 2. The concentrations of C_{22} and C_{34} are not presented as they were added to samples at the beginning of the analysis as internal standards for gas chromatography (GC) analysis. The proportions of odd-

chain n-alkanes comprised 0.93 and 0.92 from the total alkane content in the diet during wet and dry season, respectively. N-alkane such as C_{29}, C_{31} and C_{33} presented with greater than 59 mg/kg DM concentrations in the two diets and they also made up 0.86 and 0.83 of the total odd-chain proportion for wet and dry season diets in that order. Apart from these three alkanes, other alkanes were presented in low concentration in the two diets. Total n-alkane concentrations for carbon chains lengths C_{23} to C_{35} were 298.6 mg/kg DM during the wet season diet (fresh forage), and 301.5 mg/kg DM during the dry season diet (dried hay), excluding C_{34} as it was used as internal standard in the alkane analyses.

The mean fecal recoveries of n-alkanes for steers provided different levels of diets during wet and dry seasons are shown in Table 3. From combined analysis data, there were no interactions between the level of diet provision and season on fecal recovery as carbon chain length increases (C_{27} – C_{35}) except for C_{29}, but interactions were observed ($P<0.05$) for lower carbon chain lengths. Similarly, interactions were observed ($P=0.02$) between the level of diet provision and season on fecal recovery for odd to adjacent even-chain alkane pairs (C_{31}/C_{32} and C_{33}/C_{32}). Fecal recovery for higher carbon chain lengths (C_{27} − C_{35} except C_{29}) were not affected for the level of diet provision (low or high), but higher ($P<0.05$) fecal recovery were observed for the dry season compared to the wet season except for C_{32}. The mean fecal recovery rates ranged from 0.49 to 0.79 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, for low intake and high intake groups, respectively. Full recoveries were achieved from the present study for C_{31} (1.02) and C_{33} (1.05) for low intake groups during the dry season. The average ratio between the fecal recovery of dosed even-chain and adjacent odd-chain alkanes were 0.84 and 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 groups, during the wet season trial.

Table 3

Mean (SD) feces recovery of n-alkanes coefficient and the ratio of dosed and adjacent odd-chain alkanes in Zebu type Arsi steer fed natural pasture at different levels of feed provision

Alkane	Wet season		Dry season		[1]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	0.01
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	0.01	0.06
C_{29}	0.50 (0.04)	0.79 (0.08)	0.99 (0.05)	0.85 (0.12)	0.10	0.01	0.01
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	0.01	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	0.01	0.08
C_{35}	0.73 (0.03)	0.70 (0.05)	0.99 (0.07)	0.90 (0.13)	0.17	0.01	0.53
C_{31}/C_{32}	0.84 (0.06)	0.82 (0.07)	1.22 (0.06)	1.0 (0.09)	0.01	0.01	0.02
C_{33}/C_{32}	0.88 (0.04)	0.84 (0.07)	1.25 (0.06)	1.03 (0.1)	0.01	0.01	0.02

D=diet (low and high intake); HI=high intake (provided *ad libitum* at 50 g refusal per kg diet offered); LI=low intake (provided 11 g DM/kg BW); S=season (wet and dry); SD= standard deviation.

[1]P-value after combined analysis.

Table 4

Mean measured and estimated DMI (kg/day (SD)) during wet and dry seasons by using two different odd-to-even chain alkanes in Zebu type Arsi steer fed natural pasture

Parameter	Wet Season		Dry season		[1]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)	0.01	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)	0.01	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)	0.01	0.02	0.42

D=diet (low and high intake); HI=high intake (provided *ad libitum* at 50 g refusal per kg diet offered); LI=low intake (provided 11 g DM/kg BW); S=season (wet and dry); SD= standard deviation.

[1]P-value after combined analysis.

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

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

Table 4 shows the actual DMI, and the estimated DMI calculated using C_{31} and C_{33} as internal markers according to C_{31}/C_{32} and C_{33}/C_{32} pairs used for the calculations. There was no interaction between level of diet provision and season on measured and estimated DMI, whereas the level of diet provision was affected (P<0.01) for all groups as intentionally done from the beginning (low and high intake provision). Season effect was different for the measured DMI (P=0.01) and estimated DMI (P<0.05) after fecal recovery correction, but no differences were observed before fecal recovery correction. Assuming similar fecal recovery between adjacent

odd-and-even chain alkane 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 low intake group and 0.18 and 0.16 for high intake group, respectively. On the contrary, the two alkane pairs accurately predicted DMI for both low and high intake groups with only 0 to 0.01 overestimations than the measured DMI after fecal recovery correction.

During the dry season, the C_{31}/C_{33} and C_{33}/C_{32} pairs overestimated DMI by 0.22 and 0.26, respectively, for the low intake group by assuming similar fecal recovery. But, after fecal recovery correction, the two alkane pairs accurately predicted the DMI with only 0.06 over estimation from the measured DMI. On the other hand during the dry season, the two alkane pairs (C_{31}/C_{33} and C_{33}/C_{32}) accurately predicted the DMI both before and after fecal recovery correction for high intake group which did not differ from the mean measured DMI. The coefficient deviation of the estimated DMI from the measured DMI before and after fecal recovery correction followed consistent trends during both seasons for the two alkane pairs.

