Stall feeding regimen for indigenous dairy cow production in northwestern Ethiopia

(エチオピア北西部における在来種乳牛生産のための舎飼い給餌法)

Shigdaf Mekuriaw Zewdu

The United Graduate School of Agricultural Sciences

Tottori University, Japan

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The United Graduate School of Agricultural Sciences, Tottori University, in partial fulfillment of requirements for the Degree of Doctor of Philosophy in Global Arid Land Science

Shigdaf Mekuriaw Zewdu

The United Graduate School of Agricultural Sciences
Tottori University, Japan

Major Supervisor
Prof. Atsushi Tsunekawa

Co-supervisors
Prof. Toshiyoshi Ichinohe
Prof. Nigussie Haregeweyn

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ACRONYMS AND ABBREVIATIONS

ADF	acid detergent fiber
BW	body weight
BhH	b*rachiaria* hybrid hay
CH_4	methane
CP	crude protein
Co_2	carbon dioxide
CT	condensed tannin
DE	digestible energy
DM	dry matter
DMI	dry matter intake
FPCM	fat protein corrected milk
FCE	feed conversion efficiency
GE	gross energy
GHG	greenhouse gas
GDP	gross domestic product
IFTS	indigenous trees and shrubs
IVOMD	in vitro organic matter digestibility
Kg	kilo gram
ME	metabolizable energy
N	nitrogen
NDF	neutral detergent fiber

NE	net energy
NGH	napier grass hay
NPH	natural pasture hay
NUE	nitrogen utilization efficiency
OM	organic matter
PEG	polyethylene glycerol
PUN	plasma urea nitrogen
RE	retained energy
SEM	standard error of the mean
TEP	total extractable phenol
TET	total extractable tannins
TMR	total mixed ration
TTS	treated teff straw
VFA	volatile fatty acid

Chapter 1: General Introduction

1.1 Background

1.1.1 Global dairy production system and milk consumption

Globally, the dairy sector is probably one of the most important agricultural sectors (Hernández-Castellano et al., 2019). Milk production contributes to household livelihoods, food security and nutrition (Enahoro et al., 2018). Milk is a nutrient-rich, white liquid food produced by the mammary glands of mammals (Ansary, 2019). Milk also provides relatively quick returns for small-scale producers and is an important source of cash income (Wegerif and Martucci, 2019). The average per capita global milk consumption amounts is about 113 kg of milk/year, with very significant differences between countries (Figure 1.1) (Boelling, 2015). Recently, the world per capita consumption of dairy products is expected to increase over the coming decade driven by population growth, income developments and consumer preferences (OECD., 2019).

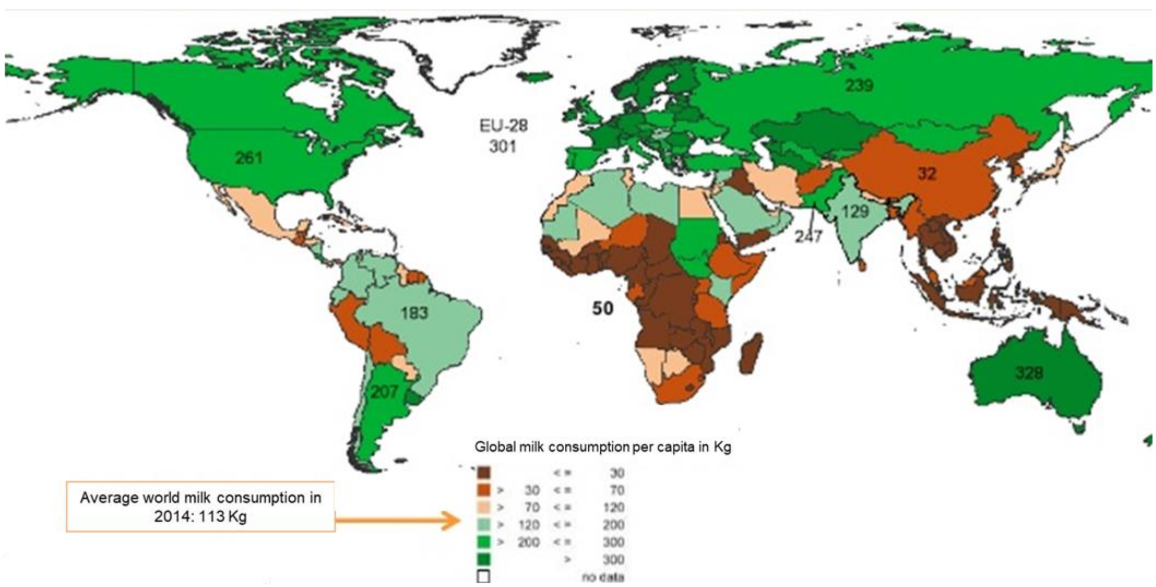

Figure 1.1 Map of global milk consumption per capita in kg (Boelling, 2015)

Dairy production systems have rapidly intensified over the past several decades at aim of increasing productivity while simultaneously decreasing the negative environmental effects of conventional farming practices (Clay et al., 2019). In recent decades, developing countries have increased their share in global dairy production and this is due to an increase in numbers of producing animals rather than a rise in productivity per head (Kapaj and Deci, 2017).

