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Quantification of the nutrition of grazing Arsi cattle through prediction of diet composition, feed intake and digestibility using plant wax markers

By

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A thesis submitted in partial fulfillment of the requirements for the degree of:

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Declaration

I, **Teklu Wegi Feyisa** declare that the thesis, which I hereby submitted for the degree

PhD: Animal Science

At the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or tertiary institution.

Mr. Teklu Wegi Feyisa

PhD candidate

Dedication

To:

My beloved wife, Abezash Bekele and my children, Sifan and Rabira Teklu for all the sacrifices that they made on my behalf

and

My brothers, Beyisa, Bekele and Kebede Wegi, who have provided me their absolute support during this study

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Preface

This thesis is based on the following chapters, which have been published, submitted or ready for submission for publication in peer-reviewed academic journals.

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Teklu Wegi, Abubeker Hassen, Melkamu Bezabih, Ajebu Nurfeta, Sintayehu Yigrem, Adugna Tolera, 2021. Estimation of feed intake and digestibility in zebu type Arsi steers fed natural pasture using the n-alkane technique. Animal Feed Science and Technology, 271: 1-8.

The thesis has been prepared in line with the **Animal Feed Science and Technology** journal referencing style in which the fourth chapter was published.

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List of Abbreviations

A/F	Abundance to frequency ratio
ABY	Aboveground biomass yield
ADF	Acid detergent fibre
ADL	Acid detergent lignin
AGP	Agricultural Growth Program
AOAC	Association of Official Analytical Chemist
AP	Available phosphorous
BW	Body weight
Ca	Calcium
CEC	Cation exchange capacity
CP	Crude protein
D	Diet
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
E	Enclosure
EC	Electric conductivity
EE	Ether extract
EGP	Effective gas production
ESAP	Ethiopian Society of Animal Production
FAO	Food and Agricultural Organization
FF	Fractionation factor
FID	Flame ionization detector
FO	Faecal output
G	Group
GC	Gas chromatograph
GIT	Gastro intestinal tract
GC-C-IRMS	Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry
GDP	Gross domestic product

GTP	Growth and Transformation Plan
H’	Shannon diversity index
HI	High intake
IAEA	International Atomic Energy Agency
IBC	International Biodiversity Conservation
ISwt	Internal standard weight
IFAD	International Fund for Agriculture Development
IVI	Important value index
K	Potassium
LCOH	Long chain alcohols
LI	Low intake
ME	Metabolizable energy
N	Nitrogen
Na	Sodium
NA	Not available
NAS	Natural and Agricultural Science
NDF	Neutral detergent fibre
O	Open access
OARI	Oromia Agricultural Research Institute
OC	Organic carbon
OMD	Organic matter digestibility
OPR	Orthogonal procrustes rotation
P	Phosphorous
PAST	Paleontological Statistics
PC	Principal Components
PCA	Principal Component Analyses
S	Season
SCFA	Short chain fatty acid
SD	Standard deviation
SDW	Sample dry weight
SE	Species evenness

SEM	Standard error of mean
SOC	Soil organic carbon
SPE	Solid-phase extraction
SR	Species richness
SRF	Standard response factor
TEC	Total even-chain
TLU	Tropical Livestock Unit
TN	Total nitrogen

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Executive summary

Grazing land resources in the central highland of Ethiopia are shrinking due to intense degradation as a consequence of expansion of crop cultivation and continuous heavy grazing. Improved management of grazing lands necessitates knowledge of the nutritional qualities of different pasture species, biomass yields and botanical composition. Estimation of diet composition, feed intake and digestibility of the diet consumed by animals is a perquisite for effective forage pasture utilizations; however, it is very difficult and complicated to estimate these parameters in free grazing animals due to limitations of available methods of measurement. The use of plant cuticular wax hydrocarbons as diet composition markers has recently received increasing acceptance as it is less-invasive and allows diet composition estimation without restricting free movement of animals. Four studies were conducted as part of the research with the objectives: (i) to access changes in vegetation composition, aboveground biomass yield and soil quality attributes in response to traditional grazing land management practices; (ii) to characterize nutritive values of key forage species from traditional enclosure area; (iii) to quantify the amount and composition of plant wax markers for different native herbaceous species and evaluate the potential of using these compounds as markers to estimate diet

composition of grazing animals; (iv) to validate the use of these plant markers for feed intake and digestibility estimation both during wet and dry seasons by using n-alkane technique.

In study one, two traditional grazing land management practices, enclosure and the adjacent open access grazing land were selected in two agro-ecologies, highland and mid highland, in the Kofele district, West Arsi Zone Oromia regional state, Ethiopia. At the two agro ecologies, 10 plots of 40 m x 40 m (five plots each for the two grazing management practices) were established. Along 10 m transects, sixteen 0.5 m x 0.5 m quadrats were nested per plot for sample collections. Mean aboveground biomass yield from enclosure area was higher ($P<0.001$) than open access grazing in the mid-highland agro-ecology with an increment of 28.8%. Principal component analyses (PCA) correlation variance of aboveground biomass yield, species richness, species evenness and Shannon diversity index showed distinct separation with PC1 accounting for 50.2% and PC2 accounting for 45.5% of the total explained variance of vegetation attributes across enclosure and open grazing areas.

In study two, chemical composition, *in vitro* gas production and methane measurement were conducted for 19 key forage species. Forage species evaluated in the present study showed large variation in the crude protein (CP) and fibre contents, gas and methane (CH_4) production. Principal component (PC) analysis showed that the first three PC (PC1 to PC3) explained 84.2% of the total variation in chemical composition and *in vitro* fermentable parameters of forage species evaluated. The first principal component (PC1), which explained 54.7% of the total variation was positively associated with CP, organic matter digestibility (OMD), metabolizable energy (ME), fractional gas production rate (c), effective gas production (EGP) and total gas production at 24 h, whereas it was negatively correlated with neutral detergent fibre (NDF), acid detergent fibre (ADF) and CH_4 production. The second PC (PC2), which explained 17.9% of the total variation was positively correlated with ADF and total gas production at 96 h, while it was negatively associated with ash, OMD and ME content. Forage species which had higher values for PC1 (*Centella asiatica* forb and all legume species evaluated) were characterized by higher CP content, fractional gas production rate (c), EGP and total gas production at 24 h, but had lower fiber contents and CH_4 production compared to forage grasses, sedges and other forbs evaluated. Similarly, forage grasses which had higher values for PC2 (*Eleusine floccifolia*,

Eragrostis botryodes, *Pennisetum sphacelatum* and *Poa leptoclada*) were characterized by higher ADF and CH_4 production. The key forage species clustered depending on their chemical composition and *in vitro* fermentable parameters into five main groups. Group I included *Centella asiatica* forb which had higher gas production at 24 h, higher fractional gas production rate (c) and higher EGP, but lower in NDF and CH_4 production compared to the rest groups. Group III, which had all legume species had on average higher CP content, higher fractional rate of fermentation (c) and higher EGP, but lower CH_4 production compared to grasses in groups IV and V. In study three, plant wax components from ten key forage species were measured in order to select promising markers for estimation of diet composition, feed intake and digestibility of the diets. Large variations in total C_{23} to C_{35} were observed between plant species, ranging from 58 mg/kg DM in *Centella asiatica* to 968 mg/kg DM in *Haplocarpha hastata*. The odd-chain n-alkanes comprised the highest proportion, being 79% of the total alkane concentration in *Ischaemum afrum* to 95% in *Haplocarpha hastata*. Large differences in the patterns of long chain alcohols (LCOH) were observed among the plant species for $C_{22}OH$ to $C_{34}OH$, which might be an indication of LCOH as species marker. Even-chain LCOH had the highest proportion of the total LCOH concentration, ranging from 92% in *Brachiaria scalaris* to 97% in *Ischaemum afrum*. Patterns of carbon stable isotope enrichments ($\delta^{13}C$) of the n-alkanes for all n-alkanes were between -19.7‰ in *Andropogon amethystinus* and -40.6‰ in *Trifolium mattirolianum*, which showed relatively large variations between forage species.

Lastly in study four, controlled in-door experiments were conducted using eight steers during wet and dry periods to validate the accuracy of the n-alkane method in intake and digestibility estimations. Randomized complete block design with 2 × 2 factorial arrangement of the treatments, two levels of diet intake (low or high) and season (wet or dry) were used. Steers were housed in individual pens with individual access to feed and water, and strees 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 diet. 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 twice daily (at 6:00 and 18:00 h) using a balling gun. The result showed that 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 and C_{29}, C_{31} and C_{33} presented with higher concentrations in the two diets. They also made up 0.86 and 0.83 of the total odd-chain proportion for wet and dry season diets in that order. The mean faecal recovery rates for C_{23} to C_{35} ranged from 0.49 for C_{24} to 0.79 for C_{32} for low intake group and 0.62 for C_{23} to 0.83 for C_{32} for high intake group during the wet season. Similarly, mean faecal recovery rates ranged from 0.68 to 1.05 and 0.61 to 0.9 for C_{23} and C_{33} in that order during the dry season for low and high intake groups, respectively. For the two groups, the two alkane pairs underestimate dry matter intake (DMI) during the wet season without faecal recovery correction. On the contrary, the two alkane pairs accurately predicted DMI for both low and high intake groups after faecal recovery correction. During the dry season, the C_{31}/C_{33} and C_{33}/C_{32} pairs overestimated DMI by 22% and 26%, respectively for the low intake group by assuming similar faecal recovery. After faecal recovery correction the two alkane pairs accurately predicted the DMI with only 6% over estimation from the measured DMI. The two alkane pairs accurately predicted the DMI regardless of faecal recovery correction for high intake group. Dry matter digestibility (DMD) was accurately estimated by using C_{35} alkane as internal marker after faecal recovery correction for the low intake group. On the contrary, DMD was different ($P<0.05$) for measured and estimated during both seasons for the high intake group. It can be concluded that the information generated from this thesis could increase the previous knowledge pool in this area which will be used to design appropriate grazing land management practices to increase feed resource availability, environmental sustainability and productivity of the farming system in the study area.

GENERAL INTRODUCTION

Ethiopia's current human population is estimated to be more than 105 million, with an annual increment of 2.5% (CSA, 2013a). The majority of the populations are concentrated on the highlands and because of this the highland areas are the most exploited and environmentally degraded (Hurni et al., 2005). As the rapidly increasing population needs increased food production, this has resulted in land use changes in the country. To meet the need for food, fibre and fuel, large areas of marginal lands are being farmed intensively, natural forests are suffering sever deforestation, and large areas of grazing lands are being overgrazed and degraded (Bewket, 2002; Nyssen et al., 2015). Agriculture adds nearly 35% of the country's gross domestic product (GDP) and 90% of its exports (FDRE, 2016), which makes it the main contributor to the Ethiopian economy. In addition, about 80% of the country's population depends on the agricultural sector for their livelihoods (Njeru et al., 2016), which makes it the main source of employment. The crop-livestock production system in the highland is described by the associated outward growth of cereal crop fields (Gryseels, 1988; Mengistu, 2002) which has increasingly reduced the realm of land allotted to grazing, thereby marginalizing the grazing lands into areas such as hilly tops, swampy areas, roadsides, and other marginal lands that have no farming potential (Mengistu, 2004).

Livestock production is one of the essential components of the agricultural sector in Ethiopia and provides meat and milk, farm power and income for mixed crop-livestock farmers (Ehui et al., 2002). According to Aklilu et al. (2013), the output obtained from the livestock industry is very low despite the large and diverse livestock genetic resources in the country. Among the major problems affecting livestock production and productivity in Ethiopia, feed shortage in terms of quantity and quality is the leading problem (Tolera, 2007). In the smallholder livestock production system, animals are reliant on different animal feed resources that differ in quantity and quality. The feed resources available in the country can be classified as natural pasture, crop residues, improved forages and agro-industrial by-products, of which the first two are the most important contributors (Tolera et al., 2012). As estimated by CSA (2013b), about 57.5%, 29.6%, 7.1%, 0.9 and 0.2% feed resources come from native pasture, crop residues, hay, by-products and improved feeds, respectively. This implies that grazing is the predominant form of ruminant

feeding system in most parts of the extensive and smallholder crop-livestock farming areas in Ethiopia (Mengistu, 2004).

Mengistu et al. (2017) indicated that the conditions of grazing lands in Ethiopia are very poor due to mismanagement, which necessitates immediate action. The productivity of natural pasture or grasslands in the major areas of Ethiopia is very low (Kebede et al., 2016a). According to Ndambi et al. (2018), the productivity of natural pasture in the country is characterized by less investment, small pasture improvement, and no fertilizer application leading to very low yields. The low production of pasturelands and overstocking have caused overgrazing of native pasturelands and reduced its productivity due to continuous heavy grazing and mismanagement practices (Ebro, 2015). Moreover, newly emerging climatic phenomena are affecting the botanical composition, yield, and nutritive content of pastures (Kitaba and Tamir, 2005; Denekew et al., 2005; Tessema et al., 2010). Generally, improved management of grazing lands necessitates good knowledge of the nutritional qualities of different pasture species, biomass yields and botanical composition for grazing herbivores. This in turn affects animal production and reproduction performances, and hence the livelihoods of smallholder farmers (Melaku et al., 2010).

Estimation of diet composition in freely grazing animals is challenging due to the invasiveness of the methods applied and associated in accuracies of simulating the natural grazing behaviour of animals. Similarly, feed intake is the most variable and difficult to accurately determine, and hence affects livestock productivity. Knowledge of the grazing behaviour of the different animal species and breeds under different circumstances is important to develop sustainable grazing land and management practices. Similarly, information about which plants grazing animals most or less frequently consume is limited, which has hampered the development of sustainable grazing land management practices. There are little studies conducted on these topics in which the availability of such information is of paramount important to understand the conditions of grazing pasture and develop sustainable grazing land management practices in the study area and beyond. The use of plant cuticular wax hydrocarbons as diet composition markers has recently received increasing acceptance as it is less-invasive and allows diet composition estimation without restricting free movement of animals.

Objective

The overall objective of this study was aimed at quantifing grazing ruminant nutrition in the central highlands of Ethiopia by using plant wax markers for diet composition, feed intake and digestibility estimation. The Specific objectives were:

- To quantify changes in vegetation composition, aboveground biomass yield and soil quality attributes in response to traditional grazing land management practices in a mixed crop-livestock production system in the central highlands of Ethiopia.
- To characterize nutritive values of key forage species and to determine the relationships between chemical compositions and *in vitro* ruminal fermentation parameters of the key forage from traditional enclosure area in the central highlands of Ethiopia.
- To quantify n-alkane, long chain alcohol and carbon stable isotope enrichments of forage species from the central highlands of Ethiopia, and to evaluate the potential of using these compounds as markers to estimate the diet composition of grazing animals.
- To measure the faecal recovery rate of n-alkanes from zebu type Arsi steers fed different levels of pasture forage from the central highlands of Ethiopia.
- To validate feed intake and digestibility estimation both during wet and dry seasons by using the n-alkane technique on Arsi steers fed different levels of pasture forage from the central highlands of Ethiopia.

CHAPTER ONE

Literature Review

1.1. Livestock populations, productivity and their importance in Ethiopia

Ethiopia has diverse agro-ecologies appropriate for livestock production and possesses the biggest livestock population in Africa in terms of headcount (CSA, 2018). Livestock populations in the counry estimated to 57 million cattle, 30 million sheep, 23 million goats, 4.8 million camels, 9 million equines and 57 million chickens (FAO, 2019). According to Birhanu (2014), 65% of the whole acreage in Ethiopia has an elevation of upper than 1400 masl. High populations of animals are raised in the highlands, where the majority of the population resides. As indicated by Hurni et al. (2010), most of the areas of the Ethiopian highland are arable lands, and as a result most of the country's human and livestock populations are occupied in this area. The highland areas have mostly a mixed crop-livestock farming system where oxen play a crucial role as draught animals.

According to the FAO (2019), agriculture supports over 80% of the population and contributes to 35% of the gross domestic product (GDP). Livestock is an important component of agriculture accounting for about 45% of the total value of agricultural production and sustain the lives of an oversized share of the population (FAO, 2019). The sector employs about 68.2% of smallholder farmers (FDRE, 2016). Within the mixed crop-livestock system, livestock play a central role within the livelihoods of numerous farmers. They serve as a source of food production (meat, milk, eggs and blood), inputs for crop production (draught power and manure), industrial raw materials, cash income and capital accumulation, transportation, export earnings, serve as a coping means against climatic shocks and uncertainties (Solomon, 2003; Tegegne, 2004; Behnke and Metaferia, 2011) and has some social and cultural roles suchs gifts etc.

The livestock production and management system is mostly extensive, where local breeds are carried on low-input/low output management practices **(**Tegegne and Feye, 2020**).** Despite the massive livestock population, the existing suitable environmental setting and the significance

of livestock to the livelihoods of numerous people, the productivity and economic contribution of this sector in Ethiopia is less than its potential because of technical, environmental, infrastructure, absence of appropriate institutions, and unsuitable development policies (Tolera, 2012; FAO, 2019**;** Tegegne and Feye, 2020).

1.2. Factors affecting livestock productivity

The overall livestock production constraints in Ethiopia were feed shortages, livestock diseases, low genetic paotential of indigenous livestock, lack of marketing infrastructure and water shortage (Tibbo, 2000). One of the most important technical constraints limiting the productivity of livestock is shortage and poor quality of feeds, and confirmed by many studies (Mengistu, 2002; Zegeye, 2003; Duguma et al., 2012). According to FAO (2018), Ethiopia incorporates a deficit of 27 million metric tons of animal feed every year and desires to fill this gap. On the other hand, high population pressure in the course of a rise in livestock numbers, and a discount of pasture land in favour of crop land, has increased the pressure on the remaining pasture (Seifu et al., 2011).

1.3. Livestock feed resources within the highlands of Ethiopia

According to CSA (2012), grazing is the major types of feed followed by crop residues, and also hay and by-products, a very small amount of improved feed and other types of feed were used as animal feed by small holder farmetrs in the rural areas of the country. The feed obtained from natural pastures is estimated to cover 80 to 90% of the total livestock feed (Benin and Pender, 2006; Mengistu, 2006). Livestock within the highlands graze on permanent pasturelands (private or communal) and fallow land during the cropping season and on croplands after harvest (Gizachew, 2002).

1.4. Status, productivity and contribution of natural pasture as livestock feed in the highlands of Ethiopia

According to Gurmessa et al. (2021), natural pastures in Western Ethiopia carry on livestock for a period of 6 months (June to December) until crop harvest and ready for stubble grazing. Similarly, Kofele district, central highlands of Ethiopia, has bi-modal rainfall distribution with small rains starting from March/April to May and the main rainy season extending from June to September/October and livestock depends on grazing for 6-7 moths based on the duration of the rain (Hussein, 2017). This conforms to the overall research report that shows natural pasture is one of the main supplies of animal feed in the country (Mengistu, 2006; CSA, 2013b). The productivity of natural pasture in the major areas of Ethiopia is very low (Kebede et al., 2016a). In the study conducted in Adaa Liben district, central highlands of Ethiopia, on poorly managed and overgrazed unimproved pasture, annual average primary production of 2 ton DM/ha was reported by ESAP (2009). According to Jutzi et al. (1987), natural pasture can produce up to 6 ton DM/ha in the highlands, but when continuously grazed it yields only 2.5 ton DM/ha. The aboveground biomass yield and nutritional quality of natural pasture is usually low (Kitaba and Tamir, 2005) because of poor management and utilization. As a result of seasonal fluctuations, there is high variability in the amount and quality of feed available from natural pastures (Funte et al., 2010). Natural pasture production is characterized by very minimal investments, little or no pasture improvement and no fertilization resulting in very low yields (Ndambi et al., 2018). The ME, CP and DM contents of those natural pastures in most cases have been reported to be below and unable to fulfill the mantainance requirement of the animals in the Bale highlands (Bogale, 2004). Similarly, there's a severe shortage of grazing resources along with a noticeable decline in the quality of the natural pasture within the highlands of the country (Kitaba, 2003).

1.5. Grazing land management practices and effects on pasture productivity, quality and vegetation status

There are differences in the owenership possession and management of grazing lands in Ethiopia. According to Gurmessa (2021) in Western Ethiopia, grazing lands are utilized and managed communally and/or privately. Gizaw et al. (2017) reported that on average about 18.9% and 81.1% of the grazing lands in East shawa (Lume and Dugda districts), in West shawa (Ejere and Ada-Berga districts and Jimma (Bora, Dedo, Kersa and Seka-Chokorsa districts) of Oromia were owned by communal and private ownership of grazing lands, respectively. Some research

findings showed that the quantity of land allocated for cultivation to grow plants has increased (Alemu et al., 2015), others suggest that croplands have been converted to woodland or forest (Yeshaneh et al., 2013). In both, the competition for land between crop and livestock production will remain as the need for the livelihood of human and animal feed resources continues to increase. Human population increment has also accelerated the conversion of grazing land into crop land (Rota and Sperandini, 2010). Other researchers (Hassen et al., 2010; Gurmessa, 2021) also reported that the increment of cultivation land is the number one reason for the declining size of grazing land at different locations.

The main traditional grassland utilization systems in either private or communal grazing lands are free grazing and enclosure (Plate 1.1). Here the enclosed area is either grazed rotationally or used for the cut-and-carry system, and in rare cases a part of the enclosed land is left aside as standing hay (Habtemicael et al., 2014; Gizaw et al., 2017; Gurmessa, 2021). The enclosure area during the rainy season refers to a selected land unit that is protected from grazing by shielded from the activities of a specific class of animals by using appropriate barriers like fencing and/or controlling the doorway of the animals following the onset of the short rainy season. On the other hand, open access grazing refers to an area of land which is exposed to unregulated and year round grazing by an oversized number of mixed livestock species. This type of grazing land may be owned privately or communally. Tether feeding on the road side was newly started by livestock keepers owning only a few livestock (Gurmessa, 2021).

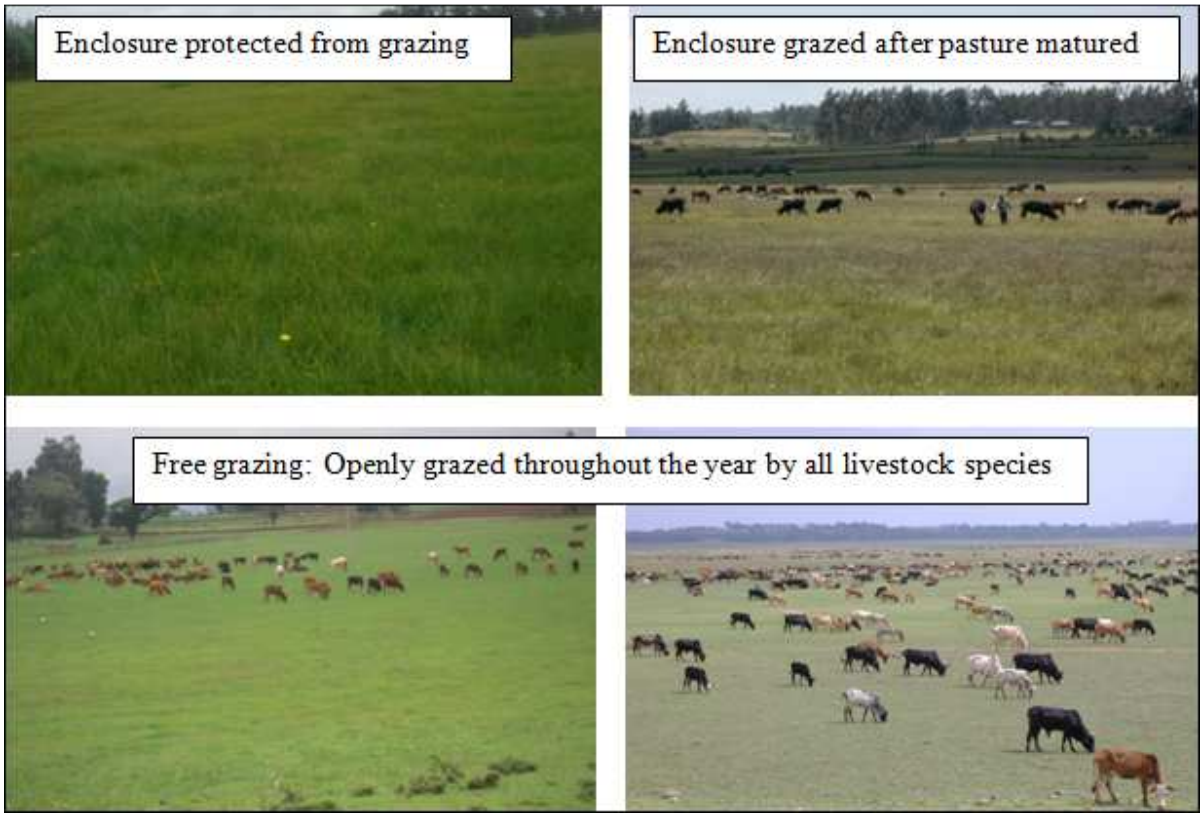


Plate 1.1. Traditional grazing land management practices (enclosure during wet season and open access grazing).