Estimated and measured DMD by using C_{35} alkane as internal marker is shown in Table 5. There was no interaction between levels of diet provision and season on the measured DMD. On the other hand, interaction between level of diet provision and season was observed ($P=0.04$) for the estimated DMD. Dry matter digestibility coefficient was higher ($P<0.01$) for the wet season compared to the dry season. Dry matter digestibility was accurately estimated by using C_{35} alkane as internal marker after fecal recovery correction which was not different from the measured DMD. On the contrary, DMD were different ($P<0.05$) for measured and estimated during both seasons for high intake group.

Table 5

Mean measured and predicted DMD (coefficient (SD)) by using C_{35} as internal marker for Zebu type Arsi steer fed natural pasture after mean fecal recovery correction

Parameter	Wet season		Dry season		[1]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	0.01	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

D=diet (low and high intake); HI=high intake (provided *ad libitum* at 50 g refusal per kg diet offered); LI=low intake (provided 11 g DM/kg BW); S=season (wet and dry); SD= standard deviation.

[1]P-value after combined analysis.

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

4. Discussion

4.1. N-alkane concentration and alkane fecal recovery

In most herbage species, large odd-chain molecules account for the bulk of n-alkanes present in the plant cuticular wax and over 0.9 of *n*-alkanes have odd-numbers of carbon atoms, with C_{29}, C_{31} and C_{33} alkanes being dominant in most pasture species (Mayes et al., 1995; Peiretti et al., 2006). Our findings agrees with the previous results in which odd-chain alkanes accounted for over 0.92 and the three odd-chain alkanes (C_{29}, C_{31} and C_{33}) were predominant hydrocarbons as reported by Dove and Mayes (1991).

The incomplete fecal recovery in n-alkanes in the current study was consistent with the previous findings (Dove and Mayes, 1996; Elwert et al., 2004; Bezabih et al., 2012). The increases in fecal recovery rate with increasing carbon chain length were in agreement with previous results (Dove et al., 2002; Dove and Mayes, 2005; Bezabih et al., 2012) for both

seasons except for few alkanes during the wet season trial. The higher fecal recovery of some alkanes in high intake group compared to low intake group during the wet season might be due to higher feed intake in the former group. The greater fecal recovery of n-alkanes in the dry season than the wet season is related to the lower digestibility of the dried hay compared to fresh pasture. This is consistent with the findings of Ferreira et al. (2005) who demonstrated a negative relationship between fecal recovery and digestibility. Full recoveries for C_{31} and C_{33} for low intake group during the dry season might be due to higher concentrations of these alkanes in the diet as compared to other alkanes as Keli et al. (2008) observed full recovery for alkanes found in highest concentrations in the diet.

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

From the present study, feed intakes were constantly underestimated during the wet season and inversely overestimated during the dry season for the low intake group when similar fecal recovery was assumed, probably because of the greater differences in fecal recovery between C_{31} and C_{33} compared to C_{32} alkane. The present result was in line with the finding of Bezabih et al. (2012) who observed underestimated actual DMI because of differences in fecal recovery between adjacent alkane pairs. From the current study, during both seasons, estimates of intake were much more accurate after fecal recovery correction for adjacent n-alkanes which confirms the previous observation (Keli et al., 2008; Bezabih et al., 2012). Different scholars used the C_{33}/C_{32} alkane pair (Mayes et al., 1986; Vulich et al., 1991; Dove and Mayes, 1996) and C_{31}/C_{32} (Ordakowski et al., 2001; Peiretti et al., 2006) based on the relative abundance of n-alkanes in the diet which dictates the type of n-alkane used for diet estimation accurately. But our results did not confirm the previous findings as C_{33}/C_{32} alkane pair estimated better diet intake during

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

The result on the use of natural C_{35} alkane as internal marker for DMD in the current study agrees with the earlier findings by Bezabih et al. (2012) who observed accurate estimate of DM and OM digestibility by using C_{35} alkane as internal marker. The present results from low intake group supports the previous finding that the natural C_{35} alkane used as internal marker to conduct digestibility in grazing animals without having to dose synthetic n-alkane. But for high intake group (fed *ad libitum*) during both seasons, the estimated DMD was different from the measured as the level of diet provision increases.

5. Conclusions

This experiment validated the accuracy of the n-alkane technique to estimate DMI and DMD in Zebu type Arsi breed steer fed on wet and dried hay pasture. Regardless of the level of diet provision and season, incomplete fecal recoveries were observed for the majority of n-alkanes evaluated. Dry matter intake was accurately estimated after fecal recovery correction during the wet season. The C_{33}/C_{32} pair accurately estimated the DMI regardless of fecal recovery correction during the dry season. Dry matter digestibility was accurately estimated by using C_{35} alkane as internal marker after fecal recovery correction for restriction feeding. Overall, the C_{31}/C_{32} and C_{33}/C_{32} pairs as n-alkane technique provided a good estimate of DMI and DMD in steer consuming different amounts of wet and dried pasture after fecal recovery correction. However, accuracy can be reduced for digestibility estimation at higher levels of intake which need further validation.

Declarations of interest

The authors certify that there is no conflict of interest related with the development of this original manuscript.

Acknowledgement

This work was funded by International Atomic Energy Agency (IAEA) research contract 20781/RO and coordinated by School of Animal and Range Sciences, Hawassa University. The first author is also grateful for the University of Pretoria for bursary award and Oromia Agricultural Research Institute (OARI) for granting him a study leave.

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