1.1.2 Dairy production in Ethiopia and current status

Dairy production is one of the key livestock sector in Ethiopia (Kassa, 2019). It is practiced almost all over Ethiopia involving a vast number of small subsistence and market oriented farms and is being practiced as an integral part of agricultural activities (Tadesse and Yilma, 2018). Milk production in the country has generally increased over the last 10 years, this increasing trend is mostly associated with an increase in the number of cows (Getabalew et al., 2019). However, the per capita milk consumption has declined from 26 kg per annum in 1980, to 22 kg in 1993, less than 20 kg in 2018 but the Food and Agriculture Organization recommends that the per capita consumption of milk be about 200 kg (Tegegne, 2018; Getabalew *et al.*, 2019). This is likely to be attributed to the mismatch between the growth rate of milk production and large human population more than 110 million people (May, 2020). The milk demand derived more with an annual growth rate of 3.5% the human population in Ethiopia that increase to about 139 million by the year 2020 (Kassa, 2019). On the other hand, Ethiopia has huge livestock population (CSA, 2017) which has one of the highest cattle populations in Africa (Berhanu et al., 2019). Unfortunately, milk production per cow is very low (Tegegne, 2018).

What challenges does the dairy sector in Ethiopia face? The major constraints affecting milk production of dairy cattle in most parts of Ethiopia are low milk production potential of indigenous breed, shortage of grazing land, infectious and parasitic diseases, shortage of land for cultivation of improved forage (Getabalew *et al.*, 2019). The local breeds provide about 1.5 kg per cow per day while in UK the average cow produces about 25 - 30 kg (Kassa, 2019). Moreover, 99% of the cattle population in Ethiopia is indigenous breeds (Yilma et al., 2011). In rural areas of Ethiopia the animals used by smallholder farmers are local breeds which aren’t selected for milk production and managed in a traditional way mostly depend on natural pasture with no supplementary feeds that leads to low quantity of milk production (Ali et al., 2019). Another key determinant of dairy production is feed, which directly affects milk yield (Knips, 2005).

Besides, the dairy production system is mainly operational in areas where the population density is high mainly found around big cities and small towns (Figure 1.2) but they may not have access to cultivable or pasture land. Recently, the Ethiopian government has identified dairy development as one of the economic drivers of the country and has taken steps to support this (Tegegne, 2018). In the future, the prospects of dairying seem to be bright because government is attempting them remedy through policies and strategies in which the dairy farmers are on the way to getting access to services and inputs that could help promote dairy production and productivity including improved forages and feeding strategies, breeding services, credit, extension, training, veterinary services, and appropriate marketing system that addresses consumers' demands (Tadesse and Yilma, 2018).

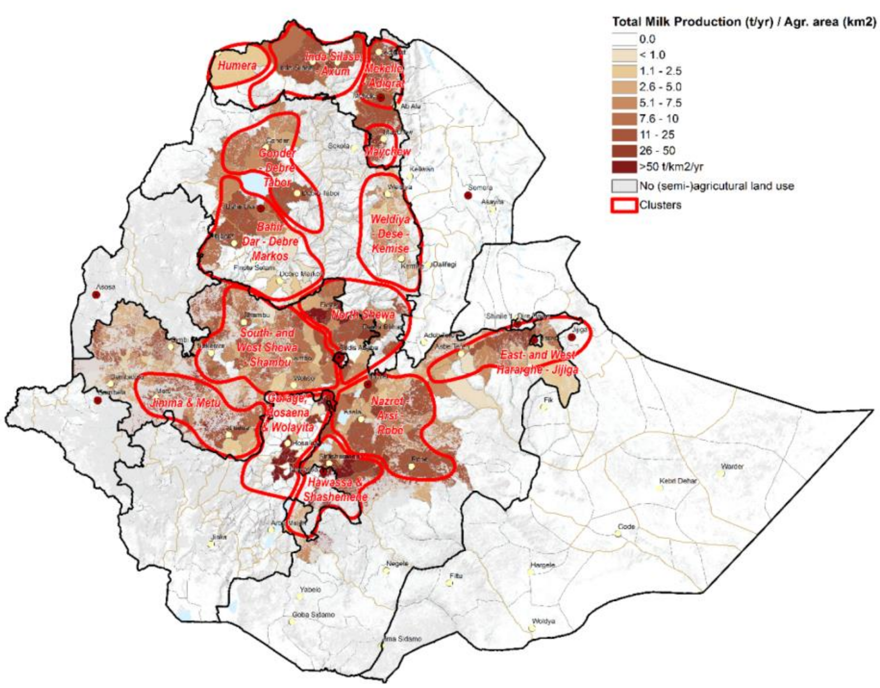

Figure 1.2 Milk mapping of total milk production (ton/year) per km^2 (Ndambi et al., 2018)