According to Habtemicael et al. (2014), herbaceous biomass production varied substantially between the three grazing management practices such as cut-and-carry, seasonal grazing and continuous grazing with almost three times the increments obtained when continuously grazed compared to the cut-and-carry system. The same author also reported that some species like *Andropogon abyssinica, Cynodon nlemfuensis* and *Heteropogon contortus* selected by farmers as extremely important completely disappeared from continuous grazing compared to seasonal grazing. The absence of desirable forage species from continually grazd area may be their lack of ability to tolerate heavy grazing than weather conditions, because the two grazing regimes were located within an identical climate. Fuhlendorf and Engle (2001) observed an identical directional effect of grazing pressure on the relative composition of short and mid-grass response groups.

Low productivity of pasturelands and enormous herd size on small grazing lands are the main causes responsible for lower productivity and overgrazing of natural pasturelands. According to Ebro (2015), low productivity of natural pasture is associated with continuous heavy grazing and weak grazing land management practices. Overgrazing is not a function of what number of animals is on a pasture alone, but it is a function of how long they continue to be within the pasture or paddock. In grazing management, time is the most significant factor that has to be considered in establishing a grazing system for sustained forage production. Grazing land management practices include observation of where, when and how much livestock graze. As a result, knowledge of the existing conditions of animal feed production is crucial so as to spot and design proper interventions to enhance feed supply and livestock productivity.

Grazing animals influence species composition and alter the biomass distribution and biodiversity of the grazing lands depending on the grazing pressure exerted (Zerihun and Saleem, 2000). Tadesse and Peden (2003) also reported that forage species composition and productivity of the pasture are highly influenced by animal species and intensity of grazing. This problem inevitably requires improving the productivity of the grazing lands within the country through appropriate management interventions so as to keep up a favourable balance within the botanical composition of the available natural pasture species.

1.6. Quantification of the nutrition of grazing animals

According to Mayes and Dove (2000), feed intake could be a top indication of animal productivity. In animals controlled in barns, intake can be calculated simply by weighing the quantity of feed offered and refused. Conversely, in grazing animals, quantification of feed intake is more complicated and it is almost not possible to calculate directly. The diverse methods available for estimating herbage intake are plant or animal based. According to Walters and Evan (1979), the plant-based intake quantification method depends on the measurement of plant aboveground biomass yield before and after grazing with corrections for plant re-growth during the quantification time. By using this method, it is impossible to estimate the intake of a single animal unless that animal is set aside in the paddock separetly. There is a consensus that animal methods used to measure pasture intake and digestibility estimation results have greater

benefit compared to plant based due to the ability to do measurements on a single animal (Mayes and Dove, 2000) and this credit that animal factors significantly contribute to variability (Keli et al., 2008).

From animal based measurement, behavioural observation relies on the animal's intake behaviour and does not need complicated technique to perform measurements. Intake estimation is either done by direct inspection or helped with equipment that estimates intake as a result of what quantity of diet the animal can take in one bite, the repetition and the total amount of time spent on feeding (Parker et al., 1993). This method has numerous drawbacks, like variations within the size and width of the animals' dental pads and also the variation of bite size over time (Oliveira and Silva, 2007). Additionally, utilization of equipment is an invasive procedure may be disturbing the animal's ingestive behaviour (Mayes and Dove, 2000). Moreover, this method is not appropriate for animals fed in groups (Macoon et al., 2003).

Weighing of animals could be another method from which the quantity of feed offered by an animal is estimated after a little period of intake, by weighing the animal's initial and final live-weights, with the difference obtained equivalent to its diet intake (Penning and Hooper, 1985). According to Mayes and Dove (2000), corrections should be needed to take into consideration weight loss because of metabolic processes (Mayes and Dove, 2000). Besides weighing the animals, the faecal collection technique is another method of pasture intake and digestibility estimation which involves the collection of the animal's fecal output. Total faecal collection may be allotted to caged animals from the ground or by employing collection equipments. Lippke (2002) hypothesized from his review that faecal collection is challenging and possibly to change the animal's normal feeding behaviour and this has impact on diet estimation. Feed intake and digestibility can additionally be measured by chemical indicators, referred to as markers. By using markers, feed intake is measured from the ratio between the total faecal output and its indigestibility (Mayes and Dove, 2000). Digestibility is often derived from the utilization of an internal marker or an *in vitro* digestibility technique. The use of markers in diet estimation is based the generalization made by Kotb and Luckey (1972), in which markers are mostly indigestible, totally recovered and no effect on alimentary tract physiology of the animals. However, up to now no marker totally fulfills these requirements (Dove and Mayes, 2005).

1.7. The impeliment of markers in nutritional studies

Markers are classified into internal or external markers. Chromic oxide is the most typical external marker implemented in animal diet estimation (Lippke, 2002). Additionally, titanium dioxide has become increasingly popular (Thompson and Wiseman, 1998). The most concern over the implementation of these markers is their possibility to cause carcinogenic effects and incomplete faecal recovery. Internal markers usually found in the diet either as distinct chemical entities or analytical products (Tamminga et al., 1989). These internal markers could pass certain criteria to be considered as markers, like being indigestible, inert, not bulky, neither affecting nor being affected by the gastro intestinal tract (GIT) (Velasquez, 2017), and mixing and remaining uniformly distributed in digesta (Pellikaan et al., 2013). However, their utilization is restricted since they are not fully indigestible (Tamminga et al., 1989).

1.8. Plant cuticular wax compounds and their use as a diet estimation marker

Plant wax which is found on the outer surface could be a complex mixture of aliphatic lipid compounds (Dove and Mayes, 2005; Charmley and Dove, 2007; Dove, 2010). Abundance and the composition of those wax layers differ within plant parts and between plant species (Ding and Long, 2010). It possesses diverse distinct chemical components like alkanes, long-chain fatty acids and alcohols (Dove and Mayes, 1991). Because plant species varies in the alkane, long chain alcohol and long chain fatty acid profiles of their wax compounds, all of those compounds can logically be used for diet estimation. A significant benefit of utilizing the plant wax marker method is that the diet estimation is in the same animals, in order that the resultant plant wax concentrations in their consumed diet will be calculated and used as entries to estimate diet intake (Mayes et al., 1986). This prevents the requirement to use oesophageal-fistulated animals to get a sample of ‘consumed diet’ and this should be taken as a significant advantage of the alkane method in terms of scientific, labour and animal ethics points of view. Similarly, the low invasiveness, accuracy and also the possibility of taking into consideration diet-animal interactions are also advantages (Bezabih et al., 2012).

Alkanes are the foremost used class of plant cuticular compounds due to their less complicated analysis compared to other classes of epicuticular wax. They are saturated hydrocarbons with major chain lengths ranging from carbon numbers 21 to 37 and exist in the wax layer of majority of plants (Dove and Mayes, 1996). Shorter chain length n-alkanes will be noticed during the analysis of plant wax markers but are usually present in minor quantities. More than 90% of n-alkanes have odd-numbers of carbon atoms, with the three carbon atoms such as C_{29}, C_{31} and C_{33} alkanes predominant in most forage species (Peiretti et al., 2006).

1.9. Use of plant cuticular wax to estimate diet composition

The species composition of the forage available and fed by grazing animals is an important variable in studies of their nutritional status. Plant wax components, particularly alkanes, have been used as internal markers for diet estimation in terms of plant species and plant parts in controlled experiments (Brosh et al., 2003; Valiente et al., 2003; Ferreira et al., 2005). The utilization of n-alkanes as faecal markers to estimate diet composition in field conditions has provided a big advance and has been used successfully to estimate the proportions of plant species in the diet of grazing animals (Dove and Mayes, 1996; Bugalho et al., 2004). As the different forage species vary within the alkane profiles, the composition of a mixed pasture diet can be obtained from the profiles of n-alkanes presented in the diet and faeces (Dove and Mayes, 1996).

The botanical composition of the forage species mixtures was estimated by applying the 'EatWhat' software through the non-negative method of least squares optimization (Dove and Moore, 1995). The biggest drawbacks to this method are the small number of n-alkanes available within distinguishable dietary components in an exceedingly complex diet (Bugalho et al., 2002). The amount of n-alkanes in the diet should be greater than or equal to the number of plant species consumed. There are a limited amount of observable dietary components in an exceedingly complex feed offered by animals, since pastures with multi-species have a limited number of n-alkanes in sufficient amount to be used as markers. According to Ferreira et al. (2011) and Bezabih et al. (2011a), to overcome this limitation utilization of other plant wax

components such as LCOH and carbon stable isotopes along with alkanes to extend the discriminatory potential in diet estimation is paramount important.

Long chain alcohols of the plant wax components are also important diet composition markers that provide distinct or additional information about plant species that was provided by n-alkanes (Bugalho et al., 2004). According to Dove and Mayes (2005), LCOH and fatty acids showed a promise for differentiating a large number of plants in the diet even though their analysis needed extra steps. Long chain alcohols are more prevalent than n-alkanes, but their analysis is more complex than n-alkanes. Combining LCOH with n-alkanes is critical for improving the accuracy of diet composition estimation because they are analyzed using the identical protocol as alkanes, and they overcome the limitation of the number of possible dietary components available to estimate plant species within the diet.

The proportions of ^{13}C and ^{12}C carbon atoms in plant tissues varies, offering an opportunity to discriminate between the amount of tropical C_4 plants which typically consist of legumes, forbs and browse, and tropical C_3 plants which comprise forage grasses. According to Mayes and Dove (2000), there are little C_4 photosynthetic pathway-based forage plants in temperate areas, and as a result, this approach is more applicable to tropical areas. Stable isotopes are utilized as tracers to evaluate the proportional contributions of numerous sources to a mix (Phillips and Gregg, 2003). The $\delta^{13}C$ for individual n-alkanes showed wide variation for some forage species at different locations (Bezabih et al., 2011b; Ferreira et al., 2014). Orthogonal procrustes rotation (OPR) results suggested that $\delta^{13}C$ values of alkanes given distinct discriminatory information from that which was provided by other markers.

1.10. Use of n-alkanes to estimate feed intake and digestibility

The n-alkane marker approach utilizes the implementation of the double n-alkane method (use of internal and external marker) to estimate feed intake of grazing animals (Mayes et al., 1986; Dove and Mayes, 1991; Mayes et al., 1994; Dove and Mayes, 2005), the digestibility of forage mixes (Dove, 1992; Unal and Garnsworthy, 1999) and botanical composition (Hameleers and Mayes, 1998) of the diet. This technique needs continuous dosing of a synthetic n-alkane

until the faecal alkane concentration of the ingested alkane stabilizes, and then diet intake is estimated using the ratio in faeces of dosed even-chain and an adjacent natural odd-chain n-alkane. The saturated hydrocarbons were at first recommended as digestibility markers (Mayes and Lamb, 1984) in individual animals as the proportion of feed consumed which is excreted in the faeces. The major constraint to obtaining precise estimate of digestibility might be the difficulty in gaining representative samples exactly consumed by grazing animals. In generall, different validation experiments found that the double n-alkane method is the most vigorous of all methods for estimating diet intake in grazing animals (Mayes and Dove, 2000; Ferreira et al., 2007a).

1.11. Challenges that may limit the estimation of diet composition and feed intake using n-alkaline

An important limitation related to the appliance of the n-alkanes approach is that the number of plant species and parts which will be distinquished within the feed. The possible number of feed components that may be distinguished using n-alkanes is restricted to the quantity of n-alkane markers obtained (Dove and Mayes, 1991), almost nine (C_{25} to C_{33}). Those are n-alkanes presented with greater concentration and lower analytical error. The capacity of n-alkanes to differentiate one species from the next decreases as the number of species in the pasture increases (Mayes and Dove, 2000). In multi-species pasture, where the number of plant species and parts present is greater than the present alkanes, Dove and Mayes (2005) propose two possible approaches to get precise herbage composition estimates. To add the number of 'discriminators' by combining the utilization of alkanes with other plant wax markers, like alkenes (Dove and Oliv´an, 1998), LCOH (Bugalho et al., 2004; Ali et al., 2005) and long-chain fatty acids (Ali et al., 2005), as well as to reduce the number of possible diet components by grouping existing plant species into statistical, functional and taxonomic groups and treating them as dietary components, in which they are treated. Recently, Bezabih et al. (2011a, 2011b) effectively utilized the $\delta^{13}C$ of n-alkanes in individual n-alkanes to estimate the botanical composition of various mixtures composed of five grass species existed in the Mid Rift Valley grasslands of Ethiopia. The data from this author indicated that $\delta^{13}C$ of n-alkanes combined with alkane data reduced the errors between estimated and measured botanical composition.

Another major consideration of the limitation of alkane as a diet composition marker is that the technique used for dosing the even-chain alkanes, commonly C_{32} or C_{36}, must not alter the diurnal pattern of faecal alkane proportion. The difficulty with markers is to gather faecal samples that are representative of the daily total output and also that reflect the dietary n-alkane proportions (Lippke, 2002). The faecal concentration proportion of dosed and diet alkanes may be disturbed by the dosing times (Mayes and Dove, 2000). Dove and Mayes (1991) argued that the dosing regimen (one or two per day) could influence the diurnal excretion pattern of the dosed n-alkanes. On the other hand, Oliv´an et al. (2007a) observed that once or twice daily dosing had no effect on the dosed n-alkane's excretion pattern. Mayes et al. (1986) and Sibbald et al. (2000) also observed a single dose was sufficient. Mann and Stewart (2003) approved that, when employing a xantham gum suspension as a carrier matrix, two doses per day schedule could be implemented.

An important point in the analysis of a plant wax marker is its recovery rate. In contrast to the earlier thoughts, n-alkanes might not be fully detectable within the gastrointestinal tract of animals but are subjected to some changes (Oliveira and Silva, 2007). They are not totally recovered in the faeces (Elwert et al., 2004). According to Dove et al. (2002) faecal recovery was in an exceedingly curvilinear fashion with chain length. The most reliable estimates of n-alkane recovery need the employment of an isolated group of housed animals with known n-alkane intake and those for which total faecal collections have been made. Previous research findings obtained by Elwert et al. (2006) and Valiente et al. (2003) in sheep fed diets of various grains, dried forages and straws, and by Brosh et al. (2003) in cattle and goats, failed to find significant differences within the faecal recoveries of the most n-alkanes used for feed estimation because of huge variation between animal variations in recovery. Nevertheless, experimenters with goats (Ferreira et al., 2005) and sheep (Ferreira et al., 2007b) ate up feed consisting of mixtures of herbaceous and woody species observed an impression of the herbage composition and its digestibility on alkane faecal recoveries. This could make it important to understand the particular alkane faecal recoveries to be used in every grazing condition. When using $\delta^{13}C$ values of n-alkanes, faecal recovery correction is not important because the results are relative values

(^{13}C and ^{12}C isotope ratios in relation to natural abundance) and this can be the greatest advantage of using $\delta^{13}C$ values of alkanes (Bezabih et al., 2011a, 2011b).

Concentrations of n-alkanes in the feed affect diet estimations. In the majority of forage species, greater odd chain molecules (C_{31}, C_{33}, C_{35}) account for the majority of n-alkanes that exist within the plant's cuticular wax (Piasentier et al., 2000) and it seems that not all n-alkane pairs are appropriate for intake estimation (Dove and Mayes, 1996). Multivariate statistical techniques like discriminant analysis or PCA can be used to ascertain whether plant species can be distinguished and which alkanes are best associated with the power to distinguish between diet components (Piasentier et al., 2000). Therefore, in selecting which alkanes to utilize, both their effectiveness in discriminating among plant components and their percentage of potential analytical error have to be considered. The C_{32}/C_{33} alkane pair has been implemented by many workers (Mayes et al., 1986; Vulich et al., 1991; Dove and Mayes, 1996) and produced reliable estimates, whereas Peiretti et al. (2006), and Ordakowski et al. (2001) selected C_{31}/C_{32} alkanes because of the relative large quantity of C_{31} in the hay diet fed to horses. According to Bezabih et al. (2012), of three evaluated alkane pairs (C_{31}/C_{32}, C_{33}/C_{32} and C_{35}/C_{36}), feed intake, DMD and OMD were predicted more precisely by using C_{35}/C_{36} compared to other pairs.

1.12. Summary and the need for future research

Natural pasture is the main source of animal feed in the highlands of Ethiopia and its herbage yield and nutritional quality are generally low due to poor management and utilization. Grazing animals influence plant species composition and pasture productivity, and change the biomass distribution and biodiversity of the grazing lands depending on the grazing pressure exerted. There is a need to document detailed information about the effect of traditional grazing land management practices on natural pasture quality and productivity to design appropriate management interventions so as to keep a favourable balance in the botanical composition of the available forage species.

Feed intake is a number one determinant of animal performance and in animals controlled in barns, feed intake can be measured by weighing the quantity of feed consumed and refused. It is

difficult for free-ranging animals as it is almost not possible to be measured intake directly. Different techniques available for estimating diet intake, whether plant or animal have been discussed in the literature. Among others, the use of plant wax components as diet composition, feed intake and digestibility estimation markers has received increasing acceptance as it is less-invasive and allows diet composition estimation without restricting free movement of animals. This technology is less common under tropical condition and the pattern of the cuticular wax profile of plant species is also influenced by environmental conditions and geographical locations. There is a need to document specific information for plant wax component profiles for natural pasture with different vegetation composition in the central highlands of Ethiopia and also to further validate the use of plant wax as internal markers under tropical conditions.

1.13. Working hypothesis

H0: There is no significant difference in vegetation composition, biomass yield and soil quality between enclosure areas and free grazing land practices.
H1: There are significant differences in vegetation composition, biomass yield and soil quality between enclosure area and free grazing land practices.
H0: There is no significant difference in chemical composition and ruminal fermentation characteristics for forage species collected from traditionally enclosed grazed pasture.
H1: There are siginificant differences in chemical composition and ruminal fermentation characteristics for forage species collected from traditionally enclosed grazed pasture.
H0: There is no significant difference in plant wax marker profile in forage species due to changes in geographical locations and environmental conditions.
H1: There are significant differences in plant wax marker profiles in forage species due to changes in geographical locations and environmental conditions.
H0: There is no significant difference in diet composition estimation by combining plant wax markers or n-alkane alone.
H1: There are siginificant differences in diet composition estimation by combining plant wax markers rather than using n-alkane alone.
H0: The plant wax procedure as internal markers does not allow feed intake and digestibility estimation to be estimated by 95% of the observed compositions.

H1: The plant wax procedure as internal markers allows feed intake and digestibility estimation to be estimated by 95% of the observed compositions.

1.14. List of studies conducted to accept or reject the working hypotheses

- Changes in vegetation composition, aboveground biomass and soil quality in response to traditional grazing land management practices in the central highlands of Ethiopia.
- Nutritive value characterization of key forage species from traditional enclosure area in the central highlands of Ethiopia.
- Evaluation of n-alkanes, LCOH, and carbon stable isotope enrichments of n-alkanes as diet composition markers for forage species.
- Estimation of feed intake and digestibility in Zebu type Arsi steers fed natural pasture using the n-alkane technique.

CHAPTER TWO

Changes in vegetation composition, aboveground biomass and soil quality in response to traditional grazing land management practices in the central highlands of Ethiopia

Abstract

Despite shrinking pastureland in the central highlands of Ethiopia, as a result of cropping, there has been little detailed work to examine the effects of traditional grazing land management practices on vegetation and soil attributes. This study aimed to quantify vegetation composition, aboveground biomass yield and soil quality as in relations to enclosure and open access management practices by using a sampling quadrat for both parameters. Aboveground biomass yield for the grass species was 17.6 and 31.2% higher, respectively, for the highland and mid-highland agro-ecologies for enclosed areas compared with open-access grazing. *Andropogon amethystinus* (Important value index (IVI) = 86.9) and *Pennisetum thunbergii* (IVI = 79.2), the most dominant and highest density species found in the enclosed areas, decreased in the open access grazing land and were partially replaced in the mid-highland area by plant species that are more resistant to continuous heavy grazing, like *Eleusine floccifolia* (IVI = 125.7). Herbaceous species richness was better in open access grazing land than the enclosed areas. Soil quality parameters such as total nitrogen (TN), available phosphorous (AP), calcium (Ca), sodium (Na) and cation exchange capacity (CEC) were significantly higher in enclosed areas than open access practice. In conclusion, enclosed areas performed better in most of the variables measured parameters considered than open access grazing land management practices at both agro-ecologies**.**

Keywords: enclosure, herbaceous species, important value index, mixed crop-livestock system**,** open access

2.1. Introduction

The mixed crop-livestock production system of the Ethiopian highlands occupied approximately by 90% of the human and 60% of the livestock populations (Hurni et al., 2010). In these production systems, livestock are dependent on a variety of feed resources that can vary in both quantity and quality throughout the year. Grazing is the predominant form of ruminant feed in most parts of the extensive and smallholder mixed crop-livestock systems of Ethiopia (FAO, 2018). Fallow lands, permanent pasturelands during cropping season and croplands after crop harvest are among the dominant grazing areas (Gizachew, 2002).

Grazing land resources are shrinking as a result of degradation as a consequence of deforestation, agricultural land expansion and continuous heavy grazing (Mengistu et al., 2005). The evidence available on the impact of grazing systems on the dynamics of natural vegetation and soils is contradictory (Vetter and Bond, 2012). Grazers change landscape heterogeneity (Belsky, 1992), rates of nutrient cycling (Frank et al., 1998), and vegetation composition and productivity (Eccard et al., 2000). Similarly, grazers affect different grassland ecosystems, including increases (Pucheta et al., 2004), decreases (Gao et al., 2008) and no changes (Frank et al., 1998) in root system biomass and below ground net primary productivity. Additionally, others consider grazing as a positive process that improves plant production and survival (Papanastasis, 2009), promotes biodiversity (O'Connor et al., 2010) and improves soil fertility. Light grazing increases aboveground biomass, canopy cover and height of the species, but from long-term experiences, moderate grazing would balance the biomass production of different species and livestock production (Wei et al., 2011; Venter et al., 2021).

According to Feyisa (2013), pasturelands in the central highland areas have significantly reduced and are limited to areas, where conditions are adverse for cropping, partly as a result of topographic, edaphic and climatic limitations. Consequently, livestock are forced to concentrate on very limited pastureland, which in turn results in reduced productivity in the long run and makes the mixed crop-livestock system unsustainable (Taddese et al., 2002). According to the same author, in the studied areas, livestock production will decline if not ecosystems are managed to feed people and protect wild species all together. The massive deforestation and the

resultant shortage of fuel wood resulted in the use of dung and agricultural residues as fuel, which prevents nutrient recycling through manure. In order to utilise the available grazing lands in a sustainable manner, it appears important to evaluate the impacts of different traditional grazing land management practices for implementing knowledge-based grazing land management strategies.

Many studies have been done on the effect of grazing pressure on plant and soil properties of grasslands, but the majority of them have been in the pastoral rangelands. Relatively, little research has been done to evaluate the effect of different grazing land management practices on vegetation and soil attributes in the highland mixed crop-livestock system of Ethiopia (Taddese et al., 2002; Habtemicael et al., 2014). The available evidence so far showed that better vegetation and soil attributes are obtained in moderate grazing than continuous heavy grazing. However, no study has been done using a phytosociological data analysis method to quantify the impact of traditional grazing land management practices on vegetation and soil parameter attributes in the central highlands of Ethiopia. Such analysis is important to provide detailed information on vegetation status and also to offer insights on grazing land ecosystem function and its restoration in general. Therefore, this study was undertaken to quantify composition in vegetation structure, aboveground biomass yield and soil quality attributes in response to traditional grazing land management practices in the mixed crop-livestock system in the central highlands of Ethiopia.