1.1.3 Feed resource and nutritive value for dairy production in Ethiopia

Natural pasture, crop residue, improved pasture and forage, agro-industrial by-products and other by-products like food and vegetable refusal are feed resources of Ethiopia (Mengistu et al., 2017b). Among the feed resources, natural pasture and crop residues from crop residues covers the major land use area of Ethiopia (Figure 1.3 and 1.4), contribute the largest source of feed (Harinder et al., 2018). The natural pasture supply the bulk of livestock feed which is composed of indigenous forage species and is subjected to overgrazing as well the availability and quality vary with altitude, rainfall, soil type, and cropping intensity (Yayneshet et al., 2009).

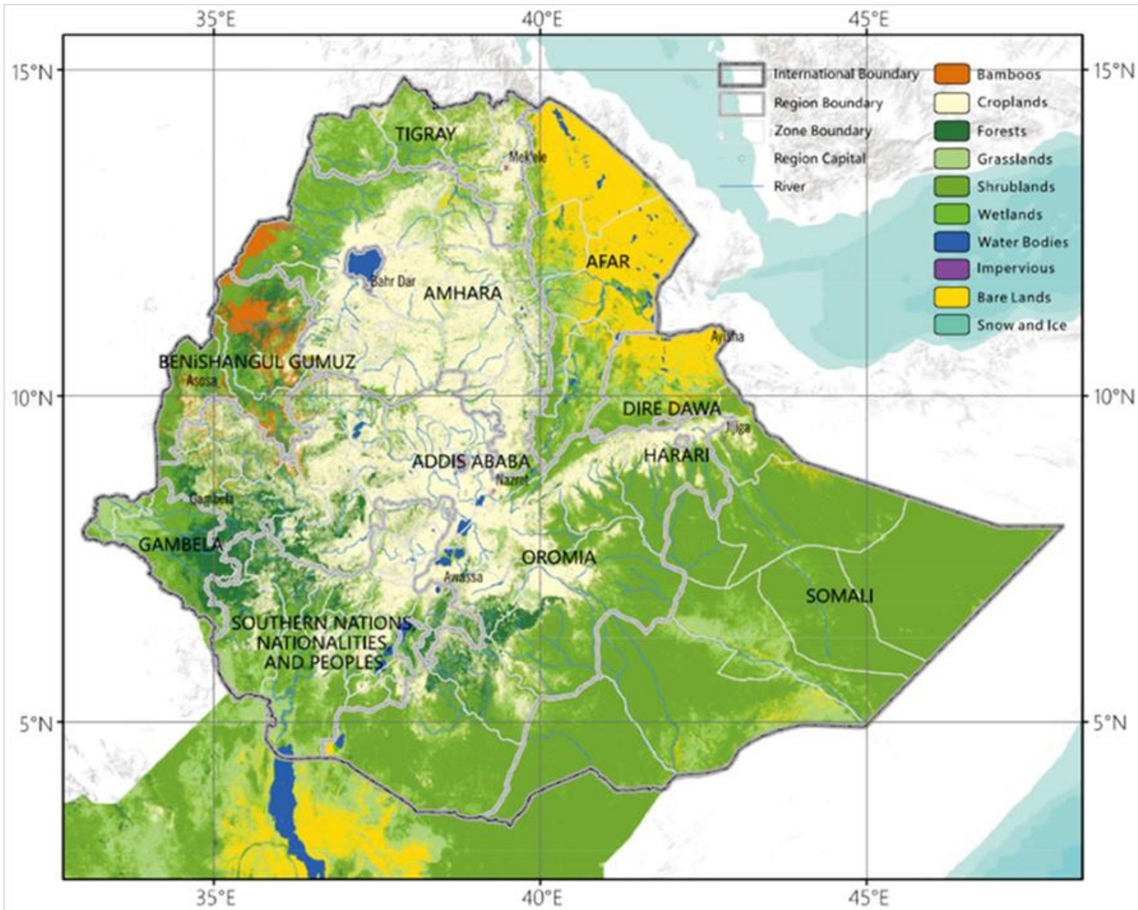

Figure 1.3 Remote sensing-based map of regional major feed resource of Ethiopia (Edoardo, 2019)

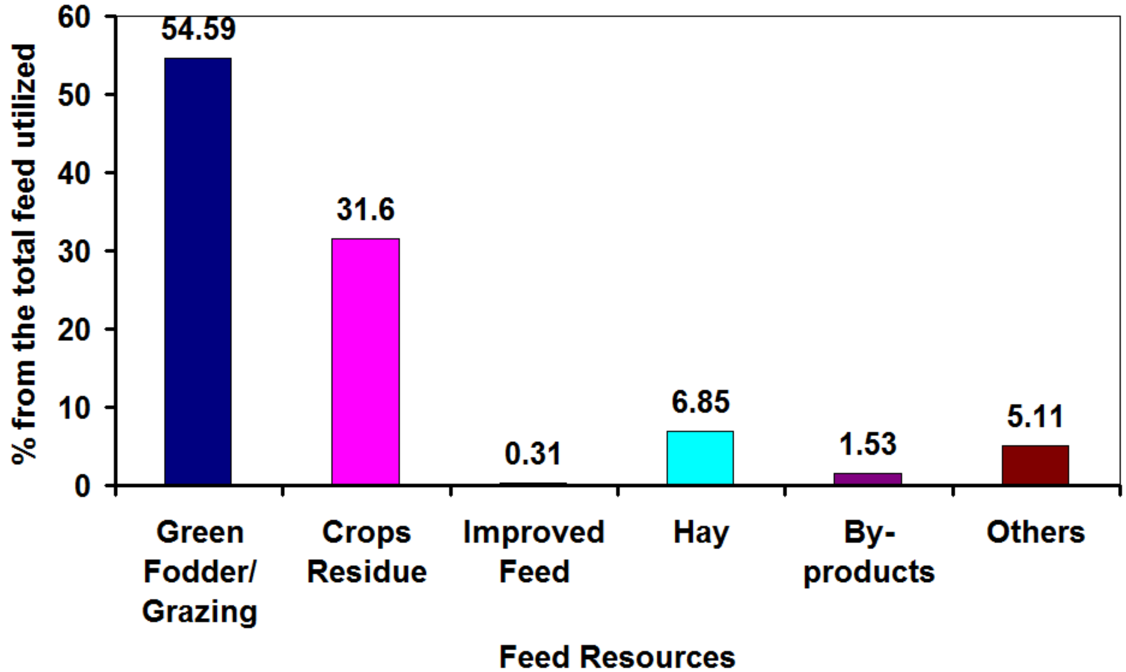

Figure 1.4 Livestock feed resources and proportion in Ethiopia (Belachew, 2019)

Reliance on a crop residue for animal feed is ever-increasing or more land is cropped to feed the fast-growing human population even though it has a lower nutritional (Birhan and Adugna, 2015). Clearly, crop residues represent an underutilized feed source; thus, improving the management of crop residues as animal feed should be one of the main priorities via physical or chemical treatment (Saylor et al., 2018).

Agro-industrial by-products produced in Ethiopia include by-products from flour milling, sugar factory, abattoir, and brewery by-products; these products are mainly used for dairy and fattening animals (Kidist, 2019). In Ethiopia, the use of agro-industrial by-products as livestock feed resources were not widely used due to high cost and unreliable supply as well as their high cost (Feyissa et al., 2013). Besides, local and commercial brewery by-products and distillers grains are also valued for lactating dairy cows because of their palatability and milk-producing property (Birhan and Adugna, 2015).

Feed and fodder availability and quality have been identified as major factors contributing to the milk production potential in Ethiopia particularity in dry season (Ndambi *et al.*, 2018). Improved forages crops must have particular features in order to warrant their inclusion in ruminant production systems aimed at strategic for better feeding of dairy cows in critical feed shortage period specifically in dry season (Shapiro et al., 2017).

Thus, preserving of forage from in excess during wet season in the form of hay and silage as means of distributing forage throughout the year is desirable to provide feed during the dry season. Thus, it needs to appropriate feeding programs and production of quality forage for dairy cow (Kassa, 2019). Alternatively, fodder trees and shrubs offer considerable potential nutrient to complement the low feeding value of crop residues and natural pastures in dry season (Melesse et al., 2019). Furthermore, investigation of their anti-nutritional factor to characterize and designing mitigation strategies of green fodders is crucial (Derero and Kitaw, 2018).

1.1.4 Mitigation of methane emissions and nitrogen excretion of dairy cattle

Globally, with the growth of livestock sector, particular ruminants, major concerns have been raised about the environmental consequences of methane (CH_4) emission for global warming and the low nitrogen utilization efficiency (NUE) on the pollution of potential protein wastage (Wanapat et al., 2015). Cattle account for 77% of the global enteric CH_4 emissions in which the emission intensities not only vary across the regions (Figure 1.5) but also between and within production systems (Carolyn, 2017).