2.2. Materials and Methods

2.2.1. Study area

The study was conducted in the Kofele district of the West Arsi Zone of the Oromia Regional State, Ethiopia, located at 7°06′ N to 7°07′ N, 38°48′ E to 38°49′ E for the highland and 7°00′ N to 7°02′ N, 38°48′ E to 39°00′ E for the mid-highland (Figure 2.1). The Kofele district is located at 305 km South of Addis Ababa. The agro-ecologies of the district are highland (90%) and mid-highland (10%) having loam soil in the highland and sandy loam in mid-highland (District Agricultural and Natural Resource Management Office 2017, unpubl. data). The district

is found within 2,200 to 3,200 masl and receives an average rainfall of 1,800 mm y^{-1} and has bimodal rainfall distribution with the short rain starting from March to May and the main rainy season extending from June to September/October. The average temperature of the distric is 19.5 °C. The area is characterized as high potential for crop-livestock farming and cattle (4.41 TLU) and sheep (0.45 TLU) are the most predominant livestock species (Gizachew, 2002). The distric have an averge crop land of 1.27 ha and farmers in the study area grow crops such as barely (*Hordeum vulgare*), wheat (*Triticum aestivum*), maize (*Zea mays*) and *enset* (*Ensete ventricosum*) as food crops, and potato (*Solanum tuberosum*), head cabbage (*Brassica oleracea*), beetroot (*Beta vulgaris*) and carrot (*Daucus carota*) as cash crops (Hussein, 2017).

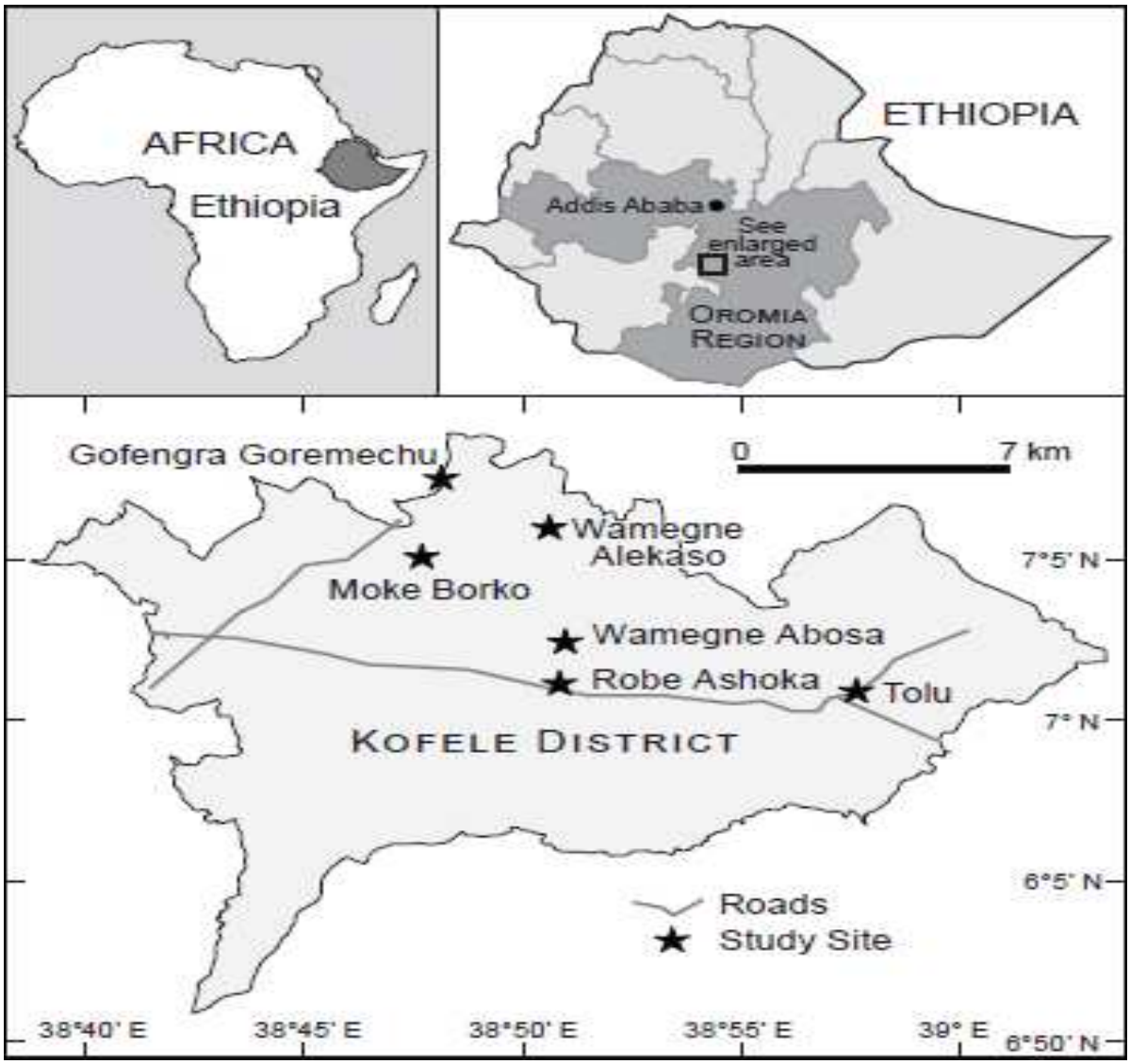


Figure 2.1. Map of the study area, Kofele district, central highlands of Ethiopia

2.2.2. Traditional grazing land management history

The study area is historically (before 60 years) known for livestock rearing, and cultivation of crops is a relatively recent phenomenon. The major land use includes farmlands and grazing lands, which in turn consist of enclosed areas during rainy season, which is traditionally known as *kelo* and an openly grazed area (free grazing) throughout the year. The enclosed areas refer to a specific land unit that is protected from the activities of animals by using appropriate barriers such as fencing and/or controlling the entrance of the animals following the onset of short rainy season. This particular type of grazing land is used for rotational stocking during the short and main rainy seasons, a cut-and-carry system and in rare cases is used as conserved standing hay for use during the dry period. Open access grazing refers to an area of land that is exposed to unregulated and year round grazing by a large number of mixed livestock species. It is free to all communities if the areas are communal, but not if owned privately especially during the rainy season. According to the information obtained from elder farmers in the study area, these traditional grazing land management practices have been evident for more than 30 years and such areas were purposively selected for the current study.

2.2.3. Site selection and study design

Two traditional grazing land management practice sites, enclosure during wet season and the adjacent open access grazing land, were systematically selected based on the similarity in landscape and soil to minimise variability in the abiotic determinants of grassland vegetation composition and functioning. At both agro-ecologies, 10 plots of 40 m × 40 m (five plots each from enclosure and the adjacent open access area) were established. All plots were enclosed uniformly from the entrance of animals for five months from May 2018 to September 2018 for data collection. For sample collection, sixteen 0.5 m × 0.5 m quadrats were nested per plot, set 10 m apart along the transects and 4 m from the plot border (Figure 2.2). In total, 320 quadrats (two agro-ecologies i.e. highland and mid-highland × two management systems × five plots × 16

quadrats) were used to collect pasture species samples. To avoid any edge effects, the plots were laid 50 m away from the boundaries of the enclosure and the adjacent open access, respectively.

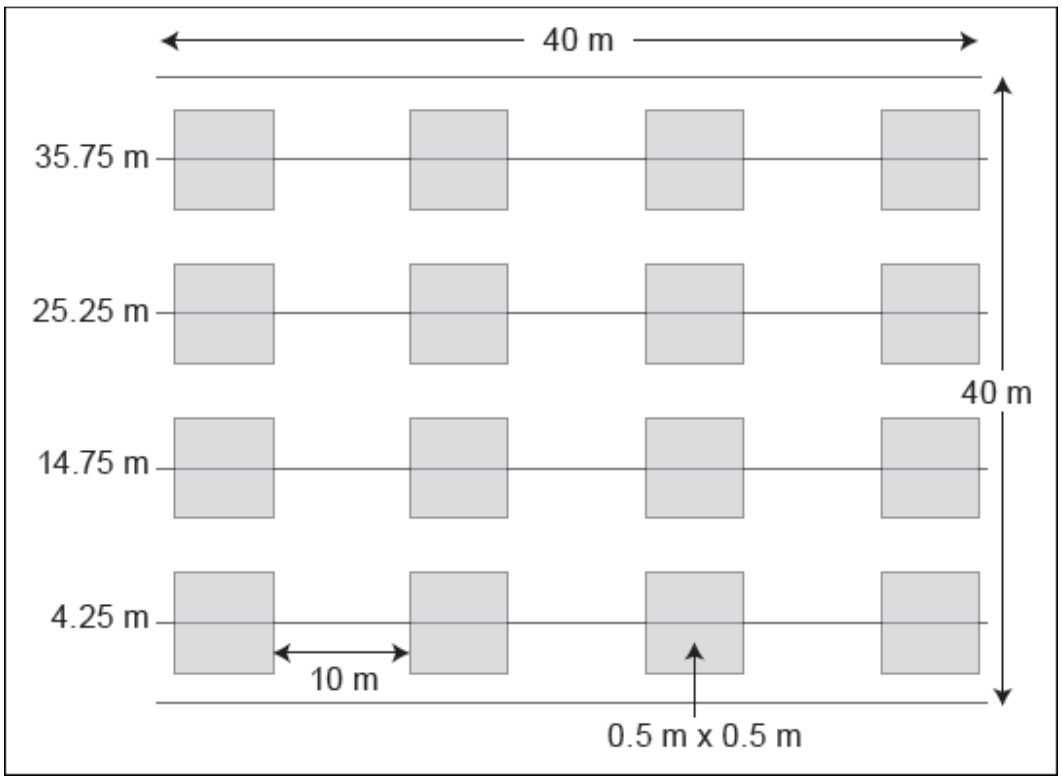


Figure 2.2. Sampling design and plot layout of the experimental site used for herbaceous species and soil sampling.

2.2.4. Vegetation sampling and analysis

Vegetation sampling in the individual enclosure and the adjacent open access grazing land was done in September 2018 at the end of main rainy season at 50% flowering and easily identified (Plate 2.1). Herbage biomass yield was determined by mowing the pasture at 5 cm aboveground, using a sickle, from an area of 0.25 m^2 quadrats and sorted by species and pooled to obtain enough quantities of individual species for determination of nutritive values. Fresh biomass was immediately weighted by using a scale to know total biomass yield from a quadrats. Forage species were identified using guidebook (Froman and Persson, 1974) on site. For those difficult to identify at the field their local names were recorded and herbarium specimens were

collected, pressed and dried properly (Plate 2.2) by using a plant presser, and identified and confirmed at the national herbarium, Addis Ababa University, Ethiopia. Individual forage species in each quadrat were counted and weighed fresh to estimate total biomass yield and converted to dry matter yield after dry matter determination. Vegetation composition was evaluated by analysing the frequency, density, abundance and important value index (IVI) by using the following formulae given by Mishra (1968) and Curtis and McIntosh (1951):

$$\text{Frequency} = \frac{\text{Total number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Relative Frequency} = \frac{\text{Frequency of a species}}{\text{Frequency of all species}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of a species}}{\text{Total number of quadrats studied}}$$

$$\text{Relative density} = \frac{\text{Number of individuals of a species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Abundance} = \frac{\text{Total number of individuals of a species}}{\text{Total number of quadrats in which the species occurred}}$$

$$\text{Relative Dominance of Herbaceous Species} = \frac{\text{Basal area of a species}}{\text{Basal area of all the species}} \times 100$$

IVI = Relative frequency + Relative density + Relative dominance

The distribution of species similarity between enclosed area and open access grazing land were computed by using the index of similarity which was calculated by applying the formula given by Jaccard (Zobel et al., 1987):

$$IS_J = (C/A+B-C) \times 100$$

Where IS_J = Jaccard's Index of Similarity, C= the number of species common to both grazing management practices; A = the number of species unique to enclosure area; B= the number of species unique to open access grazing area.

From pooled forage species across plots, 20% of above ground biomass was taken and oven-dried to a constant weight at 60 ^{0}C for 48 hours and used for dry matter determination. Species richness was estimated by counting all species within the sample quadrats. Species diversity was computed for each grazing land management practice using the Shannon–Wiener diversity index (*H'*) (Shannon and Wiener, 1963):

$$H' = -\sum_{i=1}^{s} Pi\ In\ Pi$$

Where s= number of species; pi = proportion of individuals or abundance of the i^{th} species expressed as a proportion of total cover; and ln = log base n.

Equitability or species evenness was calculated using the formula given by Pielou (1969):

Equitability (J) = H'/H'$_{max}$

Where H'$_{max}$ = Maximum possible diversity, H' = Shannon–Wiener diversity index.

Plate 2.1. Partial view of field data collections.

Plate 2.2. Partial view of field forage species identifications.

2.2.5. Soil sampling and analyses

Soil samples were collected from the centre of 0.25 m^2 quadrats after aboveground biomass was removed using an auger at depths of 0 to 10, 10 to 20, 20 to 30, 30 to 40 and 40 to 50 cm. The soil samples at each plot were pooled to form one composite soil sample per sampling plot yielding a total of 20 soil samples for each depth (two management practices × 10 sample plots) to determine the cumulative effect of traditional grazing land management on soil attributes. Samples were air dried and taken to the laboratory, grind and passed through a 0.5 mm and 2 mm

mesh size sieve. Soil pH was determined using a pH meter in 1:2.5 soil water:suspension ratios (Lewis and Freitas, 1984). Electrical Conductivity (EC) was determined in a 1:2.5 soil water suspension following the steps and procedures suggested by Chopra and Kanwar (1976). Total nitrogen (TN) was determined using the Kjeldahl procedures suggested by Bremner and Mulvaney (1982). Soil organic carbon (SOC) was determined following the method recommended by Walkley and Black (1934). Soil organic matter (SOM) was calculated by multiplying the percentage of the organic carbon by a factor of 1.724 following the method recommended by Brady and Weil (1999). The level of available phosphorus (AP) was determined by using the methods and procedures specified by Olsen et al. (1954). Calcium was determined by atomic absorption spectrometry and potassium (K) and Na were determined by flame photometry. Texture was determined using the hydrometer method (Bouyoucos, 1962).

2.2.6. Statistical analyses

The effect of traditional grazing management practices (enclosure area vs. open access grazed) i.e. the independent variable, on herbaceous aboveground biomass yield and proportions of different botanical composition, species frequency, abundance, density, richness and evenness and soil properties (dependent variables) were analysed using ANOVA (SAS 9.1) using the General Linear Model Procedure (SAS, 2001). Twenty sampling plots and 16 quadrats per plot were used as replicates to assess the impact of traditional grazing land management practices on aboveground biomass yield, and twenty sampling plots were used as replicates for soil quality parameter analyses. Tukey's HSD test with $p<0.05$ was employed for comparison of means. The data were tested for homogeneity of variance using a Shapiro–Wilk test and, where necessary, transformed before analyses (Steel and Torrie, 1980).

A principal component analysis (PCA) was used to explain the total variance of analysed soil and/or vegetation characteristics across the two grazing land management practices. The PCA axis correlation coefficient was used to explain the location of the vegetation and soil attributes across the enclosure and open grazing and the analysis was done using the Paleontological Statistics (PAST) software package version 4.02 (Hammer et al., 2001).

2.3. Results

2.3.1. Vegetation biomass yield

Aboveground biomass yield (mean) for enclosed areas was higher (p<0.001) than open access grazing in the mid-highland agro-ecology (Table 2.1) with an increaments of 28.8%. The proportion of grasses among the botanical groups was 76.3 and 69.6% from highland and 92.9 and 83.7% from mid-highland for enclosed and open access areas respectively, and the highest share among the botanical component groups. Mean aboveground biomass yield for grasses species from the enclosed areas was higher (p<0.001) than open access grazing at both agro-ecologies with an increments of 17.6 and 31.2%, respectively, in the highland and mid-highland agro-ecologies. Mean aboveground biomass yield for legumes and forbs in open access grazing was higher (p<0.001) than the enclosed areas at highland agro-ecology.

Table 2.1. Total aboveground herbaceous biomass yield and proportions for botanical groups on dry matter basis (t/ha) from enclosure and open acces grazing practices (mean ± SEM).

Vegetation variables	Agro ecology			
	Highland		Mid-highland	
	Enclosure	Open access	Enclosure	Open access
Total aboveground biomass	3.26 ± 0.19	3.07 ± 0.16	6.62 ± 0.36[a]	4.71 ± 0.47[b]
Herbaceous components				
Grasses	2.61 ± 0.20[a]	2.15 ± 0.20[b]	6.18 ± 0.36[a]	4.25 ± 0.47[b]
Legumes	0.12 ± 0.04[b]	0.42 ± 0.12[a]	0.27 ± 0.04	0.28 ± 0.06
Sedges	0.23 ± 0.05	0.14 ± 0.03	0.17 ± 0.03	0.16 ± 0.03
Forbs	0.39 ± 0.04[b]	0.56 ± 0.07[a]	0.17 ± 0.03	0.21 ± 0.03

[a,b] Different superscripts within the row for each agro-ecology show significant difference (P<0.05). SEM = standard error of mean.

2.3.2. Herbaceous species composition

In the highland agro-ecology, a total of 29 herbaceous species were recorded of which 22 and 27 herbaceous species were recorded for the enclosure and open access grazing areas, respectively (Table 2.2). From identified herbaceous species, *Andropogon amethystinus* (IVI = 86.9) was the most dominant, and the highest density perennial grass. *Pennisetum thunbergii* (IVI = 79.2) and *Centella asiatica* (IVI = 35.8) were the codominant forage species in the enclosed areas. In the open access grazing area, *Pennisetum thunbergii* (IVI = 100.6) was the most dominant with the highest density and *Andropogon amethystinus* (IVI = 44.2) and *Centella asiatica* (IVI = 36.5) were the codominant herbaceous species. Four grass species (*Cynodon dactylon, Pennisetum humile, Pennisetum sphacelatum* and *Sporobolus pyramidalis*) and two forbs (*Cerastium octandrum* and *Crepis schultzii*) that were recorded in open access grazing were absent in the enclosed areas. *Pennisetum thunbergii* and *Andropogon amethystinus, Cyperus rigidifolius*, and *Centella asiatica* and *Uebelinia abyssinica* were the most frequently occurring species among others.

Table 2.2. Frequency (%), abundance and density (0.25 m^2 quadrats) and important value index (IVI) of forage species collected from the enclosure and open access grazing practices in the highland agro-ecology.

Species scientific name	Frequency		Abundance		Density		A/F		IVI	
	E	O	E	O	E	O	E	O	E	O
Grasses										
Andropogon amethystinus	81.3	60.4	798.0	305.9	648.4	184.8	9.8	5.1	86.8	44.2
Brachiaria scalaris	2.1	2.1	66.0	10.0	1.4	0.2	31.7	4.8	0.4	0.4
Cynodon dactylon	0.0	43.8	0.0	71.6	0.0	31.3	0.0	1.6	0.0	11.9
Eragrostis botryodes	2.1	8.3	11.0	55.8	0.2	4.6	5.3	6.7	0.7	1.7
Eragrostis tenuifolia	6.3	10.4	14.3	36.2	0.9	3.8	2.3	3.5	1.0	2.1
Helictotrichon elongatum	25.0	10.4	36.3	93.2	9.1	9.7	1.5	8.9	0.5	3.9
Ischaemum afrum	2.1	12.5	24.0	88.2	0.5	11.0	11.5	7.1	0.4	5.9
Pennisetum humile	0.0	12.5	0.0	34.7	0.0	4.3	0.0	2.8	0.0	3.1
Pennisetum sphacelatum	0.0	12.5	0.0	54.0	0.0	6.8	0.0	4.3	0.0	4.7
Pennisetum thunbergii	95.8	97.9	471.0	401.1	451.4	392.7	4.9	4.1	79.2	100.6
Sporobolus pyramidalis	0.0	4.2	0.0	40.5	0.0	1.7	0.0	9.7	0.0	1.58
Legumes										

Trifolium cryptopodium	16.7	8.3	53.6	79.8	8.9	6.6	3.2	9.6	3.2	2.0
Trifolium mattirolianum	12.5	12.5	69.2	87.7	8.6	11.0	5.5	7.0	4.4	2.9
Trifolium simense	4.2	18.8	128.0	141.0	5.3	26.4	30.7	7.5	7.1	5.6
Trifolium tembense	20.8	50.0	50.9	59.2	10.6	29.6	2.4	1.2	4.1	12.7
Sedges										
Cyperus rigidifolius	81.3	50.0	74.5	40.7	60.5	20.3	0.9	0.8	18.8	12.5
Cyperus rubicundus	0.0	4.2	0.0	20	0.0	0.8	0.0	4.8	0.0	0.7
Cyperus papyrus	6.3	6.3	178.0	24.0	11.1	1.5	28.5	3.8	1.8	1.4
Scleria hispidula	37.5	4.2	71.0	108.0	26.6	4.5	1.9	25.9	7.6	1.2
Scleria schimperiana	20.8	0.0	190.7	0.0	39.7	0.0	9.2	0.0	6.4	0.6
Forbs										
Alchemilla pedata	4.2	18.8	62.0	50.8	2.6	9.5	14.9	2.7	0.8	3.9
Centella asiatica	93.8	75.0	314.7	259.9	295.0	195.0	3.4	3.5	35.8	36.5
Cerastium octandrum	0.0	4.2	0.0	10.0	0.0	0.4	0.0	2.4	0.0	1.1
Crepis schultzii	0.0	25.0	0.0	49.2	0.0	12.3	0.0	2.0	0.1	5.9
Cyanotis barbata	4.2	0.0	29.0	0.0	1.2	0.0	7.0	0.0	0.8	0.0
Haplocarpha hastata	47.9	22.9	19.0	11.8	9.1	2.7	0.4	0.5	8.5	5.8
Oldenlandia monanthos	12.5	14.6	104.5	23.7	13.1	3.5	8.4	1.6	3.3	2.7
Satureja paradoxa	4.2	6.3	27.0	20.7	1.1	1.3	6.5	3.3	0.8	1.1
Uebelinia abyssinica	85.4	89.6	55.0	48.0	47.0	43.0	0.6	0.5	22.7	23.3

A/F = abundance to frequency ratio; E = enclosure; O = open access; IVI = important value index

In the mid-highland agro-ecology, a total of 26 herbaceous species were recorded, of which 19 and 22 herbaceous species were recorded for the enclosure and open access grazing areas, respectively (Table 2.3). Among the identified species from enclosed areas, *Eleusine floccifolia* (IVI = 119.5) was the most dominant abundant species with the highest density followed by *Andropogon amethystinus* (IVI = 67.3), *Trifolium tembense* (IVI = 23.8), *Sporobolus pyramidalis* (IVI = 19.5) and *Cyperus rigidifolius* (IVI = 16.7) as codominant species. Similarly, in open access grazing areas, *Eleusine floccifolia* (IVI = 125.7) was the most dominant abundant species with the highest density with codominant species, such as *Andropogon amethystinus* (IVI = 29.6), *Pennisetum thunbergii* (IVI = 25.7), *Cyperus rigidifolius* (IVI = 18), *Sporobolus pyramidalis* (IVI = 15.6) and *Cerastium octandrum* (IVI = 15). Two annual legumes (*Trifolium*

mattirolianum and *Trifolium rueppellianum)* and three annual sedges (*Cyperus papyrus, Scleria hispidula*, and *Scleria schimperiana)* that were recorded in open access grazing were absent from enclosed areas. Three perennial forbs present in enclosed areas (*Agrocharis melanantha*, *Alchemilla pedata* and *Centella asiatica*) were not recorded in open access grazing areas.

Table 2.3. Frequency (%), abundance, density (0.25 m^2 quadrats) and important value index (IVI) for forage species collected from the enclosure and open access grazing practices in the mid-highland agro-ecology.