The CH_4 emissions from ruminant livestock in Ethiopia amounted to an annual growth rate of nearly 2%, which is higher than the global average experienced between 1961 and 2010 (0.95%); of which cattle (dairy and non-dairy) were the largest source of enteric CH_4 contributing to 88% of emissions in 2011 and 87% in 2017 (Berhanu *et al.*, 2019)

Alternatively, there are many ways dietary mitigation strategies for methane emission in feeding dairy cows (IPCC, 2014). For example, feeding balanced rations reduce enteric CH_4 emissions by 15-20 % per kilogram of milk produced and increased efficiency of microbial protein synthesis (Garg and Makkar, 2012). Dietary factors are known to affect the community of microorganisms in the digestive tract that affect the quantity of methane produced which suggests that we can manipulate CH_4 production using diet (Hristov et al., 2013). Smallholder production systems contribute over 65% of milk production and hence targeting smallholder farmers should be the priority (FAO, 2012). Therefore, large-scale implementation of such an efficient feeding method can help improve the productivity of livestock and reduce the greenhouse gas emissions raised by smallholder farmers. For instance, the treatment of crop residues with urea results in an emission intensity reduction of 44% while urea treated molasses block reduces 20-27% of lactating cows (Carolyn, 2017).

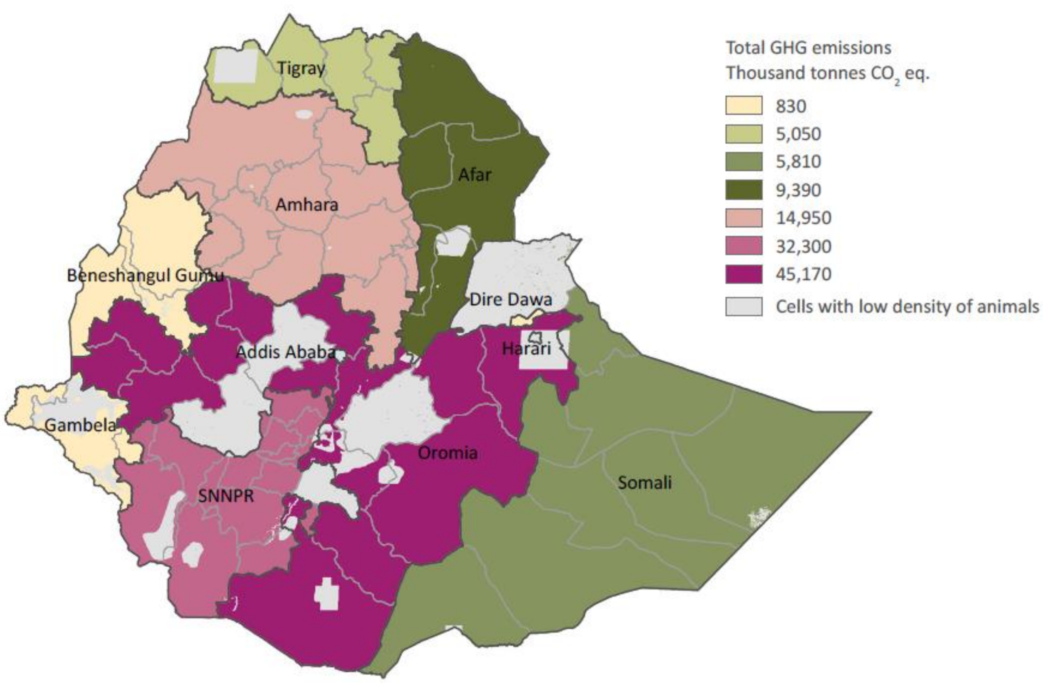

Figure 1.5 Regional distribution of regional greenhouse gas emission from milk production (FAO, 2017)

In addition to GHG emissions from the livestock sector, negative effects of low NUE of ruminants attract great attention that more than 70% of feed nitrogen (N) is excreted (such as in feces and urine) from livestock farming into the environment (Ghelichkhan et al., 2018). Inefficient utilization of nitrogen by dairy cows indicates that about 72% of consumed nitrogen is excreted in faeces and urine (Castillo et al., 2000). Moreover, approximately 60 to 80% of total N intake (NI) was excreted in the urine, which has great potential to aggravate NH_3 emissions, and only 20 to 40% was excreted in feces (Kebreab et al., 2009). Therefore, the development of a diet that can improve the NUE and reduce enteric CH_4 emissions is highly needed and beneficial to both the dairy animal and global environmental challenges (Aboagye et al., 2018). This is due to the strong inverse correlation between the emission intensity and the milk yield per animal in dairy production systems (Figure 1.6) *(*Wilkes et al., 2020*)*.

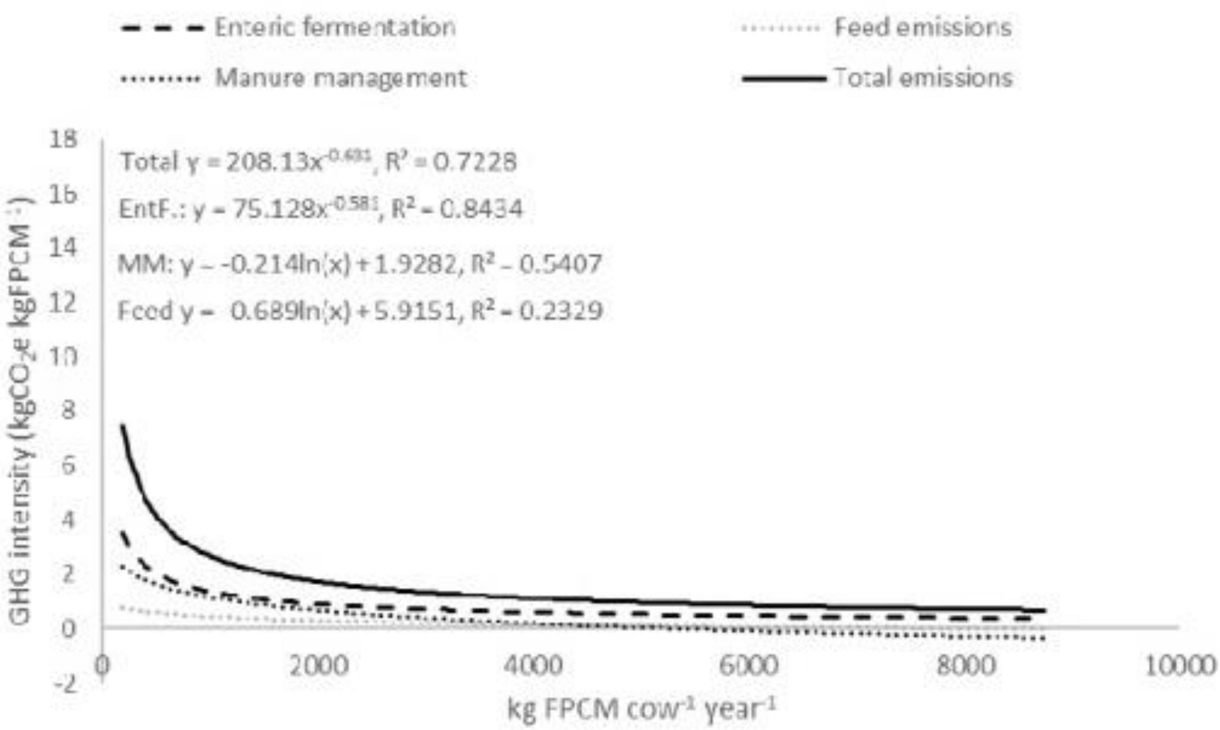

Figure 1.6 Relationship between milk yield and GHG intensity (Wilkes *et al.*, 2020).

1.1.5 Optimal dietary formulation and stall feeding dairy production system

In most sub-Saharan African countries, communal grazing lands are important sources of livestock feed and overstocking is identified to primarily drive degradation of rangelands, declining of vegetation productivity and eventually livestock productivity (Valbuena et al., 2012). Indeed, overgrazing is reported to cause about half (49%) of the land degradation in tropical region followed by deforestation (27%) (Kirui and Mirzabaev, 2014). Despite natural grazing lands are deficient in terms of nutrition quality and quantity due to drought, cattle farming is still heavily dependent on free grazing in Ethiopia (Giday et al., 2018). Improving feed qualities through the use of new technologies such as forage, rotational and stall feeding are suggested as not only economically viable but also ecologically sustainable in mitigating feed shortages (Hadush, 2017).

Recently, the Ethiopian government has instituted a policy shift regarding communal grazing and bush land, from which livestock is now prohibited from entering, as a means to rehabilitate and restore the ecosystem due to overgrazing (Amare et al., 2017). This is in connection with the integration of livestock and crop development in the context of sustainable land management that increase productivity and avoid animal induced land degradation. The sustainable benefits of these practices were also apparent when the Soil and Water Conservation structures are combined with biological interventions, which brought about immediate benefits for farmers in the form of food, feed and income (Gidey, 2015). As prospect, farmers allowed only to cut and carry the available forage feed from the such protected grazing land and enclosure areas for their animals. This promotes the cut and carry feeding for confined dairy cattle production for farmers (Alemayehu et al., 2007). Thus, there is a concept of "changing feeding system", from free grazing to “stall feeding system” in case of dairy production in Ethiopia (Shapiro *et al.*, 2017).

However, the farmers practice is giving the feed for dairy cows arbitrarily without balancing the diet which reduces the potential of their milk and meat production. In these regions, imbalanced nutrition is common in most smallholder dairy feeding systems as many farmers are unskilled in preparation and feeding of balanced diets that leads not only for low livestock productivity but also produce more methane per kg of milk (IPCC, 2014). In the case of Ethiopian dairy production, the diet is largely made of low quality feed products such as crop residues (30-35% of the ration in the rural mixed crop livestock system) and native pastures of poor nutritive value (56% in the rural mixed crop-livestock system and 90% in the agro-pastoral and pastoral system) (Figure 1.7) (Carolyn, 2017).