Species scientific name	Frequency		Abundance		Density		A/F		IVI	
	E	O	E	O	E	O	E	O	E	O
Grasses										
Andropogon amethystinus	52.1	47.9	425.7	146.1	221.7	70.0	8.2	3.0	67.3	29.6
Cynodon dactylon	10.4	4.2	8.6	15.5	0.9	0.6	0.8	3.7	2.2	0.9
Eleusine floccifolia	97.9	66.7	224.9	304.8	220.2	203.2	2.3	4.6	119.5	125.7
Eragrostis botryodes	0.0	8.3	0.0	214.5	0.0	17.9	0.0	25.7	0.0	6.0
Pennisetum humile	10.4	4.2	17.4	2.5	1.8	0.1	1.7	0.6	2.5	0.8
Pennisetum thunbergii	4.2	41.7	47.5	156.7	2.0	65.3	11.4	3.8	1.4	25.7
Poa leptoclada	39.6	25.0	61.6	30.8	24.4	7.7	1.6	1.2	13.1	7.2
Snowdenia polystachya	4.2	0.0	42.5	0.0	1.8	0.0	10.2	0.0	1.4	0.0
Sporobolus pyramidalis	50	31.3	59.6	57.1	29.8	17.9	1.2	1.8	19.5	15.6
Legumes										
Trifolium cryptopodium	20.8	22.9	25.3	52.4	5.3	12.0	1.2	2.3	5.1	7.8
Trifolium mattirolianum	0.0	8.3	0.0	60.5	0.0	5.0	0.0	7.3	0.0	3.2
Trifolium rueppellianum	0.0	12.5	0.0	27.8	0.0	3.5	0.0	2.2	0.0	4.0
Trifolium simense	14.6	6.3	74.9	70.0	10.9	4.4	5.1	11.2	4.7	2.5
Trifolium tembense	64.6	31.3	78.6	56.2	50.8	17.6	1.2	1.8	23.8	10.2
Sedges										
Cyperus rigidifolius	62.5	62.5	30.4	33.4	19	20.9	0.5	0.5	16.7	18
Cyperus papyrus	0.0	12.5	0.0	8.5	0.0	1.1	0.0	0.7	0.0	2.7
Scleria hispidula	0.0	4.2	0.0	44	0.0	1.8	0.0	10.6	0.0	1.2
Scleria schimperiana	0.0	22.9	0.0	51.9	0.0	11.9	0.0	2.3	0.0	7.1
Forbs										
Agrocharis melanantha	14.6	0.0	12.0	0.0	1.8	0.0	0.8	0.0	3.2	0.0

Alchemilla pedata	8.3	0.0	23.0	0.0	1.9	0.0	2.8	0.0	2.0	0.0
Centella asiatica	4.2	0.0	2.0	0.0	0.1	0.0	0.5	0.0	0.8	0.0
Cerastium octandrum	16.7	54.2	12.9	27.4	2.1	14.8	0.8	0.5	3.7	15
Cyanotis barbata	0.0	22.9	0.0	16.1	0.0	3.7	0.0	0.7	0.0	6.1
Haplocarpha hastata	10.4	6.3	24.0	43.0	2.5	2.7	2.3	6.9	2.5	1.8
Satureja paradoxa	25.0	6.3	46.5	16.0	11.6	1.0	1.9	2.6	7.4	1.4
Uebelinia abyssinica	12.5	27.1	17.5	25.8	2.2	7.0	1.4	1.0	3.1	7.4

A/F = abundance to frequency ratio; E = enclosure; O =open access; IVI=important value index

2.3.3. Species richness, diversity and similarity indexes

Herbaceous species richness was higher in open access grazing areas than the enclosed areas for both agro-ecologies. Although the herbaceous species evenness and the Shannon–Wiener diversity index was similar between the grazing management practices in the highland agro-ecology, the values were slightly higher in open access grazing than enclosed areas in the mid-highland agro-ecology. The overlaps of herbaceous species across the two grazing management practices were similar for both agro-ecologies, as indicated by the Jaccard Index of Similarity (Table 2.4).

Table 2.4. Herbaceous species richness, evenness, diversity and similarity indexes across the two traditional grazing land management practices in the central highlands of Ethiopia.

Index	Agro ecology			
	Highland		Mid-highland	
	Enclosure	Open access	Enclosure	Open access
Species richness	22	27	19	22
Species evenness	0.54	0.52	0.54	0.67
Shannon diversity index (H')	1.68	1.71	1.59	2.07
Maximum possible diversity (Hmax)	3.09	3.29	2.94	3.09
Jaccard's Index of Similarity (IS_J)	68.9		57.7	

2.3.4. Soil physico-chemical properties

The mean soil physico-chemical properties under the two traditional grazing land management practices are presented in Table 2.5. In the highland agro-ecology, soil TN and cation exchange capacity (CEC) were higher (p<0.05) for enclosed areas than open access grazing areas. Similarly, from the mid-highland agro-ecology, available P, CEC, Ca and K were greater (p<0.05) for enclosed areas than open access grazing areas.

Table 2.5. Soil physico-chemical properties (mean ± SEM, 0-20 cm depth) for enclosure and open access grazing land management practices

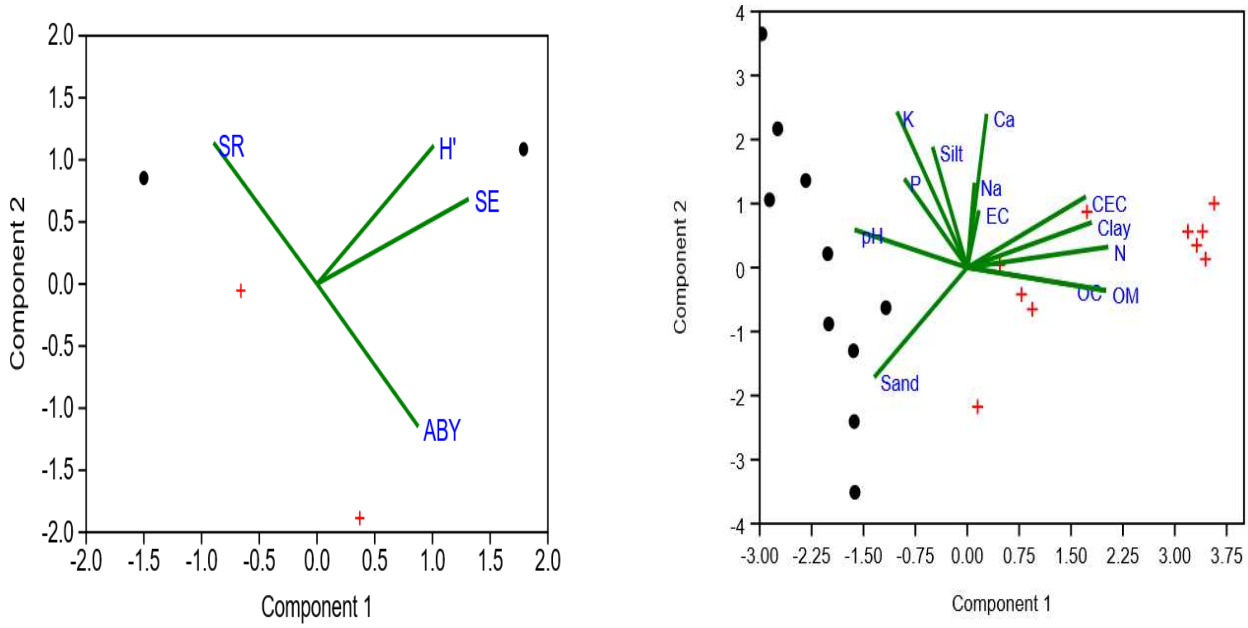
Parameters	Highland		Mid-highland	
	Enclosure	Open access	Enclosure	Open access
pH	5.4 ± 0.2	5.3 ± 0.2	5.8 ± 0.18	5.9 ± 0.04
EC (ds/m)	0.012 ± 0.009	0.001 ± 0.0001	0.008 ± 0.007	0.009 ± 0.006
SOC (%)	9.5 ± 1.9	7.6 ± 1.1	5.6 ± 1.0	5.9 ± 1.2
SOM (%)	16.4 ± 3.2	13.1 ± 1.9	9.7 ± 1.8	10.3 ± 2.1
AP (mg/kg)	9.6 ± 1.3	11.3 ± 3.5	21.9 ± 2.1^{a}	10.0 ± 3.3^{b}
TN (%)	0.51 ± 0.09^{a}	0.42 ± 0.09^{b}	0.35 ± 0.04	0.35 ± 0.04
CEC (Cmol(+)/Kg)	34.1 ± 2.6^{a}	28.7 ± 1.1^{b}	27.2 ± 1.1^{a}	20.2 ± 1.7^{b}
Ca ($Cmol_k$/kg)	44.0 ± 6.5	36.9 ± 6.2	44.3 ± 3.6^{a}	36.2 ± 4.3^{b}
K ($Cmol_k$/kg)	9.4 ± 1.1	10.3 ± 1.0	12.4 ± 0.7^{a}	9.9 ± 1.3^{b}
Na ($Cmol_k$/kg)	21.6 ± 1.1	21.5 ± 1.0	21.6 ± 0.1	21.2 ± 0.4
Texture				
Sand (%)	39.8 ± 2.9	45.5 ± 4.4	50.3 ± 4.3	49.8 ± 3.1
Silt (%)	21.2 ± 2.2	21.5 ± 2.9	23.3 ± 4.6	23.2 ± 2.3
Clay (%)	39.0 ± 2.2	33.0 ± 3.6	26.3 ± 1.7	27.8 ± 2.3

[a, b,] Different letters within each row indicate significant difference for the two grazing land management practices (p<0.05) for each agro-ecology.

2.3.5. Principal component analyses

The PCA correlation variance of aboveground biomass yield, species richness, species evenness and the Shannon–Wiener diversity index indicated clear separation (Figure 2.3a) with

principal component 1 accounting for 50.21% (eigenvalue = 2.00) and principal component 2 accounting for 45.54% (eigenvalue = 1.82) of the total explained variance in relation to the open grazing and enclosures areas. Similarly, PCA of soil parameter characteristics showed distinct separation (Figure 2.3b) between the open grazing and enclosed areas with principal component 1 accounting for 44.33% (eigenvalue = 5.76), principal component 2 accounting for 20.18% (eigenvalue = 2.62) and principal component 3 accounting for 12.83 (eigenvalue = 1.66).



(a) (b)

Figure 2.3. Average principal component analysis correlation (% variance) diagram, based on vegetation and soil parameter attributes from highland and mid-highland agro-ecologies.
Enclosure area = filled plus shape; open access area = dot shape; (a) vegetation attributes: ABY=aboveground biomass yield; SR=species richness; SE=species evenness; H' = Shannon diversity index and (b) soil parameter attributes

2.4. Discussion

2.4.1. Vegetation biomass yield

The lower aboveground biomass yield in the grass species in an open access grazing land in the current study showed the consequences of overgrazing, as a result of year round stocking, which is the principal driving forces for land degradation if open access grazing practice is continuous. Many previous findings (Gebremedhin et al., 2017; Mekuria et al., 2018; Tiscornia et al., 2019) observed that overgrazing (human-induced processes) was huge driving force for land degradation, which is the direct effect of land use change. The result from the mid-highland agro-ecology agrees with the findings of Tadesse et al. (2002), Yayneshet et al. (2009a) and Habtemicael et al. (2014) who found higher aboveground biomass yield in the enclosed areas, compared with free grazing in the highland mixed crop-livestock farming system of Ethiopia, mainly as a result of reduced grazing disturbance by livestock. Similarly, Gebregergs et al. (2019) observed greater aboveground biomass yield from cut-and-carry than open grazing lands in semi-arid areas of the Tselemti district in the north-western Tigray region of Ethiopia. The higher aboveground biomass yield recorded for the *Trifolium* species in open access compared to enclosed areas at the highland agro-ecology in the present study might be associated to the stimulatory effect of grazing on the growth of palatable leguminous plant species, as observed by Taddese et al. (2002) who found more of this species in an area where there is livestock grazing rather than non-grazing practices. The higher proportion of forbs species recorded in freely grazed areas compared to seasonally grazed areas in the highland agro-ecology in the current study was in agreement with Bilotta et al. (2007), who observed that heavily grazed areas were dominated by annual forbs and weedy species. Similarly, the finging of Holechek et al. (2005) explained that overgrazing allows the invasion of annual forbs and grasses.

2.4.2. Herbaceous species composition across

Analysis of IVI in the two traditional grazing land management practices in the present study represented different combinations of species with different dominants and codominants. The higher domination with highest IVI for few species in enclosed areas at both agro-ecologies in the current study agrees with Hailu (2017), who showed that variation of herb distribution in the enclosed areas may be attributed to the survival and reproduction of maximum species, as a result of moderate level of species competition during early regeneration. This has resulted in the domination of only a few species in his study done in the Harishin Rangelands of Eastern

Ethiopia. Similarly, Grime (1973) noted that with an increase in environmental stress, the species adapted to low levels of environmental stress lose their competitive advantage, whereas those that are more resistant to environmental stress can increase in abundance.

Andropogon amethystinus, the most desirable and dominant perennial grass species in the enclosed areas in the highland agro-ecology declined when open access grazing was practiced. This might be as a result of the inability of the species to tolerate heavy grazing than in other climatic conditions. The dominance of *Eleusine floccifolia*, a perennial grass species with a tough branching rhizome, in both grazing practices in the mid-highland agro-ecology might be as a result of longer dry season period compared with the highland agro-ecology where both grazing areas were grazed freely for a longer period until the onset of short rainy season, when the enclosed areas were protected from the entrance of the animals. The higher proportions of annual *Trifolium* species recorded in open access grazing compared to enclosed areas in the mid-highland agro-ecology for this study agrees with the finding of Taddese et al. (2002) who recorded *Trifolium* species only in grazed plots, compared with non-grazed plots in mixed crop-livestock system in the western highlands of Ethiopia. This indicates that livestock grazing is more advantageous in the growth of palatable leguminous plant species, compared with non-grazed plots. Similarly, the absence of the majority of annual sedge species in the enclosed areas in the current study in the mid-highland agro-ecology showed the dominance of annual species in open access grazing than in enclosed areas, which confirms the previous finding (Bilotta et al., 2007). However, many of the annual plants promoted by heavy grazing have low production potential because of their small size and short growing cycle (Woldu and Mohamed Saleem, 2000). Nevertheless, grazing can severely alter the species composition of grazing systems overtime (Milchunas and Lauenroth, 1993). The presence of longer time overgrazing over many years (Bard et al., 2000) in the mixed crop-livestock system of northern Ethiopia has altered the species composition of the grazing lands in ways that greatly differed from the seasonal grazing and the cut-and-carry regimes.

2.4.3. Species richness, diversity and similarity indexes

Greater herbaceous species richness was observed in the current study for open access grazing areas compared to enclosed areas at both agro-ecologies, where a slightly higher Shannon–Wiener diversity index, maximum possible diversity and species evenness were recorded for open access grazing land practices at mid-highland agro-ecology. This finding agrees with the finding of Pokharel et al. (2007), who observed higher species richness in open plots in two seasons compared to controlled plots in their study done in Trans-Himalayan Rangeland. Similarly, the findings of the present study are in agreement with the generalization made by Sternberg et al. (2000), who reported that continuous grazing increased species richness but was reduced by heavy grazing. Many previous studies (Mwendera et al., 1997; Taddese et al., 2002; Tessema et al., 2011) reported that light or moderate grazing increases species richness when, compared with non-grazing and heavy grazing practice. The overlap in enclosed areas and open access grazing land was uniform at both agro-ecologies, as measured by the Jaccard Index of Similarity, which indicates that the two grazing regimes share many of the same species.

2.4.4. Soil physico-chemical properties

The higher soil TN found in this study in the highland agro-ecology for enclosed areas compared to the open access area, agrees with the finding of Habtemicael et al. (2014) who reported the highest soil TN in areas under cut-and-carry or seasonal grazing management practices than continuous stocking in eastern zone of Tigrai, northern Ethiopia. Similarly, Jeddi and Chaieb (2010) obtained greater TN in a 12-year exclosure study compared to continuous grazing from their study done in degraded arid environments of South Tunisia. According to Augustine (2003), urine and faeces depositions increase soil N and P in many grazed systems, but this was not observed in the current study for areas under open access grazing management practices where livestock freely graze on a year-round basis. The possible explanation might be collection of dung by children for fuel, which was a case reported earlier in the mixed crop-livestock system in the highland areas by Taddesse et al. (2002). Cation exchange capacity was greater in enclosed areas than in open access areas, and this might be as a result of higher concentration of SOC in enclosed areas than in open access grazing (Table 2.5).

The reduction in soil available P, CEC, Ca and K in the open access grazing land could be the results of heavy grazing year-round and have a consequence on pasture productivity. The higher soil available P observed for enclosed areas compared to the open access grazing area in the mid-highland agro-ecology in the present study, did not agree with the findings of the previous studies (Jeddi and Chaieb, 2010; Teague et al., 2011; Habtemicael et al., 2014) that showed insignificant difference in available P because of different grazing land management practices. The current results in soil Ca and K concentration were in line with Jeddi and Chaieb (2010) who showed greater soil Ca and K in exclosure areas than for free grazing. Similar findings were observed by Hailu (2017) who reported higher soil available K in enclosed areas than for open access grazing land management practices (Table 5). Soil organic carbon and SOM were not significant ($p>0.05$), as a result of grazing land management practices at both agro-ecologies. This disagrees with the earlier result reported by Hailu (2017) who found higher SOC and SOM for enclosed areas than open access grazing.

2.5. Conclusion

The results from the present study showed that enclosure and open access grazing land management practices differentially affected the evaluated parameters. Aboveground biomass yield decreased from open access grazing area as a consequence of overgrazing due to continuous uncontrolled stocking. The abundance and density of some palatable and dominant perennial grass species in the enclosed areas decreased in open access grazing land because of the inability of the species to tolerate continuous stocking. On the other side, herbaceous species richness was higher in open access grazing land than the enclosed areas. The stress of continuous stocking in an open access grazing land also decreased soil quality parameters, such as TN, AP, Ca, Na and CEC, which in turn affected grazing land productivity. It can be concluded that enclosures have a positive effect in increasing grazing land productivity and maintain soil fertility. Therefore, within the declined grazing land as a result of different driving forces, mainly human population coupled with expansions of cultivated land, implementation of enclosure area practices play an important role in increasing feed resource availability, environmental sustainability and productivity of the farming system in the study area.

CHAPTER THREE

Nutritive value characterization of key forage species from traditional enclosure area in the central highlands of Ethiopia

Abstract

This study was conducted with the aim to characterize key forage species from traditional enclosure area in the central highlands of Ethiopia in terms of their chemical composition and ruminal fermentation production. By using quadrats, samples of 19 key forage species were collected at 50% flowering. Results showed significant variations in crude protein (CP) and fibre contents, gas and methane (CH_4) production among the evaluated species. Multivariate analysis was conducted to investigate the relationship between the key forage species and nutritive values of the traits. Principal component (PC) analysis showed that the first three PC (PC1 to PC3) explained 84.2% of the total variation in the dataset. The first PC (PC1), which explained 54.7% of the total variation was positively correlated with CP and total gas production at 24 h, whereas negative correlation was observed for the fibre contents and CH_4 production. The second PC (PC2), which explained 17.9% of the total variation was positively correlated with ADF and negatively associated with the digestibility of the feed. The key forage species clustered based on their chemical composition and *in vitro* fermentable parameters into five main groups. *Centella asiatica* forb was superior in gas production at 24 h, fractional gas production rate (c) and EGP and lower in NDF and CH_4 production and separated from the others in cluster analysis. Forage legumes were clustered close to each other and had on average higher CP content, higher fractional rate of fermentation (c) and EGP production compared to forage grass species. Forage grasses except *Eragrostis tenuifolia* were characterized by higher CH_4 production compared to forage legumes, sedges and forbs in cluster analysis. Therefore, appropriate management practices that maintains balanced proportions of herbs such as *Centella asiatca*, forage legumes (such as *Trifolium rueppellianum* in group III) and forage grasses (such as *Eragrostis tenuifolia* in group II and *Poa leptoclada* in group IV) may result in improved ruminal fermentation of the available diet while reducing methane emissions by ruminants fed on native pasture. Further studies should be conducted on *Centella asiatica* forb regarding its yield, anti-nutritional and

actual feeding values to ruminant animals in order to utilize widely as a possible forage crop in the feeding system of ruminant animals in the central highlands of Ethiopia.

Keywords: chemical composition; crop-livestock; evaluation; fermentation; multivariate technique.

3.1. Introduction

Livestock production plays a crucial share in the economy of Ethiopia (Duressa et al., 2014). Despite high livestock population, the present livestock contribution is less than its potential that may affect sustainability of crop-livestock production systems due to complex and inter-related factors like feed shortage and disease (Selamawit et al., 2017). Feed shortage is more severe in the highlands of the country where greater than 75% of human as well as livestock populations are concentrated (CSA, 2013b). In the mixed crop-livestock farming system, the feed requirement for livestock is mainly derived from natural pasture, crop residues and stubble grazing (Tolera, 2009; Duressa et al., 2014; Selamawit et al., 2017). Feed resources derived from natural pastures has declined in the highlands because of shrinking pastureland due to the expansion of cropping, reduced quantity and quality of pasture associated with over utilization and land degradation (Melaku et al., 2010). The increasing pressure on available pasture lands and newly emerging climatic phenomena is affecting the botanical composition, biomass yield and nutritive value of pastures (Kitaba and Tamir, 2005; Denekew et al., 2005; Tessema et al., 2010). This in turn affects animal production and reproduction performances and hence the livelihoods of smallholder farmers (Melaku et al., 2010). Therefore, it is necessary to understand the full life natural pastures are going through by exploring the changes in terms of botanical composition and nutritive value parameters. Such information could be important to devise appropriate grazing land management practices and livestock feeding systems which help to manage the ecosystem to feed people while protecting biodiversity simultaneously.

It is well know that some of the nutrients such as protein content of forage species is low during the dry season, leading to a prolonged periods of under-nutrition of livestock reared under dry season (Yayneshet et al., 2009b). Even though natural pasture is the first most important source of feed for livestock in the highland and mid-altitude agro-ecologies (Alemu et al., 2019), with the exception of some improved forages (Kebede et al., 2016b) and fodder trees (Assefa et al., 2015), there is little information on its nutrient content and *in vitro* ruminal fermentation parameters for the different species that describes and identifies native pasture from the central highland of Ethiopia. Research conducted in the highland areas so far were focused on majorly feed resourcs characterization works and determination of chemical composition based on a few

proximate parameters such as DM, CP and fibre contents of key species (Geremew et al., 2017; Husein, 2017). This existing knowledge pool does not present enough information to determine the true nutritive values and information that complements the indigenous knowledge of farmers by scientific data. To assess the nutritive value of forage at minimum cost, there are a number of *in vitro* techniques present so far (Giger-Reverdin et al., 2002). According to Abdalla et al. (2012), *in vitro* gas production can be used as a quick evaluation tool to evaluate nutritional quality of forages and it also helps to better quantify nutrient use, and its accuracy in describing digestibility in animals has been validated in several experiments (Getachew et al., 2004). In addition, many driving forces such as human population growth and climatic change that alter the biophysical settings of the available grazing lands in the highland area resulted in loss of soil fertility and thus land degradation which necessitated the need for regular monitoring of pasture composition, productivity and quality.

Therefore, the objectives of this study were (i) to characterize chemical composition and *in vitro* ruminal fermentation parameters of the key forage species, (ii) to determine the relationships between chemical compositions and *in vitro* ruminal fermentation parameters of the key forage species and (iii) investigate the relationship between the key forage species and nutritive values of the traits using multivariate analysis. Such information assists to develop appropriate grazing land management practices and feeding systems.

3.2. Materials and Methods

3.2.1. Experimental site

Forage collection was conducted in Kofele district of West Arsi Zone of Oromia Regional State, Ethiopia and located at $7^0$00’ to $7^0$07’ N and $38^0$48’ to $39^0$00’ E. The district is located with a altitude within 2200 to 3200 masl and receives an average rainfall of 1800 mm per annum and has an average temperature of 19.5 ^{0}C. It has a bi-modal rainfall distribution with short rains starting from March to May and the main rainy season extending from June to September/October. The area is characterized as high potential for crop-livestock farming in which cattle and sheep are the most predominant livestock species (Hussein, 2017).

3.2.2. Forage collection and processing

Forage sample collection was conducted as described by Wegi et al. (2020). Briefly, a total of 20 plots of 40 m x 40 m were established on pasture land (locally described as '*Kelo*') in the highland and mid-highland agro-ecologies. The pasture land was enclosed for five months from May 2018 to September 2018. Sixteen quadrats (0.5 m x 0.5 m) were randomly laid per plot for forage sample collection. Forage samples within each quadrat were harvested at 50% flowering by mowing the pasture at 5 cm aboveground from the area of 0.25 m^2 quadrats. The harvested pasture was sorted by species and pooled to obtain enough quantities of the individual species in the sampling area for analysis. Sample collection was done purposely to coincide with period of utilization of the enclosure area (*kelo*) by farmers as per the information collected from elder farmers in the study area. A total of 34 species were identified in the process out of which 19 were selected for the present study based on their rank in frequency, dominance, abundance, IVI and elder farmers' judgments as reported by Wegi et al. (2020). Samples of composite forage species were taken and oven-dried to a constant weight at 60 ^{0}C for 48 hours and then ground in a Willey mill to pass through a 1 mm sieve for chemical composition analysis.

3.2.3. Chemical analysis

All analyses were done in triplicate and analysis for DM, ash and N contents were done according to AOAC (2006). Nitrogen content was determined by using a standard Kjeldahl method and CP was calculated as N x 6.25. Neutral detergent fibre content was determined according to method of Van Soest et al. (1991), whereas ADF and acid detergent lignin (ADL) contents were determined according to the method of Van Soest and Robertson (1985). Heat stable amylase was not added to NDF, and both NDF and ADF were expressed inclusive of residual ash. Organic matter digestibility (OMD) and Metabolizable energy (ME) content of the experimental feeds were estimated using the equation of Menke and Steingass (1988).

3.2.4. In vitro ruminal fermentation parameters

The *in vitro* gas production was determined according to Menke and Steingass (1988). Three rumen-cannulated male sheep were used as rumen inoculum donors and rumen fluid was collected from the sheep prior to morning feeding in to a thermos flask that had been pre-warmed to a temperature of 39 °C and pooled and filtered through cheese cloth. Oven dried and ground forage samples (200 mg) were accurately weighed and carefully dropped in to 100 ml glass syringes. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution, and two blanks containing 30 ml of medium (inoculums and buffer) only were incubated at the same time. The syringe was tapped and pushed upward by the piston in order to eliminate air in the inoculum. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of gas. The syringes were placed in a rotor inside the incubator at 39 °C (Plate 3.1), and the samples were incubated once in a triplicate, since the data were meant to be used for multivariate analysis. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. The volume of a gas produced was recorded at 3, 6, 12, 24, 48, 72 and 96 h and the average of the volume of gas produced by the blank against each incubation time were deducted from the recorded values from each sample of incubated bottles.

A parallel experiment was conducted at the same time for methane measurement, as it was not possible to measure gas production thereafter due to the addition of sodium hydroxide to the incubated materials. At the end of the 48 h incubation period and recording the final gas volume in the syringe, 4 ml of (10 M) sodium hydroxide was introduced from the latter into the incubated contents, thereby avoiding gas escape. Sodium hydroxide was added to absorb CO_2 that was produced during the process of fermentation and the remaining volume of gas was recorded as methane (Fievez et al., 2005). The average volume of gas produced from the blanks was deducted. The *in vitro* ruminal gas production work was carried out at Animal Feed and Nutrition laboratory of Hawassa University, Ethiopia and the care, handling and maintenance of cannulated sheep were done in accordance with animal welfare regulations of the animal ethics committee of the University of Pretoria (NAS086/2019). The post incubation parameters such as ME (MJ/kg DM) and OMD (%) of the incubated samples were estimated according to equation of Menke and Steingass (1988) by using 48 h gas production data, whilst short chain fatty acid (SCFA, µmo) at 24 h post incubation was computed from gas volume using the linear equation by Makkar (2005).

ME (MJ/kg DM) = 2.2+0.136 GV +0.0057 CP

OMD (%) = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash

SCFA (µmol) = 0.0222GV- 0.00425

Plate 3.1. Partial view of total gas and methane production measurements at animal nutrition laboratory of School of Animal and Range Sciences, Hawassa University.

3.2.5. Gas production characteristics

Gas production patterns were evaluated by the non linear model developed by Ørskov and McDonald (1979), as modified by Osuji et al. (1993):

$$Y = b\ (1\text{-}e^{-ct})$$

Where Y = the cumulative volume of gas produced at time t; b = the asymptotic gas volume; c = fractional fermentation rate; t = incubation time and e = base of natural logarithm. The intercept is not included in the model as there is no gas production from unfermented feed, as explained by Osuji et al. (1993). Effective gas production (EGP) is calculated using the equation:

$$EGP = (bc/(c+k)),$$

Where k is outflow rate from the rumen, assumed to be 5%/h.

3.2.6. Statistical analysis

Values for chemical content and *in vitro* gas production were presented in graph and table for visual comparison. Multivariate analysis was conducted using the chemical composition and *in vitro* fermentable parameters data in order to evaluate the key forage species in terms of chemical composition and gas production characteristics and systematically group them into different groups to investigate variations between and within the group. The correlation matrix was used for the calculation, after the data was mean centered and standardized for principal component (PC) analysis. The first three principal components (PC1 to PC3) that explain the greater amount of variation were plotted graphically in order to identify forage species with similar characteristics. Points scattered close to each other on the graph represent species that are similar in their nutrient content and *in vitro* fermentation parameters. Data for PCA was analysed using SAS software, and for cluster analysis, Paleontological Statistics test (PAST) was used (Hammer et al., 2001). Correlations between the characteristics were computed on the mean values of the forage species during cluster analysis.

3.3. Results

3.3.1. Chemical composition of selected pasture species

Chemical composition of the selected key forage species are shown in Table 3.1. Crude protein (g kg^{-1} DM) content of the grass species varied widely from 45 to 99 with the lowest for

Eragrostis tenuifolia and the highest for *Cynodon dactylon*. The CP content for forage legumes ranged from 174 to 202 and comparable except for *Trifolium simense* (139), which was the lowest in CP content among others. The CP content for sedge and forb ranged from 83 to 137 and the higher CP content was observed for *Centella asiatica* forb. The neutral detergent fibre (g kg^{-1} DM) for the grass species in the current study varied from 592 to 698 except for *Eragrostis tenuifolia* (459), while those for legumes varied from 487 to 537 with the lowest for *Trifolium tembense* and the highest for *Trifolium cryptopodium*. The NDF contents of sedge and forbs varied from 606 to 664 except for *Centella asiatica* forb (343). Acid detergent fibre (g kg^{-1} DM) varied from 300 to 454, 316 to 392 and 257 to 394 and ADL (g kg^{-1} DM) varied from 38 to 77, 70 to 97 and 49 to 67 for grasses, legumes and others (sedge and forbs), respectively. In general, grasses contained greater NDF and ADF components and less ash and CP contents compared to legumes. Higher IVI for the selected forage species such as *Eleusine floccifolia*, *Andropogon amethystinus* and *Pennisetum thunbergii* were observed in that order as indicated in Table 3.1.

Table 3.1. Chemical composition of selected forage species from traditional enclosure area of Kofele district, West Arsi Zone, Oromia, Ethiopia

Forage species	IVI (%)[a]	DM	Ash	CP	NDF	ADF	ADL
			g kg^{-1} DM				
Grasses							
Andropogon amethystinus	57.0	921	122	67	608	334	56
Cynodon dactylon	3.8	915	138	99	592	300	38
Eleusine floccifolia	122.6	922	83	45	698	454	74
Eragrostis botryodes	2.1	914	64	50	654	383	56
Eragrostis tenuifolia	1.6	902	98	45	459	330	77
Helictotrichon elongatum	2.2	923	89	62	642	373	54
Pennisetum sphacelatum	2.4	927	104	48	682	446	68
Pennisetum thunbergii	51.7	924	114	51	624	343	49
Poa leptoclada	10.2	915	75	67	637	356	54
Sporobolus pyramidalis	9.2	919	75	58	632	374	57
Legumes							
Trifolium cryptopodium	4.5	913	140	202	537	348	86

Trifolium mattirolianum	2.6	898	121	194	491	328	77
Trifolium rueppellianum	2.0	904	97	174	491	352	70
Trifolium simense	5.0	901	111	139	488	392	97
Trifolium tembense	12.7	902	139	195	487	316	82
Others (sedge and forbs)							
Cyperus rigidifolius	16.5	909	74	118	664	394	67
Cerastium octandrum	5.0	921	109	83	622	351	50
Uebelinia abyssinica	14.1	913	136	102	606	337	62
Centella asiatica	18.3	894	139	137	343	257	49

ADF=acid detergent fibre; ADL=acid detergent lignin; CP=crude protein; DM=dry matter; IVI=important value index; NDF=neutral detergent fibre.

[a]Average IVI obtained from highland and mid-highland agro-ecologies from enclosure and open access grazing land for each species as observed by Wegi et al. (2020).

3.3.2. In vitro fermentation parameters and gas production characteristics

Total gas and methane production and predicted parameters are shown in Table 3.2. For all studied species, the collective gas production increased during the incubation period. The volume of gas produced by the grass species varied widely from species to species and the variations were not constant for various incubation times. Gas produced by the grass species at 24 h (ml/g DM) ranged from 27.3 to 155.7 with the lowest for *Pennisetum sphacelatum* and the highest for *Poa leptoclada.* On the other side, gas produced by legumes (ml/g DM) at 24 h varied from 145.1 to 210.3 with the lowest for *Trifolium cryptopodium* and the highest for *Trifolium rueppellianum.* Gases produced by *Centella asiatica* forb at 24 h of incubation (237.8 ml/g DM) was the highest from other species studied. Average gas produced from legumes (179.9 ml/g DM at 24) was higher than gas produced from the grass species (99.4 ml/g DM at 24). Fractional gas production rate "c" and EGP showed wide variability for the evaluated forage species regardless of their botanical composition. The fractional gas production rate "c" varied from 0.001 to 0.025 h^{-1} for grasses, and 0.027 to 0.044 h^{-1} for legumes. The volume of EGP ranged from 19.4 ml/g DM for *Pennisetum sphacelatum* to 125.7 ml/g DM for *Poa leptoclada*. The highest EGP volume of 190.9 ml/g DM was observed for *Centella asiatica* forb. The gas production patterns

for the key grass species are shown in Figure 3.1 while for legumes and others (sedge and forbs), it is shown in Figure 3.2 and Figure 3.3, respectably.

Methane produced (ml/g DM) by the grass species at 48 h of the incubation period varied from 27.8 to 130.3 with the lowest for *Eragrostis tenuifolia* and the highest for *Eleusine floccifolia* (Table 3.2). Methane produced (ml/g DM) by forage legumes ranged from 33.0 to 49.8, and sedge and forbs ranged from 16.5 to 33.5. The lowest methane production was recorded from *Cyperus rigidifolius* sedge, which is the second dominant species in the highland area during the short rainy season next to *Centella asiatica* forb. On average, grasses produced 43.9% higher methane than legumes in the current study. Short chain fatty acid (SCFA) produced (µmol) at 24 h from the species studied varied widely from 0.59 to 5.26 with the lowest for *Pennisetum sphacelatum* grass and the highest for *Centella asiatica* forb. The *in vitro* OMD in the grass species ranged from 47.4 to 59.1%, from 56.5 to 60.4% in legumes and from 55.7 to 66.4% in sedge and forb species. Metabolizable energy (MJ/kg DM) concentrations in the grasses ranged from 7.1 to 8.7, in legumes from 7.4 to 8.4 and from 7.8 to 9.5 in sedge and forbs.

Table 3.2. *In vitro* total gas and gas production characteristics (ml/g DM), methane (CH_4, ml/g DM), SCFA (µmol) production, OMD (%) and ME (MJ/kg DM) contents of forage species from traditional enclosure area of Kofele district, West Arsi Zone, Oromia, Ethiopia

Forage species	Total gas		Gas production characteristics		CH_4	SCFA	OMD	ME
	24 h	96 h	c	EGP	48 h			
Grasses								
Andropogon amethystinus	32.6	122.2	0.001	24.6	73.3	0.70	52.4	7.6
Cynodon dactylon	112.0	270.4	0.017	90.9	60.3	2.46	56.5	7.9
Eleusine floccifolia	132.8	325.3	0.013	97.7	130.3	2.93	48.4	7.2
Eragrostis botryodes	95.7	287.1	0.005	72.8	123.0	2.10	54.7	8.4
Eragrostis tenuifolia	133.1	277.3	0.025	107.2	27.8	2.93	59.1	8.7
Helictotrichon elongatum	120.2	363.2	0.012	110.1	43.5	2.65	50.9	7.4
Pennisetum sphacelatum	27.3	79.3	0.002	19.4	95.8	0.59	47.4	7.1
Pennisetum thunbergii	54.1	248.9	0.002	80.5	70.3	1.18	52.5	7.8
Poa leptoclada	155.7	311.5	0.025	125.7	71.0	3.44	53.3	7.8
Sporobolus pyramidalis	130.5	326.3	0.016	107.0	57.3	2.88	51.0	7.6
Legumes								
Trifolium cryptopodium	145.1	295.8	0.027	121.5	33.0	3.20	60.3	8.0
Trifolium mattirolianum	194.9	300.7	0.041	148.6	41.8	4.31	60.4	8.2
Trifolium rueppellianum	210.3	312.7	0.043	162.2	49.8	4.65	59.7	8.4
Trifolium simense	183.1	269.2	0.044	139.2	39.0	4.04	56.5	7.9
Trifolium tembense	166.4	260.6	0.04	130.4	47.3	3.67	57.5	7.4

Others (forb and sedges)								
Cyperus rigidifolius	79.8	291.5	0.002	60.0	16.5	1.75	55.7	7.8
Cerastium octandrum	59.7	190.1	0.005	47.3	24.3	1.30	56.8	8.3
Uebelinia abyssinica	43.8	246.5	0.002	39.5	33.0	0.95	56.5	8.1
Centella asiatica	237.8	293.7	0.059	190.9	33.5	5.26	66.4	9.5

DM= dry matter; ME= metabolizable energy; OMD= organic matter digestibility; SCFA= short chain fatty acid; c= fractional gas production rate (%/h). EGP is the effective gas production calculated using the equation EGP = (bc/(c+k)), where k is outflow rate from the rumen, assumed to be 5%/h. DM=dry matter.

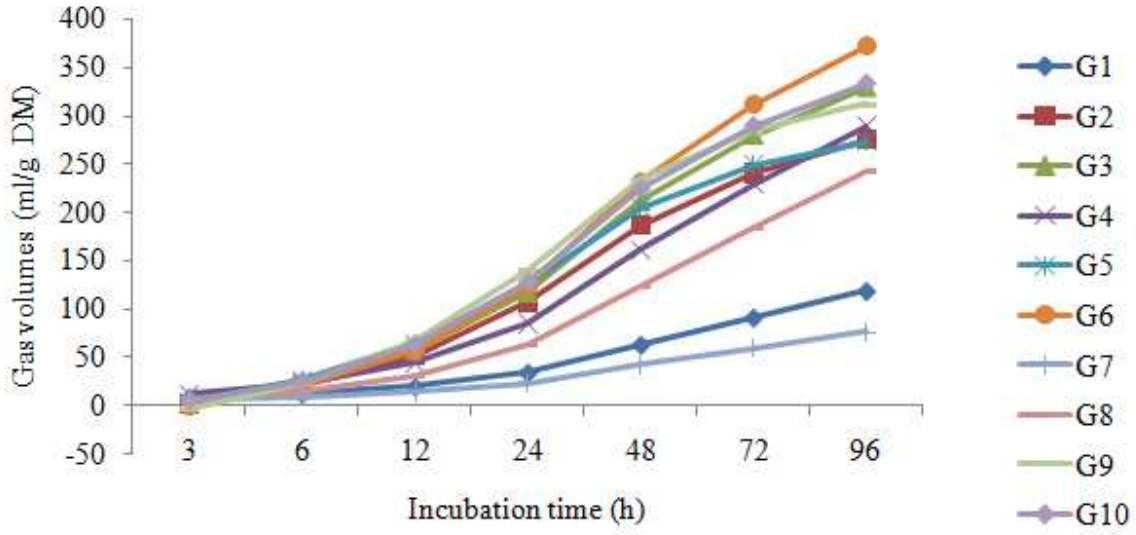


Figure 3.1. Gas production patterns for the tropical grasses.

G1=*Andropogon amethystinus*; G2=*Cynodon dactylon*; G3=*Eleusine floccifolia*; G4=*Eragrostis botryodes*; G5=*Eragrostis tenuifolia*; G6=*Helictotrichon elongatum*; G7=*Pennisetum sphacelatum*; G8= *Pennisetum thunbergii*; G9=*Poa leptoclada*; G10= *Sporobolus spyramidalis.*

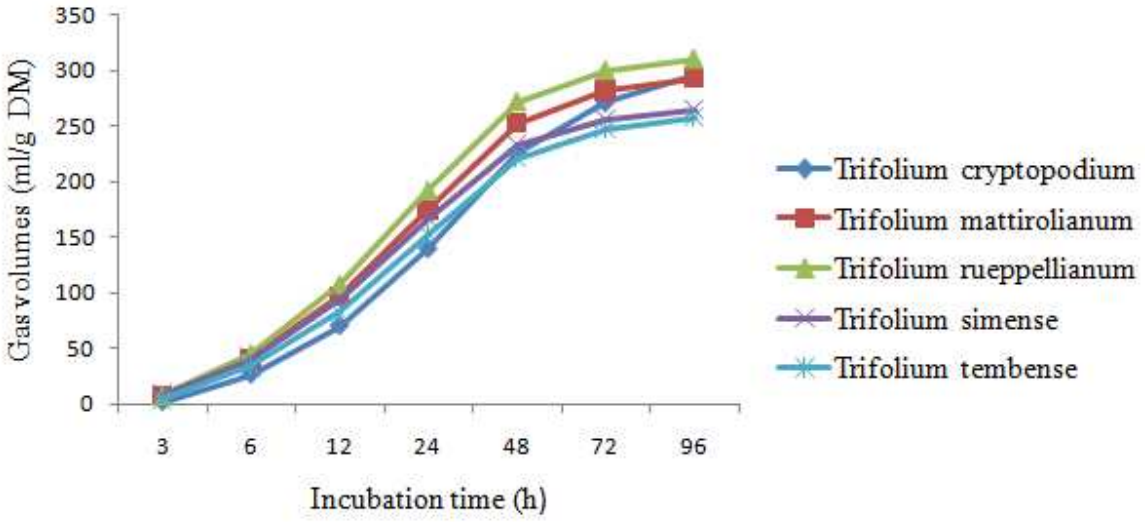


Figure 3.2. Gas production patterns for forage legumes

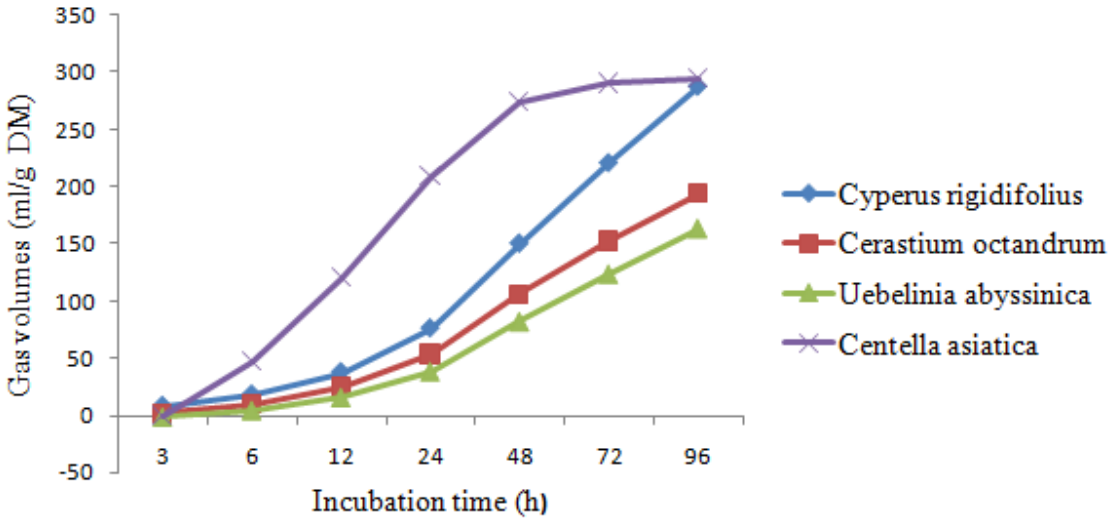


Figure 3.3. Gas production pattern for sedge and forb species

3.3.3. Principal component analysis

From the PCA correlation variance, the first three principal components (PC1 to PC3) had eigenvalue greater than 1 (Table 3.3), and these three components explained 84.2% of the total variation of the data. In particular, the first principal component (PC1), which explained 54.7% of the total variation, was positively associated with CP, OMD, ME, fractional gas production rate (c), EGP and total gas production at 24 h, whereas it was negatively correlated with NDF, ADF and CH_4 production. The second PC (PC2), which explained 17.9% of the total variation was positively correlated with ADF and total gas production at 96 h, while it was negatively associated with ash, OMD and ME content. The third PC (PC3) which explained 11.6% of the total variation was positively correlated with CP and ADL, and was negatively correlated with ME and total gas production at 96 h. Data structure of forage species in the two-dimensional space was shown in Figure 3.4 for PC1 vs. PC2 and in Figure 3.5 for PC1 vs. PC3. Forage species which had higher values for PC1 (*Centella asiatica* forb and all legume species evaluated) were characterized by higher CP content, fractional gas production rate (c), EGP and total gas production at 24 h, but had lower fiber contents and CH_4 production. Similarly, forage grasses which had higher values for PC2 (*Eleusine floccifolia*, *Eragrostis botryodes*, *Pennisetum sphacelatum* and *Poa leptoclada*) were characterized by higher ADF and CH_4 production. As

well, legume species such as *Trifolium simense* and *Trifolium rueppellianum* had also greater total gas production along PC2. *Pennisetum sphacelatum* grass which was characterized by higher ADF, and forage legumes such as *Trifolium tembense*, *Trifolium cryptopodium* and *Trifolium simense* were characterized by higher CP and lignin content and these had higher correlation values with PC3.

Table 3.3. Eigenvector coefficient of 13 chemical composition and *in vitro* fermentable parameters for the first three principal components with Eigenvalue of the total variance.