On the other hand, balanced nutrition is a concept for optimal total mixed ratios (TMR) of feeds, which is based on the physiological conditions of the animal and could contribute to improving animal output as well as to reducing cost of production and methane emissions per unit of animal product produced (Hristov *et al.*, 2013; Gerber et al., 2013). Previous studies reported that TMR rations has also been shown to have several advantages such as a decrease in feed loss, higher nutrient availability, lower enteric CH_4 production and higher animal performance over feeding ingredients separately (Carolyn, 2017), which is conventionally practiced in most African countries. However, there is limited information and practical experience of TMR stall feeding system in developing countries such as Ethiopia. Moreover, the concept of dietary formulation for dairy cows' production is based on NRC (2001) system since there is no feeding standards for Ethiopian dairy production. Hence, practical application and validation of optimal diet formulation in dairy cow feeding is crucial at this time. Linear programming model is certainly the most popular method for diet formulation that can solve for a complicated set of nutrient requirements to give a relatively well-balanced ration (Ou et al., 2019; Moraes et al., 2015).

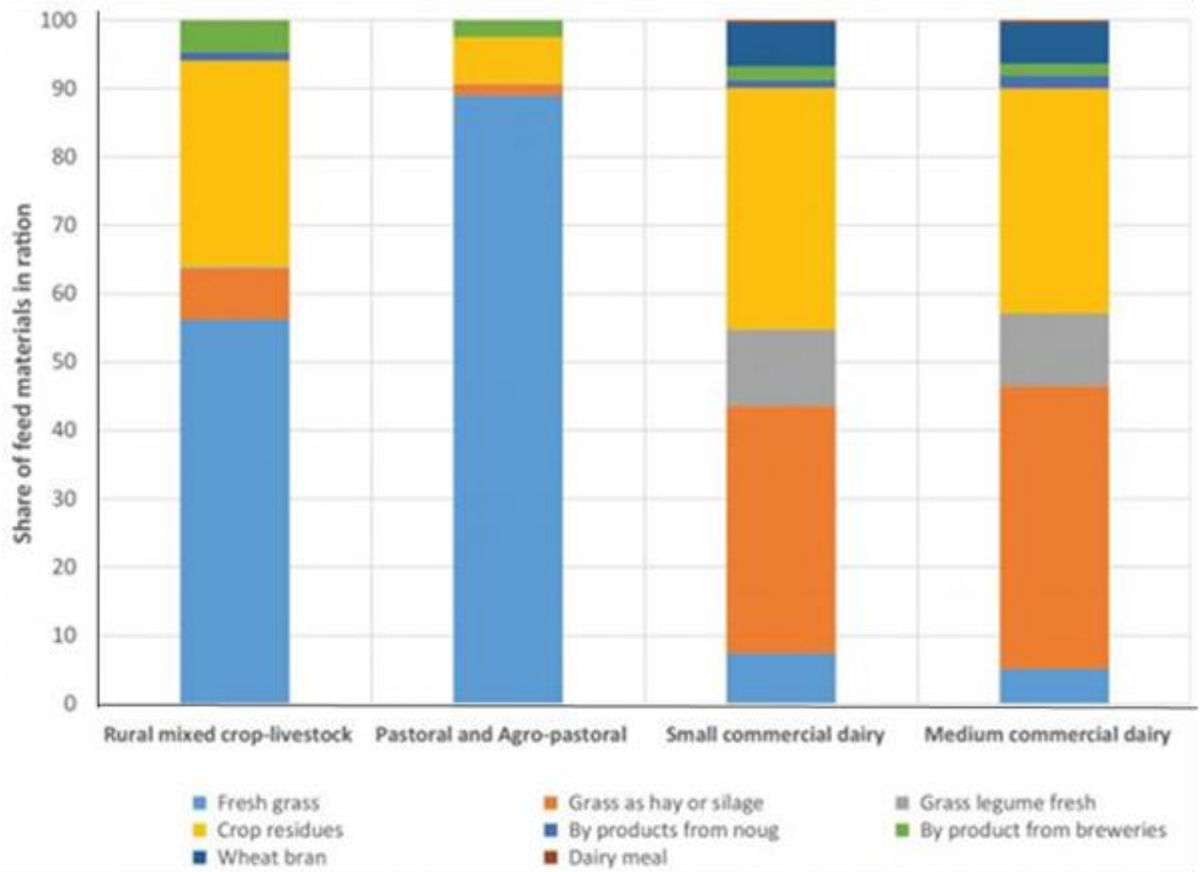

Figure 1.7 Feed composition of dairy rations in Ethiopia (Carolyn, 2017).