Variables	Principal Component		
	First	Second	Third
Ash	0.163	−0.435	0.309
Crude Protein	0.28	−0.05	0.402
Neutral detergent fibre	−0.346	0.14	−0.045
Acid detergent fibre	−0.248	0.393	0.251
Acid detergent lignin	0.112	0.244	0.638
Total gas production at 24 h	0.333	0.282	−0.051
Total gas production at 96 h	0.175	0.384	−0.305
Methane production	−0.198	0.291	−0.1
Short chain fatty acid	0.333	0.286	−0.052
Organic matter digestibility	0.333	−0.227	−0.065
Metabolizable energy	0.251	−0.213	−0.378
Fractional gas production rate (c)	0.351	0.136	0.079
Effective gas production	0.336	0.256	−0.093
Eigenvalue	7.108	2.331	1.51

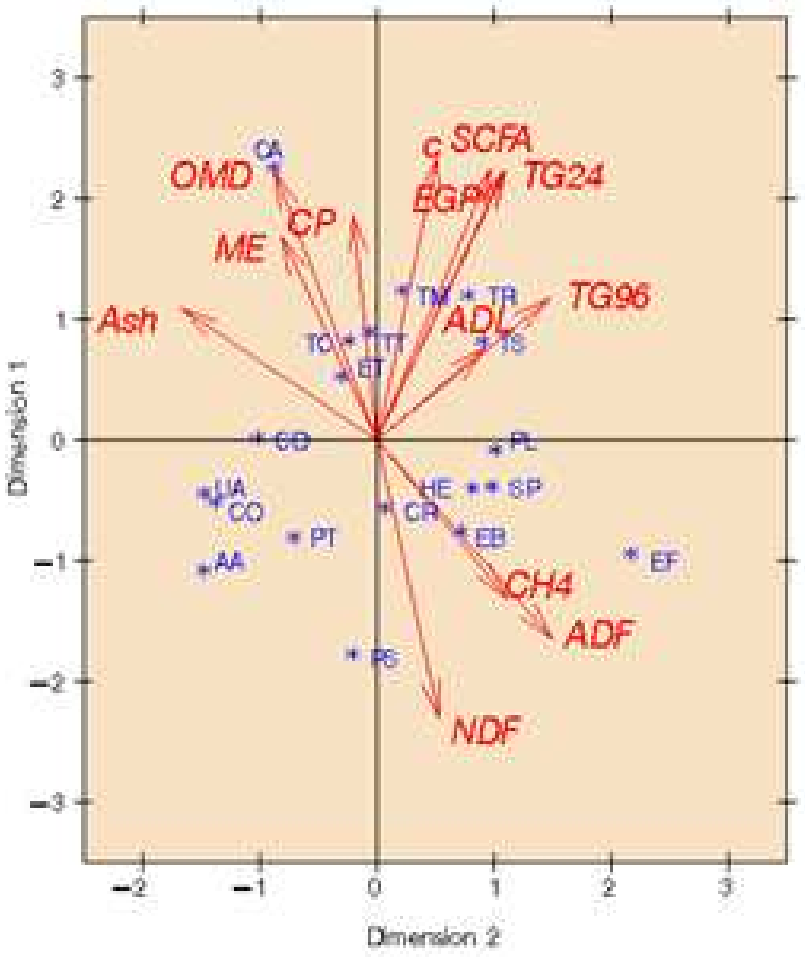


Figure 3.4. Principal component analysis for PC1 vs PC2 showing the clustering and separation of forage species depending on chemical composition and *in vitro* ruminal fermentation parameters.

AA= Andropogon amethystinus; CD= Cynodon dactylon; EF= Eleusine floccifolia; EB= Eragrostis botryodes; ET= Eragrostis tenuifolia; HE= Helictotrichon elongatum; PS= Pennisetum sphacelatum; PT= Pennisetum thunbergii; PL= Poa leptoclada; SP= Sporobolus pyramidalis; TC= Trifolium cryptopodium; TM= Trifolium mattirolianum; TR= Trifolium rueppellianum; TS= Trifolium simense; TT= Trifolium tembense; CR= Cyperus rigidifolius; CO= Cerastium octandrum; UA= Uebelinia abyssinica; CA= Centella asiatica.

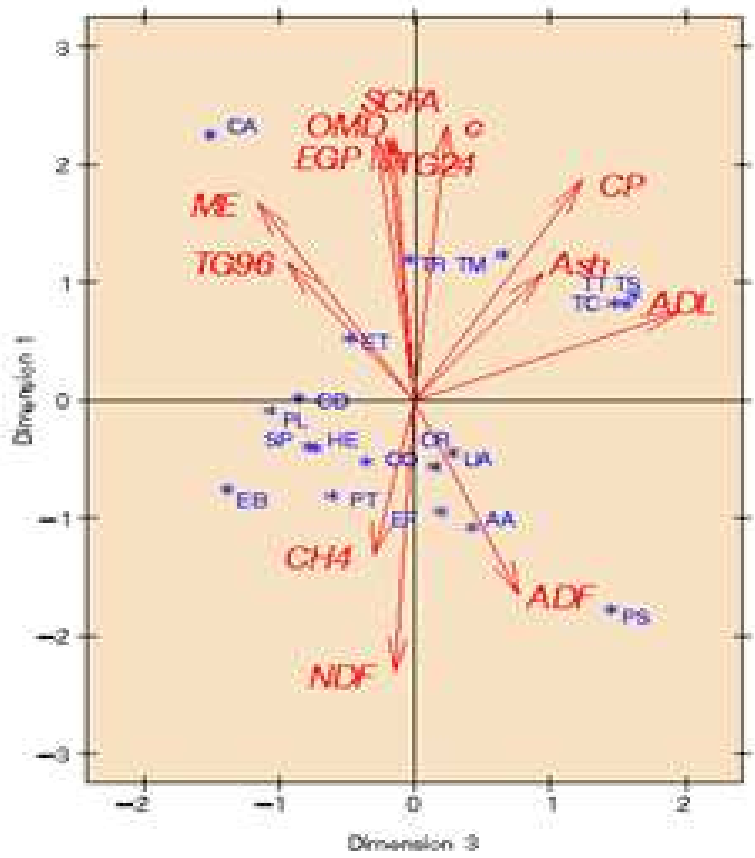


Figure 3.5. Principal component analysis for PC1 vs. PC3 showing the clustering and separation of forage species depending on chemical composition and *in vitro* ruminal fermentation parameters.

AA= Andropogon amethystinus; CD= Cynodon dactylon; EF= Eleusine floccifolia; EB= Eragrostis botryodes; ET= Eragrostis tenuifolia; HE= Helictotrichon elongatum; PS= Pennisetum sphacelatum; PT= Pennisetum thunbergii; PL= Poa leptoclada; SP= Sporobolus pyramidalis; TC= Trifolium cryptopodium; TM= Trifolium mattirolianum; TR= Trifolium rueppellianum; TS= Trifolium simense; TT= Trifolium tembense; CR= Cyperus rigidifolius; CO= Cerastium octandrum; UA= Uebelinia abyssinica; CA= Centella asiatica.

3.3.4. Cluster analysis

The finding from cluster analysis is presented in Figure 3.6. The graph showed that the key forage species were classified in to five groups with a possibility to divide group IV into two sub

groups (IVa and IVb). Group I included *Centella asiatica* forb which had higher gas production at 24 h, higher fractional gas production rate (c) and higher EGP, but lower in NDF and CH_4 production compared to the rest groups. Group II is represented solely by *Eragrostis tenuifolia* grass and group III contains all legumes, which differed from groups IV and V that had all grass species except *Eragrostis tenuifolia.* Group III, which had all the legumes have on average higher CP content, higher fractional rate of fermentation (c) and higher EGP, but lower CH_4 production compared to grasses in groups IV and V. Group II which contain *Eragrostis tenuifolia* grass differed from group III (all the legume species) by having lower CP content and lower CH_4 production and lower fractional rate of fermentation (c) when compared to average values of group III. Group IV (most of the grass species) differed from group V (*Eleusine floccifolia* and *Pennisetum sphacelatum* grasses*)* by producing lower CH_4 production as compared to the average values recorded for group V.

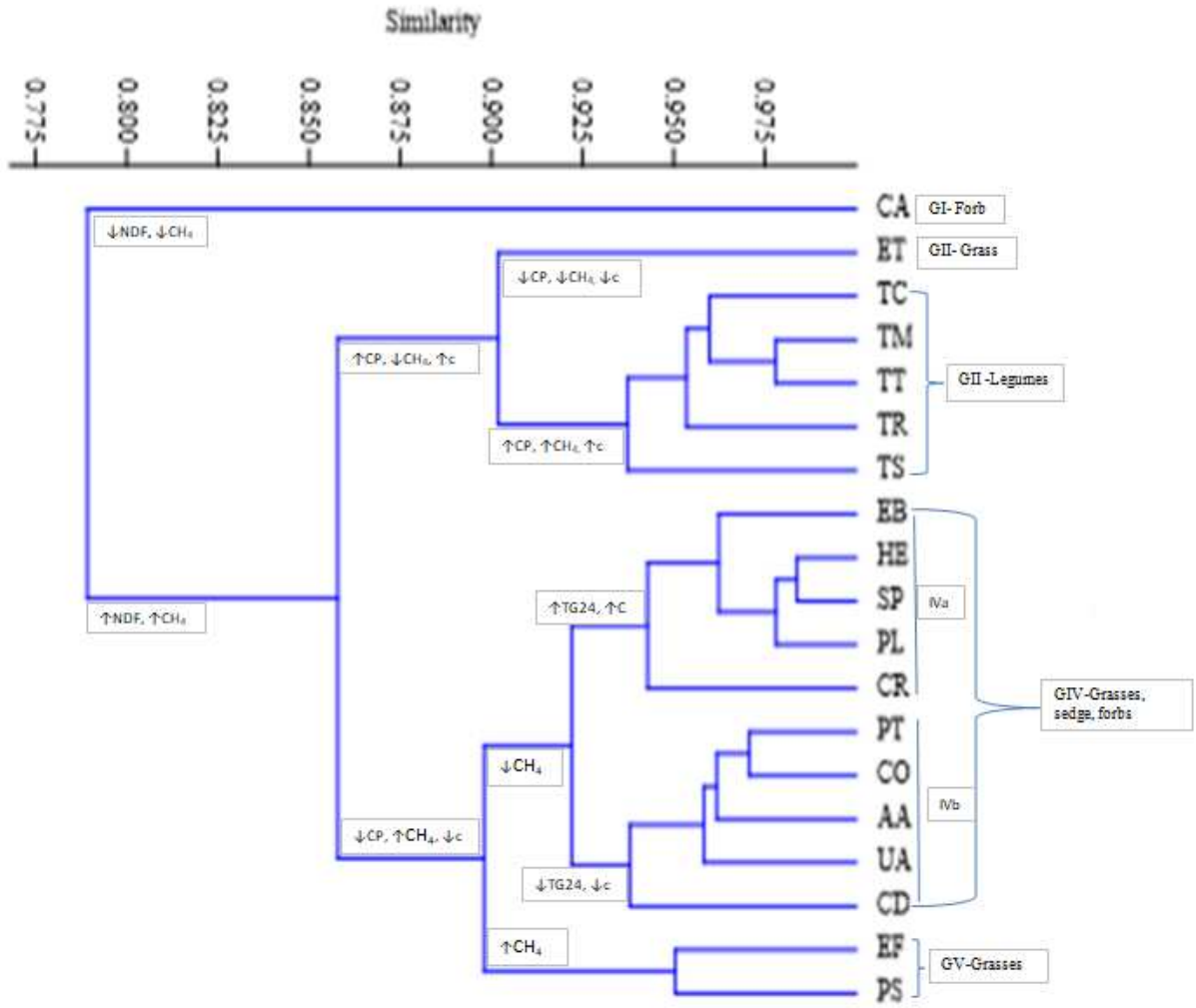


Figure 3.6. Multivariate cluster analysis plot presenting groups of different forage species depending on similarities in chemical composition and *in vitro* ruminal fermentable parameters. Grasses: *AA= Andropogon amethystinus; CD= Cynodon dactylon; EF= Eleusine floccifolia; EB= Eragrostis botryodes; ET= Eragrostis tenuifolia; HE= Helictotrichon elongatum; PS= Pennisetum sphacelatum; PT= Pennisetum thunbergii; PL= Poa leptoclada; SP= Sporobolus pyramidalis,* legumes: *TC= Trifolium cryptopodium; TM= Trifolium mattirolianum; TR= Trifolium rueppellianum; TS= Trifolium simense; TT= Trifolium tembense,* sedge: *CR= Cyperus rigidifolius,* forbs: *CO= Cerastium octandrum; UA= Uebelinia abyssinica; CA= Centella asiatica,* c= fractional gas production rate; CP= crude protein; G= group; NDF= neutral detergent fiber; TG24= gas production at 24 h.

3.4. Discussion

3.4.1. Chemical composition of selected forage species

Most grasses except *Cynodon dactylon* had CP below 70 g/kg DM, a CP content below the minimum level required for normal rumen functioning (Van Soest, 1994). On the other side, the CP contents of legume species were above 150 g/kg DM, the least amount of CP contents for lactation and growth (Norton, 1982) except for *Trifolium simense* (139 g/kg DM). The CP contents for sedge (*Cyperus rigidifolius*) and forb species in this study were well above 70 g/kg DM, a minimum level for rumen functioning as indicted by Van Soest (1994).

Animal feeds with more than 650 g/kg DM NDF were considered as low quality feed (Singh and Oosting, 1992), and 70% of the grass species and all legumes and forbs in the current study contained NDF below this threshold level. Neutral detergent fiber contents for the majority of the grass species in the current study were in between 613 to 877 reported for the key grass species from natural pasture in crop-livestock mixed farming system of Southern Ethiopia (Gemiyo et al., 2013). Similarly, lower fibre contents for the grass species were observed for the current study compared to sub-tropical grass species in transitional rangeland of South Africa (du Toit et al., 2018). Such variation might be observed due to stage of maturity of the grass species at harvest, as higher fibre contents resulted with increased forage species age (Mero and Udén, 1998). The moderate quality of fibre contents from the evaluated forage species might be due to stage of harvesting, as forage sample collection was done at flowering stage when farmers in the study area utilize the conserved grazing area known traditionally as "*Kelo*".

3.4.2. In vitro fermentation and multivariate analysis

Gas produced by legume species at 24 h in the current study was slightly lower than the 213 to 257 ml/g DM gas produced at the same incubation period for legume hays grown in Turkey (Suha Uslu et al., 2018). Highest gas production was recorded for *Centella asiatica* forb at 24 h of incubation period with higher CP and OMD along PC1 (Figure 3.4) compared to other forbs and grass species. This is in agreement with du Toit et al. (2018), who found the highest total gas

volume after 24 h of incubation for species higher in *in vitro* organic matter digestibility (IVOMD) and CP concentrations. Similarly, the higher gas production for legume species than grass species in the current study agrees with Singh et al. (2012), who found more gas production by legumes than cereals or grasses. This is clearly observed from cluster analysis in the present study (Figure 3.6) that all legume species clustered close to each other in the same group, and from PCA analysis (Figure 3.4), had higher total gas production at 24 h and higher EGP that was highly correlated with PC1.

As indicated by Babayemi and Bamikole (2006), CH_4 production is energy loss to the animals and also one of the sources of global warming. The differences in CH_4 production between grass species in the current study may be attributed to differences in the chemical composition (fibre components) between plant species (Table 3.1). From PCA, perennial forage grasses such as *Eleusine floccifolia*, *Pennisetum sphacelatum* and *Eragrostis botryodes* had greater NDF, ADF and CH_4 production and were strongly and negatively associated with PC1 and as a result *Eleusine floccifolia* and *Pennisetum sphacelatum* clusterd close to each other in the same group in cluster analysis (Figure 3.6). This provides an opportunity for selecting low methane emission forage species by targeting their fibre contents. Similar to the present finding, Gemeda and Hassen (2014) also found a positive correlation for methane with fibre components. The higher methane production for grass species compared to legume species agrees with previous research (Navarro-Villa et al., 2011; Melesse et al., 2017), which found higher methane production for grass species than leguminous forages. On the other hand, the lower methane production in legumes species compared to grasses could be due to less extensive *in vitro* rumen fermentation of legumes species as proposed by Navarro-Villa et al. (2011). The higher SCFA from legume species and *Centella asiatica* forb in the present study might be due to their lower fibre fractions (Table 3.1) compared to grasses, sedge and forbs, which is in line with the discovery by Van Soest (1994) of greater SCFA for species containing lower fibre fractions. This is clearly observed from PCA that forage legumes and *Centella asiatica* forb had lower fiber components (NDF and ADF) along PC1. According to Getachew et al. (1998) gas production is a reflection of the production of SCFA and the synthesis of microbial biomass (Getachew et al. 1998). Similarly, as indicated by Ajayi and Babayemi (2008), SCFA is a reflection of energy

contents in a feed. This is a possible explanation why forage legumes and *Centella asiatica* forb in the present study had greater EGP compared to forage grasses.

All grass species in the present experiment contained lower OMD than the range of OMD values of 56.1 to 79.6% reported by Melesse et al. (2017) for some common grass species grown in Ethiopia, except *Cynodon dactylon* and *Eragrostis tenuifolia*, which had a values within this range. This was clearly observed from PCA (Figure 3.4) in which all grass species evaluated (except *Eragrostis tenuifolia*) had negative loadings and association with PC1. On the contrary, all grass species studied had a value above 22.5 to 42.2% OMD obtained by du Toit et al. (2018) for selected sub-tropical grass species. Forage legumes evaluated in this study had lower OMD than was reported by Melesse et al. (2017) for some common legumes grown in Ethiopia, and by Suha Uslu et al. (2018) for some legume plants grown in Turkey. Meissner et al. (2000) observed that forages having an OMD of 70% or above are deemed to be of high quality thus all forage species in the current study, which had a value below 70% OMD were considered to below quality forage. The ME in the grass species in the present study were higher than 3.0 to 5.7 MJ/kg DM reported by du Toit et al. (2018) for selected sub-tropical grass species and in the range of ME reported for common tropical grass forages in Ethiopia by Melesse et al. (2017). On the other side, the values of ME obtained for legumes and others (sedge and forbs) were below 8.9 to 10.3 MJ/kg DM reported by Melesse et al. (2017) for common tropical legume forages in Ethiopia except for *Centella asiatica* forb recorded 9.5 MJ/kg DM.

3.5. Conclusions

The key forage species characterized in the present experiment showed significant difference in terms of chemical composition, and *in vitro* fermentable parameters such as gas and CH_4 production, EGP and fractional gas production rate (c). Multivariate analysis clustered the forage species in to five main groups and two sub-group in one of the groups based on their chemical composition (NDF, CP) and *in vitro* fermentable parameters (c, CH4 and total gas at 24 h). The PCA revealed that the fiber components were positively correlated with CH_4 production while the cluster analysis showed that forage grasses which had higher fiber contents and higher CH_4 production were clustered close to each other as compared to other species. Lower methane

emission in turn minimizes the environmental damage caused by livestock species. Similarly, the description of forage species included in this study in terms of chemical composition and *in vitro* fermentable parameters will serve as a source of information to improve the existing knowledge pool which helps design appropriate pasture based feeding system and grazing land management practices. Furthermore *Centella asiatica* forb was superior in most of the nutritive value parameters included in current study. Hence, further experiments could be conducted regarding its yield, anti-nutritional and actual feeding values to recommend this forb as possible forage crop in the feeding system of ruminants in the central highlands of Ethiopia and similar agro ecologies elsewhere.

CHAPTER FOUR

Evaluation of n-alkanes, long chain alcohols, and carbon stable isotope enrichments of n-alkanes as diet composition markers for forage species

Abstract

Plant species exhibit different patterns of plant cuticular wax profiles, which can potentially be used as diet composition markers in free grazing herbivores. The objective of this study was to evaluate the suitability of the plant cuticular n-alkanes, long chain alcohols (LCOH) profiles and carbon stable isotope enrichment ($\delta^{13}C$) of n-alkanes of forage species from the central highlands of Ethiopia to use as markers in the estimation of diet composition of grazing animals. Forage samples were collected from 100 representative quadrats of 0.5 m x 0.5 m at 10 m transects, covering 16 hectares of grazing area. Forage samples were sorted by species and pooled from different quadrats to obtain enough quantities of representative individual species for subsequent analysis. A total of ten dominantly forage species were identified and analyzed for n-alkanes and LCOH by gas chromatography (GC) and the isotopic ratio ($^{13}C/^{12}C$) by using Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS). Principal component analysis was used to explore inter-species variations in the concentration of n-alkanes, LCOH and $\delta^{13}C$ of n-alkanes. Odd-chain n-alkanes comprised the highest proportion of the total n-alkane concentration ranging from 79% in *Ischaemum afrum* to 95% in *Haplocarpha hastata*. N-alkanes C_{31}, C_{29} and C_{33} were the most abundant with an average 167, 80 and 61 mg/kg DM, in that order in all species. Even-chain LCOH comprised the highest proportion of the total LCOH concentration accounting for 92% in *Brachiaria scalaris* to 97% in *Ischaemum afrum*. The dominant even-chain LCOH were $C_{30}OH$, $C_{32}OH$, $C_{28}OH$ and $C_{26}OH$, with an average concentration of 362, 348, 266 and 237 mg/kg DM, respectively. The $\delta^{13}C$ of n-alkanes showed relatively large variations between forage species ranging from −19.7‰ in *Andropogon amethystinus* to −40.6‰ in *Trifolium mattirolianum*. The finding of the PCA presented that 81% of the total variance in the pattern of concentrations of n-alkanes was explained by the first two principal components compared to 69.3 and 82.9% in the case of LCOH and $\delta^{13}C$ of n-alkanes, respectively. It was concluded that the variations in the patterns of concentrations of n-alkanes,

LCOH and $\delta^{13}C$ of n-alkanes could be suitable as markers for diet composition estimation of grazing animals under tropical grassland conditions.

***Keywords*:** forage species profile, assessment, pasture, plant wax

4.1. Introduction

Ethiopian highlands are characterized by high plant species diversity and are considered a center of species endemism due to large elevation range, heterogenous landscape and climate (IBC, 2005). These highlands, once given by rich natural resources, have been cultivated agriculturally for many years' and now are severly degraded due to mismanagement. Numerous studies have showed that the grazing lands in Ethiopia are found in poor condition and need immediate action (Mengistu et al., 2017). Improved management of grazing lands necessitates good knowledge of the nutritional qualities of different pasture species, biomass yields and botanical composition of grazing herbivores. Estimation of diet composition in freely grazing animals is challenging due to the invasiveness of the methods applied and associated in accuracies to simulate the natural grazing behaviour of animals. The use of plant cuticular wax hydrocarbons as diet composition markers has recently received increasing acceptance as it is less invasive and allows diet composition estimation without restricting free movement of animals.

The basic prerequisite for estimating diet selection of animal using plant cuticular wax on a complex pasture is the presence of enough differentiation of marker profiles between plant species (Mayes and Dove, 2000). Various forage species have different patterns of plant wax components in their cuticular wax (Dove and Mayes, 2005) and this truth has successfully been utilized to estimate the diet composition of controlled (Charmley and Dove, 2007) and grazing animals (Piasentier et al., 2007). N-alkanes are saturated hydrocarbons found in the cuticular waxes of plants and their pattern is unique to plant species and plant parts (Dove et al., 1996; Ferreira et al., 2005). When using plant cuticular wax markers as diet composition markers, the number of plant species consumed cound be equal to or lower than the number of markers used. In dealing with multispecies plant communities, the number of plant species available to the herbivore may well be high and necessitate use of other cuticular markers in addition to n-alkanes (Ali et al, 2005; Bezabih et al, 2011b). This is due to the limited number of n-alkane markers available in high enough concentrations to be used for diet composition calculations (Brosh et al., 2003; Dove and Mayes, 2005). One means of overcoming this constraint is to include additional classes of plant wax components as markers such as $\delta^{13}C$ of n-alkanes

(Bezabih et al., 2011b; Ferreira et al., 2014) and LCOH (Dove and Charmley, 2008; López et al., 2015; Heublein et al., 2017) for distinguishing between plant species.

According to Phillips and Gregg (2003) carbon stable isotopes are utilized as tracers to find out the proportional contributions of numerous sources in a mixture. The $\delta^{13}C$ for individual n-alkanes showed wide variation for some forage species at different locations (Bezabih et al., 2011b; Ferreira et al., 2014). Orthogonal procurst rotation (OPR) findings suggested that $\delta^{13}C$ values of alkanes provided additional discriminatory information to that given by other markers. Similarly, LCOH of the plant wax components are potential diet composition markers that presented additional or complementary information about plants to those given by n-alkanes (Bugalho et al., 2004). Bugalho et al. (2004) observed that the proportional differences explained by using n-alkane, LCOH or their combination varied among plant mixtures, suggesting that marker selections are diet dependent. According to Samuels et al. (2008), environmental conditions and geographical locations could influence the pattern of the cuticular wax profile of plant species growing in different places. However, little information is available (Bezabih et al., 2011b) regarding the patterns of plant wax components in forage species in the highlands of Ethiopia.

According to Ali et al. (2005), it is necessary to collect location specific information on the n-alkane profiles of existing forage species to use widely in animal feed research. As a result, there is a need to extend the earlier work in the Mid Rift Valley rangelands (Bezabih et al., 2011b) to pasture lands with different vegetation composition in the central highlands of Ethiopia and also characterize additional plant wax components. Therefore, the aim of the present study were to quantify n-alkanes, LCOH profiles and $\delta^{13}C$ of n-alkanes of forage species from the central highlands of Ethiopia, and to evaluate the potential utilization of these compounds as markers to estimate diet composition of grazing animals.

4.2. Material and Methods

4.2.1. Research site

The study was conducted in Kofele district, West Arsi Zone of Oromia Regional State, Ethiopia. It is situated at 7°07'N and 38°48'E with an altitude of 2660 masl with a predominantly loam soil type. The area has bi-modal rainfall distribution with short rains lasting from March to May and the main rainy season extending from June to September/October. The long term average annual rainfall is 1800 mm and the average daily temperature is 19.5°C.

4.2.2. Plant sampling and processing

Forage species samples were collected from 2 hectares of Gurmicho Primary School at the grazing land in October, 2017 from the highland area. Forage sampling was done from 100 representative quadrats of 0.5 m x 0.5 m at 10 m transects drown in a perpendicular manne in the grazing land area. Forage sampling was done at 50% flowering stage when it was possible to easily identify the species, which coincided with the period when farmers start to use the pasture from enclosure areas known locally as *"kelo"*. From a quadrat, whole plant species were mowed at 5 cm aboveground, sorted by species and weighed to determine the dominance of a species from a mixture. Forage species were pooled from different quadrats to obtain sufficient quantities of individual species in the sampling area for plant wax analysis. Forage species were identified by using guidebooks on the site and for those plant species that were difficult to identify on site, their local names were recorded and herbarium specimens were collected, pressed and dried properly by using a plant presser and identified and confirmed at the national herbarium, Addis Ababa University, Ethiopia. A total of 10 dominant available forage species as they appeared naturally in the pasture (data not shown) consisting of grasses (4 species), legumes (3 species) and forbs (3 species) were selected for further analysis (Table 4.1.). The individual forage species samples were oven-dried to a constant weight at 60 ^{0}C for 48 hours and then ground in a Willey mill to pass through a 1 mm sieve for subsequent laboratory analysis.

Table 4.1. Details of selected forage species collected from Gurmicho Primary School grazing land, Kofele district

Botanical name	Family	Plant type	Life form	To Ethiopia
Andropogon amethystinus	Poaceae	Grass	Perennial	Indigenous
Brachiaria scalaris	Poaceae	Grass	Annual	Indigenous

Ischaemum afrum	Poaceae	Grass	Perennial	Indigenous
Pennisetum thunbergii	Poaceae	Grass	Perennial	Indigenous
Trifolium cryptopodium	Fabaceae	Legume	Perennial	Indigenous
Trifolium mattirolianum	Fabaceae	Legume	Annual	Endemic
Trifolium tembense	Fabaceae	Legume	Annual	Indigenous
Centella asiatica	Apiaceae	Herb	Perennial	Indigenous
Haplocarpha hastata	Asteraceae	Herb	Perennial	Endemic
Uebelinia abyssinica	Caryophyllaceae	Herb	NA	Indigenous

NA=not available

4.2.3. Extraction and analysis of plant wax markers

N-alkane and LCOH extraction and analysis were conducted at the isotope nutrition laboratory of James Hutton Institute, UK. Extraction and analysis of forage samples for n-alkanes was done as described by Dove and Mayes (2006) by gas chromatography (GC), running analyses in duplicate, and for LCOH a modification of the method of Dove and Mayes (2006) was used. Long-chain fatty alcohols were extracted and analysed with 1-heptacosanol ($C_{27}OH$) being used as internal standard. Crude alcohol extracts were obtained using the method of Dove and Mayes (2006). Instead of using aminopropyl solid-phase extraction (SPE) columns to purify the crude alcohol extracts (Dove and Mayes, 2006), a column-based urea adduction method (Mayes, unpublished) was adopted. To an empty SPE, fitted with polyethylene frits and closed at the bottom with a Luer syringe cap, a saturated solution of urea in ethanol was added followed by crude alcohol extract dissolved in n-heptane. After initial warming, the urea was allowed to crystallise and the solvents evaporated. The columns were placed in a positive-pressure SPE manifold and the Luer syringe caps removed. Sterols, stanols and any triterpenol impurities were removed by applying n-heptane to the columns (allowing the washings to run to waste). Water was then added in order to remove urea. The purified alcohol fraction was obtained using a second application of n-heptane. After removal of the solvent in the purified extract by evaporation, acetate derivatives of the LCOH were prepared by heating (50 ^{o}C) overnight with a mixture of acetic anhydride and pyridine. The pyridine and excess acetic anhydride were

removed by evaporation and the derivatised extract was dissolved in n-dodecane prior to analysis by GC.

For GC analysis, the derivatised extract was injected (1µl) into a Trace (Thermo Finnegan) gas chromatograph fitted with a split/splitless injector (running in splitless mode at 275^{o}C, with a splitless time of 5 min) and flame ionization detector (FID), using helium (flow rate 1ml/min) as the carrier gas. The GC column was a non-polar bonded-phase capillary type Rtx-5 MS (Restek) (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The temperature programme used for the GC column oven was: 170 ^{o}C for 5 min; 30 ^{o}C/min to 210 ^{o}C; held at 210 ^{o}C for 1min; 5.3 ^{o}C/min to 320 ^{o}C; held at 320 ^{o}C for 12 min. The fatty alcohol peaks from the detector were integrated and processed using EZChrom Elite software; the peak data was imported into an excel spreadsheet in order to calculate the fatty alcohol levels in the forage.

$$\text{Concentration alkane}_i\ (\text{mg/kg DM}) = \frac{[10 \times \text{area \% alkane}_i \times C_{34}\ \text{IS wt (mg)}]}{\text{sample wt (g)} \times \text{DM content} \times SRF_i \times FF_i}$$

$$\text{Concentration LCOH}_i\ (\text{mg/kg DM}) = \frac{[10 \times \text{area \% LCOH}_i \times C_{27}\text{OH IS wt (mg)}]}{\text{sample wt (g)} \times \text{DM content} \times SRF_i}$$

Where C_{34}ISwt and C_{27}OH ISwt is the weight of the solution containing the internal standard, and %Area is the area of n-alkane$_i$ or LCOH$_i$ calculated as the percent area of C_{34} or C_{27}OH, respectively. The DM content is the sample dry weight, SRF$_i$ is the average response factor, calculated as the percent area of n-alkane$_i$ or LCOH$_i$ in the mixed standard solution divided by the percent weight of n-alkane$_i$ or LCOH$_i$ in the mixed standard, and FF$_i$ is the fractionation factor.

For compound-specific isotope analysis, 90% of purified alkane extract obtained from each sample replicate was used and the remaining 10% was subjected to n-alkane analysis by GC. The carbon isotope composition of the alkanes was determined by fitting a GC with a split/splitless injector operated in split mode to a combustion interface which was connected to Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) enabling the δ^{13}C values in individual n-alkanes to be determined. Full-base line separation of all individual

alkanes was achieved by fitting the Trace GC with a capillary column as described for n-alkane by Dove and Mayes (2006) and using helium as carrier gas. The temperature setting of the column was identical to that described for n-alkane. The isotope ratio of the alkanes was calculated in terms of conventional delta values ($\delta^{13}C$) as follows:

$$\delta^{13}C = \frac{1000\ (R_{sample} - R_{standard})}{R_{standard}}$$

Where, R_{sample} is the abundance ratio of ^{13}C to ^{12}C in the plant sample, and $R_{standard}$ is the abundance ratio of ^{13}C to ^{12}C in the standard sample.

4.2.4. Statistical analyses

Principal Component Analysis (PCA) was used to explore the pattern of n-alkane, LCOH and $\delta^{13}C$ of alkanes by grouping species along the principal component axes. The correlation matrix was used for the calculation, after the data was mean-centered and standardised. The first two principal components (PC1 and PC2) were plotted graphically where points on the graph represent plant species. The distance between species in the scatter plots is an indication of the variations in marker profile between the species. The species that are close together in the scatter plots are the ones with a similar marker profile. On the other hand, the species that are placed far apart are expected to have huge differences in their marker profiles as explained by Bezabih et al. (2011b). Data were analyzed using GenStat for Windows (11th edition).

4.3. Results

4.3.1. Composition of n-alkanes in the whole plants

The n-alkane patterns of the forage species collected from the grazing lands are presented in Table 4.2. The n-alkanes utilized as internal standards (C_{22} and C_{34}) in the plant wax analysis are not presented. Large variations in total C_{23} to C_{35} were observed between plant species, ranging from 58 mg/kg DM in *Centella asiatica* to 968 mg/kg DM in *Haplocarpha hastata*. The odd-chain n-alkanes comprised the highest proportion, being 79% of the total alkane concentration in

Ischaemum afrum to 95% in *Haplocarpha hastata*. In all species, except *Brachiaria scalaris* and *Ischaemum afrum*, C_{31} was the most abundant in concentration, ranging from 13.2 mg/kg DM in *Centella asiatica* to 462.3 mg/kg DM in *Trifolium tembense.* Next to C_{31} alkane, C_{29} was the most abundant alkane in most species, ranging from 9.3 mg/kg DM in *Centella asiatica* to 217.1 mg/kg DM in *Haplocarpha hastate.* and the third dominant was C_{33} alkane. From the current study, *Centella asiatica* contained the lowest quantity in most alkane concentrations, with a total of 58 mg/kg DM.

Table 4.2. Concentration of n-alkanes for selected forage species collected from Gurmicho Primary School grazing land, Kofele district

Forage species	N-alkanes concentration (mg/kg DM)													
	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}	Total	TOC
Andropogon amethystinus	3.3	1.6	8.4	2.6	25.9	4.0	68.2	4.4	83.6	6.3	60.8	8.3	280	259
Brachiaria scalaris	1.5	1.0	6.4	1.5	18.3	4.9	53.4	8.6	97.3	14.6	184.8	42.8	437	405
Ischaemum afrum	6.7	6.4	34.0	8.4	52.8	8.6	28.2	9.9	36.8	15.1	24.2	6.1	240	189
Pennisetum thunbergii	2.3	1.9	4.7	2.8	19.4	4.4	51.8	5.4	93.2	8.1	81.0	10.6	288	263
Trifolium cryptopodium	11.1	11.3	17.6	11.2	45.7	14.2	143.2	14.0	292.6	12.9	31.7	3.6	613	546
Trifolium mattirolianum	2.2	1.8	6.9	2.9	27.0	5.9	54.7	8.6	107.9	7.0	13.5	2.4	243	215
Trifolium tembense	1.7	1.5	9.6	3.3	36.8	7.2	105.8	21.1	462.3	20.6	53.5	3.0	728	673
Centella asiatica	0.9	1.1	2.9	1.5	9.6	1.7	9.3	2.2	13.2	3.9	8.0	2.1	58	46
Haplocarpha hastata	1.0	1.4	3.6	1.4	52.0	6.5	217.1	21.7	419.4	16.4	145.0	79.8	968	918
Uebelinia abyssinica	1.2	1.3	5.1	2.3	29.3	4.4	65.0	5.5	61.9	4.5	8.7	1.9	193	173

TOC=total odd-chain alkanes

4.3.2. Composition of alcohol in the whole plants

The LCOH concentrations of forage species collected from the grazing lands are presented in Table 4.3. Large differences in the patterns of LCOH were observed among the plant species for $C_{22}OH$ to $C_{34}OH$, excluding $C_{27}OH$ which was used as internal standard. *Centella asiatica* forb showed the lowest total LCOH concentration (677 mg/kg DM) whereas *Trifolium mattirolianum* legume had the highest concentration (2228 mg/kg DM). Even-chain LCOH presented the highest proportion of the total LCOH concentration, ranging from 92% in *Brachiaria scalaris* to 97% in *Ischaemum afrum*. Most even-chain LCOH such as $C_{30}OH$, $C_{32}OH$, $C_{28}OH$ and $C_{26}OH$ were abundant with an average concentration of 362, 348, 266 and 237 mg/kg DM, respectively. *Trifolium mattirolianum*, *Trifolium tembense* and *Trifolium cryptopodium* legumes predominated in $C_{30}OH$ and *Andropogon amethystinus* and *Pennisetum thunbergii* grasses predominated in $C_{32}OH$.

Table 4.3. Concentration of LCOH for selected forage species collected from the central highlands of Ethiopia

Forage species	LCOH concentrations (mg/kg DM)													
	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	Total	TEC
Andropogon amethystinus	89.7	6.7	59.2	7.7	152.1	370.0	21.7	194.5	32.3	757.2	9.0	31.2	1731	1654
Brachiaria scalaris	36.3	4.4	43.8	4.4	138.4	75.7	10.5	53.9	26.1	327.1	13.0	25.5	759	701
Ischaemum afrum	23.2	2.8	51.0	4.8	297.2	454.5	10.4	78.9	9.6	119.1	2.9	3.1	1058	1027
Pennisetum thunbergii	76.4	5.9	49.9	4.9	170.5	191.8	15.6	116.6	32.2	605.8	10.1	41.2	1321	1252
Trifolium cryptopodium	68.4	10.4	87.3	10.0	155.6	129.7	30.5	572.1	50.8	457.7	18.6	44.2	1635	1515
Trifolium mattirolianum	41.4	5.8	55.4	8.6	210.9	511.0	54.7	1072.0	24.8	196.7	7.6	38.8	2228	2126
Trifolium tembense	71.6	6.7	59.6	9.7	176.6	140.6	48.7	956.5	67.0	306.7	11.9	35.2	1891	1747
Centella asiatica	17.6	2.5	40.1	8.2	178.8	208.8	14.9	113.2	12.4	74.9	1.9	3.2	677	637
Haplocarpha hastata	51.5	3.4	129.2	12.4	632.4	467.5	23.8	336.5	99.9	381.1	7.2	20.9	2166	2019
Uebelinia abyssinica	44.9	5.9	38.6	5.1	256.0	107.7	13.0	128.7	25.0	251.0	7.2	16.2	899	843

LCOH=long chain alcohol; TEC=total even-chain LCOH

4.3.3. Compositions of $\delta^{13}C$ of n-alkanes in the whole plants

The $\delta^{13}C$ values of the n-alkanes are given in Table 4.4. The isotope enrichment of C_{34} alkane was not presented as it was used as internal standard in GC-IRMS analysis. The results of the patterns of $\delta^{13}C$ of the n-alkanes ranged from -19.7‰ in *Andropogon amethystinus* to -40.6‰ in *Trifolium mattirolianum,* which showed relatively large variations between forage species. The $\delta^{13}C$ of n-alkanes for the grass species ranged from -19.7‰ for C_{29} alkane in *Andropogon amethystinus* to -38.1‰ for C_{32} alkane in *Brachiaria scalaris,* whereas it ranged from -29.2‰ for C_{24} alkane in *Trifolium tembense* to -40.6‰ for C_{32} alkane in *Trifolium mattirolianum* legume species. On average, the odd-chain alkanes tended to be greater in magnitude compared to the subsequent even-chain with 1.0 delta unit in *Brachiaria scalaris* to 7.2 delta units in *Trifolium mattirolianum* except in *Uebelinia abyssinica* in which there was no data for even-chain alkanes C_{24} and C_{32} due to too little alkane present during analysis. *Andropogon amethystinus* comprised the highest concentration of $\delta^{13}C$ for alkanes C_{23} to C_{29}, while *Trifolium mattirolianum* contained the lowest concentration as carbon length increase from C_{25} to C_{33}.

Table 4.4. Concentration of $\delta^{13}C$ for selected forage species collected from from Gurmicho Primary School grazing land, Kofele district.

Forage species	$\delta^{13}C$ values (‰) of n-alkanes											
	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
Andropogon amethystinus	−23.9	−25.9	−20.9	−22.8	−20.0	−22.9	−19.7	−25.4	−21.3	−29.1	−29.8	−24.0
Brachiaria scalaris	−29.8	−28.8	−29.7	−27.5	−30.9	−34.7	−33.0	−36.4	−34.6	−38.1	−29.9	−36.8
Ischaemum afrum	−26.8	−28.4	−22.0	−26.3	−21.1	−24.9	−22.0	−20.2	−22.7	−21.9	−29.0	ND
Pennisetum thunbergii	−27.2	−28.5	−25.4	−25.8	−21.9	−24.7	−21.1	−25.3	−21.9	−28.1	−29.7	−23.8
Trifolium cryptopodium	−30.7	−30.1	−30.4	−30.5	−33.0	−34.3	−34.6	−36.9	−35.2	−37.0	−29.9	ND
Trifolium mattirolianum	−30.4	−29.8	−31.5	−31.3	−35.0	−38.4	−37.1	−39.9	−37.8	−40.6	−30.0	ND
Trifolium tembense	−30.4	−29.2	−31.4	−33.2	−34.7	−35.5	−36.6	−37.9	−37.0	−38.3	−29.8	ND
Centella asiatica	−29.1	−31.4	−27.4	−33.1	−30.1	−32.6	−28.5	−30.3	−26.9	−30.1	−29.5	ND
Haplocarpha hastata	−29.9	−28.6	−28.8	−30.7	−33.2	−36.1	−34.9	−37.7	−35.8	−37.6	−29.9	−36.0
Uebelinia abyssinica	−30.8	ND	−27.6	−29.7	−31.6	−34.3	−35.4	−36.8	−35.6	ND	−29.7	ND

ND=not detected

4.3.4. Principal component analysis

The result of the PCA showed that 81.1% of the variance in the profile of n-alkanes was explained by the first two principal components (PC1 and PC2), whereas 69.3 and 82.9% was explained in the case of LCOH and $\delta^{13}C$ of n-alkanes, respectively (Table 4.5). The two principal component scores were used to present the position of forage species in a two-dimensional space as shown in Figure 4.1. For n-alkanes, PCA showed some of the forage species scattering along PC1 and PC2. *Haplocarpha hastata, Brachiaria scalaris* and the two legumes (*Trifolium cryptopodium and Trifolium tembense)* scattered from other species. On the other hand, the two grasses (*Andropogon amethystinus* and *Pennisetum thunbergii*), *Trifolium mattirolianum* and *Uebelinia abyssinica* clustered close to each other. When the PCA analysis was based on LCOH, a distinct pattern of cluster was observed with groupings observed between *Ischaemum afrum*, *Centella asiatica*, *Brachiaria scalaris*, and *Uebelinia abyssinica*. When the PCA analysis was depend on $\delta^{13}C$ of n-alkanes, most of the forage species studied was separated except for the three legumes which clustered close to each other. When the data from markers were combined (n-alkanes and $\delta^{13}C$ of n-alkanes, n-alkanes and LCOH, LCOH and $\delta^{13}C$ of n-alkanes and the three markers), better scattering of forage species was observed, except when n-alkanes and LCOH combined in which *Andropogon amethystinus*, *Pennisetum thunbergii* and *Trifolium mattirolianum* clustered close to each other (Figure 4.2).

Table 4.5. The variance (%) in the pattern of cuticular wax marker concentration explained by the first two principal component scores (PC1 and PC2) for each data set

	% Variance explained by:		
	PC1	PC2	Total
n-alkanes	47.6	33.5	81.1
LCOH	41.4	27.9	69.3
$\delta^{13}C$ of n-alkanes	65.0	17.9	82.9

LCOH=long chain alcohol; PC=principal components

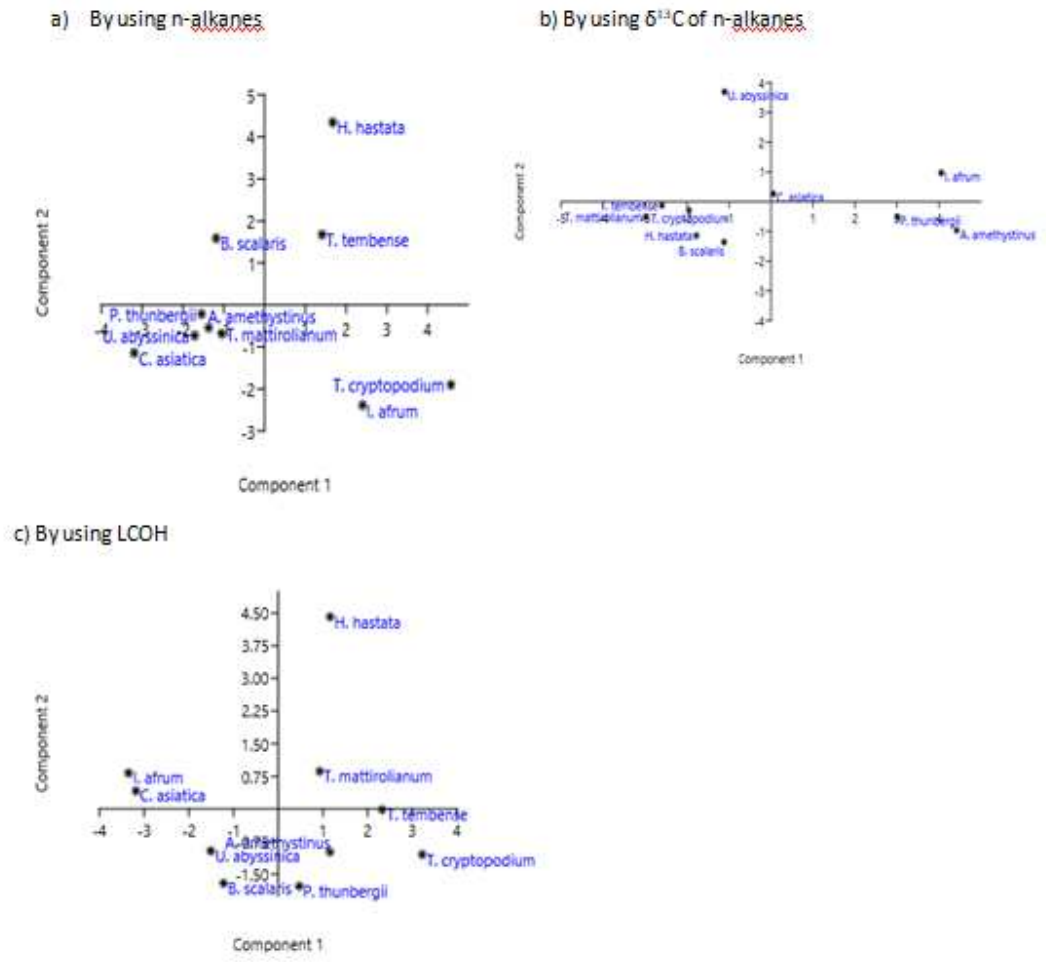


Figure 4.1. Two-dimensional scatter plots of pasture species from principal component scores (PC1 and PC2) based on individual markers.

Grasses: *A. amethystinus= Andropogon amethystinus*; *B. scalaris= Brachiaria scalaris*; *I. afrum= Ischaemum afrum*; *P. thunbergii=Pennisetum thunbergii*; legumes: *T. cryptopodium= Trifolium cryptopodium*; *T. mattirolianum= Trifolium mattirolianum*; *T. tembense= Trifolium tembense*; forbs: *C. asiatica= Centella asiatica*; *H. hastata= Haplocarpha hastata; U. abyssinica= Uebelinia abyssinica.*

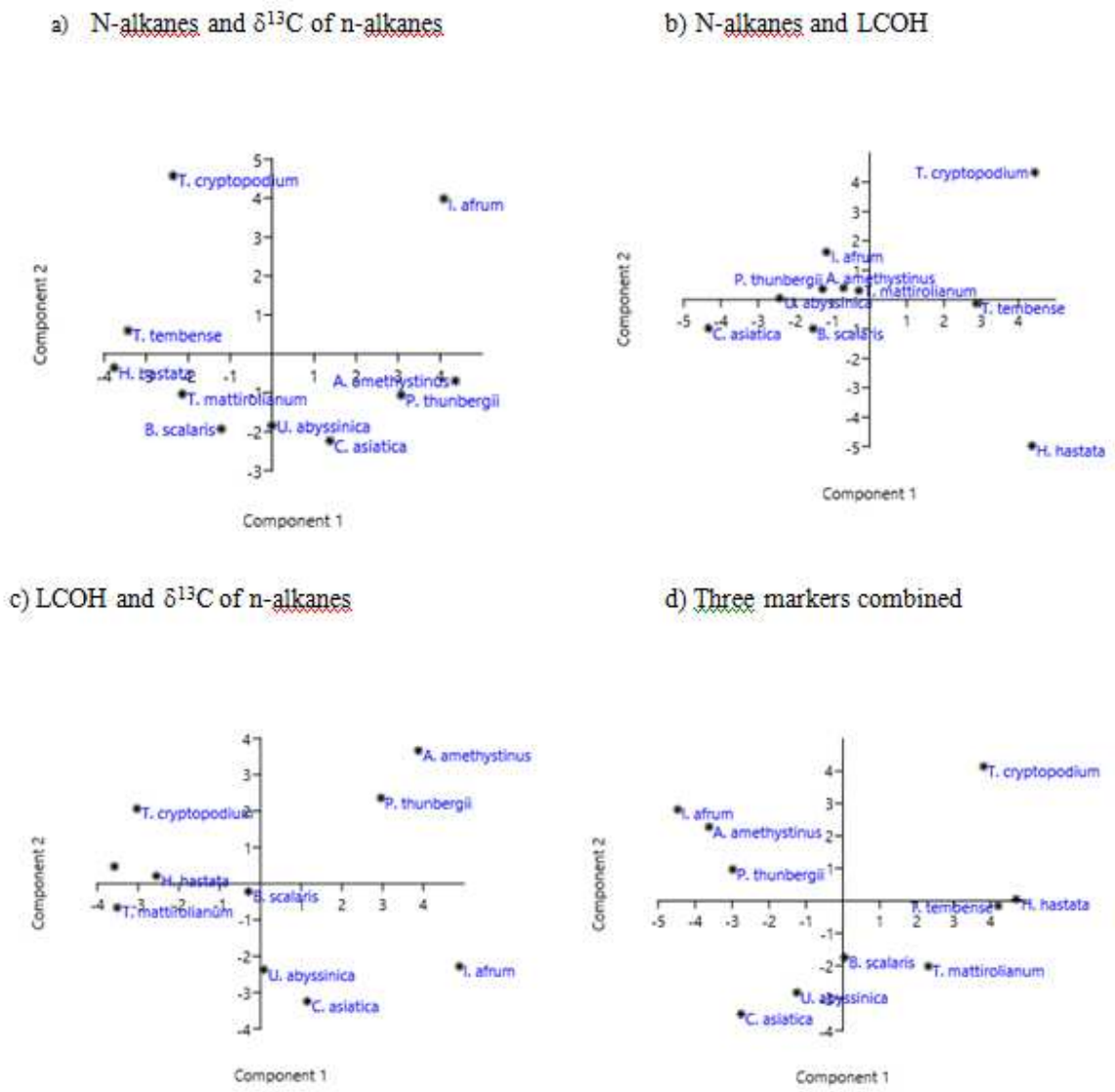


Figure 4.2. Two-dimensional scatter plots of pasture species from principal component scores (PC1 and PC2) based on marker combinations.

Grasses: *A. amethystinus= Andropogon amethystinus*; *B. scalaris= Brachiaria scalaris*; *I. afrum= Ischaemum afrum*; *P. thunbergii=Pennisetum thunbergii*; legumes: *T. cryptopodium= Trifolium cryptopodium*; *T. mattirolianum= Trifolium mattirolianum*; *T. tembense= Trifolium tembense*; forbs: *C. asiatica= Centella asiatica*; *H. hastata= Haplocarpha hastata; U. abyssinica= Uebelinia abyssinica*

4.4. Discussion

4.4.1. Composition of n-alkanes, LCOH and $\delta^{13}C$ of n-alkanes in the whole plants

The plant wax concentrations in the most species analyzed in the present experiment have not been reported in the literature as most of them are endemic and indigenous to Ethiopia (Table 4.1). The n-alkane profile of the plant species has been documented in the literature (Ferreira et al., 2013; López et al., 2015; Heublein et al., 2017). Also Bezabih et al. (2011b) evaluated the n-alkane and $\delta^{13}C$ of n-alkanes of forage species collected from the Mid Rift Valley rangelands of Ethiopia. In all cases, considerable variability was observed in the n-alkane profile as well as the isotopic enrichment of n-alkanes among the studied species. As Ali et al. (2005) observed, it is necessary to collect location specific information on the marker profiles of forage species as 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; Bezabih et al., 2011b).

Odd-chain n-alkanes recorded in a greater concentration compared to even-chain alkanes in the current study in line with others finding (Ali et al., 2005; Dove and Charmley, 2008; Bezabih et al., 2011b). Laredo et al. (1991) suggested 50 mg/kg DM to be the minimum requirment fort any n-alkane to be used as a marker. The alkanes C_{29} and C_{31} except for *Ischaemum afrum* and *Centella asiatica* fulfilled this minimum requirement for evaluated forage species. Following the two alkanes, C_{33} alkanes recorded more than 50 mg/kg DM for three grasses (*Pennisetum thunbergii*, *Andropogon amethystinus* and *Brachiaria scalaris*), *Trifolium tembense* legume and *Haplocarpha hastate* forb. The dominances of alkanes C_{29}, C_{31} and C_{33} for most species in the forage species conforms the earlier findings (Ferreira et al., 2007b; Bezabih et al., 2011b), and these alkanes tended to be suitable for diet composition, feed intake and digestibility estimation. The enrichment levels of $\delta^{13}C$ of alkanes for forage legume species in the current study (−29.2‰ to −40.6‰) are in agreement with the values obtained for C3 plant species as observed by Bezabih et al. (2011b) and Ferreira et al. (2014). The lower enrichment level of $\delta^{13}C$ of alkanes (−19.7‰ to −29.8‰) for forage grasses, except *Brachiaria scalaris*, in the current study supports the earlier findings by Schweizer et al. (1999), and is typical of C4 plants.

In agreement with the previous findings (Ferreira et al., 2013; López et al., 2015), huge variability in LCOH concentration were found between forage species in the current study which indicates the usefulness of these compounds as plant wax markers in diet composition estimation. Similarly, predominant even-chain compared to odd-chain LCOH reported by others (Dove and Mayes, 2006; Ferreira et al., 2013) were observed from the present study. The observed higher concentration of total LCOH compared to n-alkane (Table 2 and 4) is in line with previous reports (Ferreira et al., 2005; Oliv'an et al., 2007b).

Some species which have lower concentrations of n-alkane had higher concentrations in LCOH. For example, *Brachiaria scalaris* grass which was the second lowest in total LCOH become higher in total n-alkane concentration compared to others. On the other side, *Trifolium mattirolianum,* which was the highest in magnitude in total LCOH concentration, had lower total n-alkane concentration. This is an important observation, as it suggests the value of using a combination of markers to improve the discriminatory power of the wax profiles in diet composition estimation (Charmley and Dove, 2007). As a result, it was possible to choose marker types (alkanes or LCOH) for diet estimation based on their concentration in the available plant species (Ferreira et al., 2013). *Centella asiatica*, *Uebelinia abyssinica* and *Ischaemum afrum* had lower concentrations of both total n-alkane and LCOH, which may necessitate evaluation of another marker to avoid lower accuracy in diet estimation due to their lower concentrations. The predominance of C_{30}OH concentrations for *Trifolium mattirolianum, Trifolium tembense* and *Trifolium cryptopodium* agrees with Body (1974) who observed prevalent C_{30}OH alcohol in white clover. Similarly, greater concentrations of C_{30}OH in dicotyledons than monocotyledons were obserbed by Oliv´an et al. (1999). This indicates the usefulness of C_{30}OH alcohol in differentiating between the dicotyledons and monocotyledons in forage legumes.

4.4.2. Principal component analysis

The majority of the forage species showed distinct positions along PC1 and PC2, which showed that the level of separation between species based on the respective marker profiles.

From the present study, $\delta^{13}C$ of n-alkanes showed relatively larger variation as explained by the first two principal components and this indicated that $\delta^{13}C$ of n-alkanes had the greatest variability among the studied forage species. According to Mayes (1998), for any compound to meet the requirements as a diet composition marker, large differences in the pattern of marker concentration should exist between plants, and in many instances plant wax hydrocarbons have proved to be suitable.

The evaluated plant wax markers scattered and clustered forage species differently which favours multiple markers for diet composition estimations. For instance, the three legumes (*Trifolium cryptopodium*, *Trifolium mattirolianum* and *Trifolium tembense*) clustered close to each other when $\delta^{13}C$ of n-alkanes was used as a marker, but much wider when either n-alkanes or LCOH were used. Similarly, the two grasses (*Andropogon amethystinus* and *Pennisetum thunbergii*), and *Trifolium mattirolianum* and *Uebelinia abyssinica* showed resemblance when n-alkanes were used, but scattered when either LCOH or $\delta^{13}C$ of n-alkanes were used. This trend is in agreement with Kelman et al. (2003) and Ali et al. (2005) for alkanes and alcohols and Bezabih et al. (2011b) for alkanes and $\delta^{13}C$ of n-alkanes, who observed different plant wax markers clustering and scattering of species differently. Dove and Mayes (1996) postulated that the use a single marker type for diet estimation existed in multi-species pasture is possibly to result less reliable diet composition estimates compared to the utilization of multiple marker types**.** Similar trends were observed in the current study as combining markers resulted in more scattering of forage species compared to individual markers. When many species are present in a mixture, the chance of the presence of species with similar marker profile of markers would increase.

4.5. Conclusions

The results from the current study showed huge variability in the patterns of concentration of n-alkanes, LCOH and $\delta^{13}C$ of n-alkanes among forage species studied from the central highlands of Ethiopia. Odd-chain n-alkanes were found to be greater when compared to the subsequent even-chain alkanes, even though the reverse was observed for LCOH. Most of the inter species variances in studied forage species were explained by the first two principal

components, and the studied plant wax marker scattered and clustered forage species differently, which favoured multiple markers for diet composition estimations. This will allow a better discrimination between plant species than using one marker alone, which shows the potential use of multiple plant wax markers for diet composition estimations. It can be concluded that the variations in the patterns of concentrations of n-alkanes, LCOH and $\delta^{13}C$ of n-alkanes in the current experiment could make them suitable as markers for diet composition estimation of grazing animals.

CHAPTER FIVE

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

Abstract

An experiment was conducted to validate the use the of n-alkane 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 a low intake (11 g DM/kg BW) or high intake (*ad libitum* at 50 g refusal per kg diet offered) pasture (wet season) and hay (dry season) diet. The steers were housed in individual pens, and each steer was dosed twice daily for 15 days with a paper bung containing 400 mg C_{32} alkane 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 faecal 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 faecal 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 faecal recovery corrections, whereas that for the high intake level was accurate both with and without faecal recovery corrections. Moreover, using C_{35} alkane as internal marker provided an accurate estimate of DM digestibility (DMD) during both seasons for the 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

5.1. Introduction

Livestock serve as a source of income and food security and are 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 reduced to about 57% (CSA, 2013b). 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 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, which is 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 faecal 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 faecal 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 faecal 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 using the n-alkane technique. The information generated would help to build on the pool of knowledge available for wider application under tropical conditions.

5.2. Materials and Methods

5.2.1. 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 masl during wet (December, 2017) and dry (April, 2018) seasons on Gurmicho Primary School grazing land (Plate 5.1). 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 the 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 of 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.*

Plate 5.1. Partial view of Gurmicho Primary School grazing land enclosed for five months for controlled experiments.

5.2.2. 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 Zebu type Arsi steers aged about 48 months were purchased from the local market and used during each of the trial periods (Plate 5.2). For the wet season trial, the average initial live weight of the steers was 148 ± 9.2 kg 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 (Plate 5.3). The shed was partitioned into 2 m × 1.5 m pens which contained 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).

Plate 5.2. Zebu type Arsi steers purchased from local market used for controlled experiments.

Plate 5.3. Partial view of experimental barn constructed at Gurmicho Primary School grazing land for controlled experiments during wet and dry seasons.

5.2.3. 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 5.1). For the 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 quadrats for each species composition determination during the 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.

5.2.4. 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 diet and housed in individual pens with individual access to feed and water (Plate 5.4). 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 faecal outputs were recorded during the last 5 days of the feeding period. The amounts of feed offered were weighed 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 by using watering troughs (Plate 5.5) 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.

Plate 5.4. Partial view of controlled experiments at Gurmicho Primary School grazing land.

Plate 5.5. Feeding and watering troughs used during controlled experiments.

5.2.5. 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 had 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 faeces were collected twice daily (days 13 to 15), weighed and after being homogenized, subsamples 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 (Plate 5.6). Faecal 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 faeces 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 pastic bags.

Plate 5.6. Partial view of field individual species, feed and faeces weighing.

5.2.6. Chemical analysis

Chemical compositions of feeds, refusals and faeces samples were analyzed for DM, ash, NDF, ADF and ADL at the animal nutrition laboratory of Hawassa University. Crude protein 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 the Kjeldahl method according to AOAC (2006). Organic matter (OM) content was determined as 100 − %ash. Neutral detergent fibre content was determined according to the 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 faeces samples were extracted and analyzed in duplicate by 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.

5.2.7. 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.

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

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

Where R_i is the faecal recovery rate of $alkane_i$, FO is the daily faecal output (kg DM), F_i is the concentration of $alkane_i$ in faeces (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 faecal and H_i the herbage odd-chain alkane$_i$ concentrations (mg/kg DM), F_j resembles the faecal 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 estimates were calculated using natural C_{35} alkane as an internal marker according to the following formula:

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

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

5.2.8. Statistical analysis

The effect of feeding level, season and their interaction on the n-alkane faecal recoveries, feed intake and DMD estimates were assessed by analysis of variance using the General Linear Model (GLM) Procedure of Statistical Analysis System (SAS, version 9.0). Block effect was initially included in the model but 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 DMD, paired *t*-tests were performed.

5.3. Results

5.3.1. Proportions and chemical composition of the experimental diets

Chemical composition and diet proportions used during wet and dry season experiments are presented in Table 5.1. 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* 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 5.1. 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 fibre	697.1	712.4

Acid detergent fibre	374.0	409.5
Acid detergent lignin	58.1	68.0

DM=dry matter

5.3.2. N-alkane concentrations and faecal recovery

The n-alkane profiles of the diets used during the experiments are shown in Table 5.2. 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 GC analysis. The proportions of odd-chain n-alkanes comprised 0.93 and 0.92 of 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 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.

Table 5.2. 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

The mean faecal recoveries of n-alkanes for steers provided different levels of diets during wet and dry seasons are shown in Table 5.3. From combined analysis data, there were no interactions between the level of diet provision and season on faecal recovery as carbon chain length increases (C_{27} to 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}). Faecal recovery for higher carbon chain lengths (C27 to C35 except C_{29}) were not affected by the level of diet provision (low or high), but higher ($P<0.05$) faecal recovery was observed for the dry season compared to the wet season except for C_{32}. The mean faecal 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 faecal 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 5.3. Mean (SD) faecal 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; LI=low intake; S=season (wet and dry); SD= standard deviation.

[1]P-value after combined analysis.

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

Table 5.4 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 faecal recovery correction, but no differences were observed before faecal recovery correction. Assuming similar faecal 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 of the measured DMI after faecal 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 faecal recovery. After faecal 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 faecal recovery correction for the 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 faecal recovery correction followed consistent trends during both seasons for the two alkane pairs.

Table 5.4. 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 faecal 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 faecal 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; LI=low intake; 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.

Estimated and measured DMD by using C_{35} alkane as internal marker is shown in Table 5.5. 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. The 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 faecal recovery correction which was not different from the measured DMD. On the contrary, DMD was different (P<0.05) for measured and estimated during both seasons for high intake group.

Table 5.5. Mean measured and predicted DMD (coefficient (SD)) by using C_{35} as internal marker for Zebu type Arsi steer fed natural pasture after mean faecal 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; LI=low intake; 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.

5.4. Discussion

5.4.1. N-alkane concentration and alkane faecal recovery

According to different scholars, the concentrations of plant cuticular wax hydrocarbon majorly composed of odd-chain molecules over 0.9, 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 agree with the previous results in which odd-chain alkanes accounted for over 0.92, and C_{29}, C_{31} and C_{33} were predominant hydrocarbons as reported by Dove and Mayes (1991).

The incomplete faecal 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 faecal 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 faecal recovery of alkanes except for C_{33} and C_{35} in the high intake group compared to the low intake group during the wet

season might be due to higher feed intake in the former group. The greater faecal recovery of n-alkanes in the dry season than the wet season except for C_{32} 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 faecal recovery and digestibility. Full recoveries for C_{31} and C_{33} for the low intake group during the dry season might be due to higher concentrations of these alkanes in the diet as compared to other alkanes. Similarly Keli et al. (2008) observed full recovery for alkanes found in highest concentrations in the diet.

5.4.2. 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 faecal 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 faecal recovery between adjacent alkane pairs. From the present experiment, during both seasons, estimates of intake were much more accurate after faecal recovery correction for the adjacent n-alkanes which confirms previous observations (Keli et al., 2008; Bezabih et al., 2012). Many researchers 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) depending on the relative abundance of n-alkanes in the diet which dictates the type of n-alkane used for accurate diet estimation. Our results did not confirm the previous findings as the 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 for low intake group agrees with the earlier findings by Bezabih et al. (2012) who observed an accurate estimate of DM and OM digestibility by using C_{35} alkane as internal marker. For the high intake group (fed *ad libitum*) during both seasons, the estimated DMD was different from the measured as the level of diet provision increases.

5.5. 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 faecal recoveries were observed for the majority of n-alkanes evaluated. Dry matter intake was accurately estimated after faecal recovery correction during the wet season. The C_{33}/C_{32} pair accurately estimated the DMI regardless of faecal 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 use of C_{31}/C_{32} and C_{33}/C_{32} pairs as n-alkane technique provided a good estimate of DMI and DMD in steers consuming different amounts of wet and dried pasture after faecal recovery correction. However, accuracy can be reduced for digestibility estimation at higher levels of intake, which need further validation.

CHAPTER SIX

General conclusion, recommendation and critical evaluation

6.1. General conclusion

This study aimed at characterizing the vegetation status in terms of botanical composition, potential nutritive value (chemical composition and ruminal fermentation characteristics) of key forage species and subsequently quantifying the nutrition of grazing cattle in terms of diet composition, feed intake and digestibility in the central highlands of Ethiopia by using vegetation inventory, proximate anlaysis and gas production, and plant wax markers, respectively. The second chapter dealt with changes in vegetation structure and aboveground biomass yield in response to traditional grazing land management practices to obtained detailed information on vegetation status in the study area. The third chapter evaluated chemical composition and ruminal fermentation characteristics of selected forage species from the traditional seasonal enclosure area. The second and third chapters were used as basic background information to the subsequent chapters, which dealt with plant wax characterization for key forage species and evaluating the suitability of the wax profile as markers to estimate diet composition, feed intake and digestibility in freely ranging animals. The following conclusions can be drawn from this research for the study area:

- Mean above ground biomass yield for the grass species was significantly lower in the open access grazing area due to continuous uncontrolled stocking compared to the enclosure area.
- The number of species recorded in the enclosure and open access grazing land area was affected due to management practices.
- Lower crude protein and higher fibre contents were observed for forage grasses than for legumes, sedge and forbs.
- The key forage species characterized in the present trial showed obserbable differences in terms of chemical composition and *in vitro* fermentable parameters such as gas and CH_4 production, EGP and fractional gas production rate (c).

- The volume of gas produced by the grass species varied widely from species to species and the differences in pattern between species were not consistent for different incubation times.
- Characterization of forage species in this study in terms of chemical composition and *in vitro* fermentable parameters will serve as a source of information to improve existing knowledge pool which helps to design appropriate pasture based feeding system and grazing land management practices.
- Huge variability in the patterns of concentration of n-alkanes, LCOH and $\delta^{13}C$ of n-alkanes among forage species were observed.
- The concentrations of odd-chain n-alkanes and even-chain LCOH were found to be greater compared to their subsequent even-chain alkane and odd-chain LCOH, respectively.
- Most of the inter species variances in forage species studied were explained by the first two principal components (PC1 and PC2) and the studied plant wax marker scattered and clustered forage species differently, which favours multiple markers for diet composition estimations.
- Incomplete faecal recoveries were observed for the majority of n-alkanes evaluated and faecal recovery correction is of paramount importance to use n-alkanes as diet estimation markers.
- Dry matter intake was accurately estimated by using the C_{31}/C_{32} and C_{33}/C_{32} pairs after fecal recovery correction.
- Dry matter digestibility was accurately estimated by using C_{35} alkane as internal marker after faecal recovery correction for restriction feeding (low intake group).
- This experiment validated the accuracy of the n-alkane technique to estimate DMI and DMD in Zebu type Arsi breed steers fed on wet forage and dried hay from natural pasture after faecal recovery correction.

The following recommendations are made depending on the results of the present study:

- In order to maintain palatable forage species, which are unable to tolerate heavy and continued grazing, and reduce the dominance of a coarse unpalatable forage species,

implementations of enclosure area management practices, whether rotationally grazed, cut-and-carry or standing hay, should be of paramount importance.

- The traditional practice of seasonal enclosures plays an important role in increasing feed resource availability, environmental sustainability and productivity of the farming system in the study area. This is particularly important as pressure increases on the grazing land because of fastly increasing population numbers and expansion of cropping into traditional grazing areas.
- Some forage species evaluated such as the forb *Centella asiatica* are in higher CP, OMD, ME, SCFA content and lower in NDF, ADF and ADL than other evaluated forage species in the study area. However, further studies should be conducted regarding yield, anti nutritional factor and actual feeding value of this species to recommend it as a possible forage crop for cultivation inorder to improve animal nutrition.
- The plant wax components evaluated in the current study such as LCOH and $\delta^{13}C$ of n-alkanes as the diet composition estimations could be validated in controlled experiments.
- As observed from the current study, faecal recovery correction for the adjacent carbon chain (dosed even- and internal odd-chain) is mandatory for accurate dry matter intake estimation.
- For accurate digestibility estimation by using internal markers such as C_{35} alkane, faecal recovery correction is inevitable.
- Accuracy of the n-alkane approach as digestibility estimation can be reduced at higher levels of feed intake, which needs further validation.

6.2. Critical Evaluation

It is necessary to take in to account some important issue that were not included in the present study, but could have improved the findings and conclusions of this study, and would also be important for similar research that may be conducted in the future on similar topics using the methodologies used in this study.

- According to the information obtained from elder farmers in the study area, the two traditional grazing land management practices (enclosure during wet season and the

adjacent open access grazing land) have been practiced for more than 30 years. Longer periods of grazing practices would be necessary to obtain clear differences due to grazing regime for some quality parameters such as soil quality parameters to obtain significant change.

- Vegetation sampling in individual enclosures and the adjacent open access grazing land were done at the end of the main rainy season at 50% flowering. In practice, not all botanical compositions (grass, legume, forb and sedge), and annual and perennial forage species reached 50% flowering at the same time suggesting that there is a need for repeated sampling and identifications.
- Individual forage species in each quadrat were counted and weighed fresh to measure biomass yield and vegetation composition, and were evaluated by analysing the frequency, density, abundance and important value index (IVI). The hardest part of this work was separating forage species harvested from a quadrat into individual species and counting each species to obtain total numbers and total numbers of each species. It is necessary to provide adequate training for data collectors before sampling to obtain reliable and accurate results.
- For methane measurement during *in vitro* ruminal fermentations, 4 ml of (10 M) sodium hydroxide was added from behind into the incubated contents, to avoid gas escape at the end of 48 h of incubation period and after recording the final gas volume in the syringe. Sodium hydroxide was added to absorb CO_2 that was produced during the process of fermentation and the remaining volume of gas was recorded as methane. Parallel experiments were conducted at the same time for methane and total gas measurements, as it was not possible to measure gas production thereafter at different time intervals due to addition of sodium hydroxide to the incubated materials. This has its own effect on the accuracy of the measurements and comparisons done among the two parameters. For a better result, another methodology in which a small number of samples are taken during gas production measurements and used for methane measurement from the same incubated sample is of paramount importantance.
- During controlled experiments, feed intake and digestibility estimation was done by using plant wax markers in this study, and 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 during the wet season trial. During the dry period experiment, precautions should be taken as it is difficult to separate dried forage species (hay) into different species components.

- In the current study, the heart girth tape measurement was used to predict the live weight of steers in order to know the initial and final live weight due to the lack of a large electronic scale that can be used at a field and infrastructure such as electricity for the use of an electric scale in the grazing area. An electronic scale is the most precise and commonly used technique to determine the weight of a live animal.

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