1.2 Problem statement

The major constraints affecting milk production in Ethiopia are attributed to the dominant free grazing feeding practice of poor-condition natural pasture feed sources combined with lack of improved feeding practice which is influenced by feed shortage both in quality and quantity. Free grazing cause low productivity, requires extra effort, spread of disease, overgrazing communal land and aggravate land degradation (Kairis et al., 2015; Hennessy et al., 2020). As result, inadequate and unbalanced feeding in free grazing result in low milk yield (Garg et al., 2018). On the other hand, stall-feeding system increase potential productivity (Mwendia, 2015) and showed higher milk yield than the grazed animals (Mbugua et al., 1999) as well as reduce land degradation by avoiding grazing (FAO, 2012). Even, the degraded lands converted to exclosures and farmers are allowed to cut and carry forage for their animals to feed in stall-feeding system. Thus, the key research gaps identified in this study are two folds: lack of information on the type, nutritive value, anti-nutritional factors of locally available or improved feedstuffs and feeding regimen, an optimal diet that specifies the amount and schedule of nutritional intake. Moreover, there is no balanced diet feeding for indigenous lactating dairy cows towards stall feeding system and lack of knowledge and skills on nutritionally balanced rations. Furthermore, quantification of methane emission for such stall feeding system wasn't addressed in the stallholder dairy production in Ethiopia.

What is more, feeding of poor quality roughage for dairy cows is common practice in tropical regions that decrease productivity and increase methane emissions. For that reason, introduce an alternative feeding strategies, feeds such as nutritionally improved forages or bio-chemically treated straw silage based diet in total mixed ration form are essential for improving milk yield, dietary nitrogen utilization and reducing enteric methane emission from dairy cows. Besides, there is scarce information on milk yield, N utilization and methane emission of balanced diets composed of improved grasses of new varieties and treated straw using total mixed ration system. Hence, finding a diet with an appropriate proportion of quality feed ingredients improved utilization and beneficial for sustainable dairy production is crucial in tropical regions dryland environments. Consequently, to enhance dairy production improving feeding and designing appropriate feeding strategies are crucial. Therefore, this research was attempt to quantify the livestock feed resources and propose stall feeding scheme for dairy production compatible with climate change adaptation and mitigation.

1.3 Objectives of the study

To improve the productivity of indigenous dairy cows through formulating stall-feeding regimen taking a case study of the Fogera breed in northwest Ethiopia.

The specific objectives were:

- To identify the available feedstuffs and evaluate the nutritive values;
- To improve feed quality through mitigating the anti-nutritional factors;
- To formulate optimal diet for lactating dairy cows for dry season; and
- To evaluate and validate the effect of some selected diets containing improved grasses hays and treated teff straw silage on milk yield.

1.4 Important terminologies definition

- *A **balanced ration*** is a 24-hour feed allowance that provides an animal with appropriate amounts and proportions of all nutrients required for a given level of performance such as growth, maintenance, lactation or gestation.
- ***Dairy diet formulation*** is the process of quantifying the amounts of feed ingredients that need to be combined to form a single uniform mixture (diet) for dairy that supplies all of their nutrient requirements they need to stay healthy and optimize production (White and Capper, 2014).
- ***Feedstuff*** is a food provided for cattle and other livestock
- ***Feeding regimen*** is a plan that specifies a diet, amount and schedule of nutritional intake
- ***In vitro (Latin for "in the glass")*** refers to a feed sample that is digested in test tubes or tested outside the animal.
- ***In vivo (Latin for "within the living")*** refers to when research is done with or within living organism.
- ***Nutrient requirements*** - the minimum amounts of nutrients (energy, protein, minerals and vitamins) necessary to meet an animal's needs for maintenance, growth, reproduction, lactation or work; does not include a margin of error in ration formulation.
- **Premix** - a uniform mixture of one or more micro-ingredients and a carrier, used to facilitate uniform dispersion of micronutrients into a larger mixture.
- ***Regimen*** is a plan, or course of action such as a diet e.g a low-salt diet is a regimen
- ***Stall feeding*** is to keep and feed (an animal) in a stall (especially as an intensive method). In addition, Stall Feeding defined as the practice of feeding some or all animals in a restricted open homestead land in full year or season (Hadush, 2017).
- ***Total Mixed Ration (TMR)*** is a way to feed the cows by combining all ingredients that typically combine roughages (forages) and concentrates into a single mix feed to optimize animal performance.

1.5 Description of the study area

The location map of the study areas of Aba Gerima, Guder and Andassa in Amhara Region and Dibatie in Benishangul Gumz region, in north western Ethiopia (Figure 1.8). The major land use types in the study area are cultivated lands, grazing lands, and degraded bushlands (Table 1.1). In all sites, cultivated lands proportion is larger than grazing lands and degraded bushlands. Livestock types are more or less similar across the three sites. In all sites, the farming system is mixed crop–livestock, characterized by small scale, rained and continuous cropping practices. Major crops include tef (*Eragrostis tef*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and potato (*Solanum tuberosum*) at the Guder site, whereas finger millet (*Eleusine coracana*), maize (*Zea mays*), and tef are the major crops at Aba Gerima and Dibatie sites. Moreover, these sites are placed in one of the major dairy milk shed areas in northwestern Ethiopia in which stall feeding is highly encouraged to reduce free grazing due to sustainable land management practice (Berihun et al., 2019).

Table 1.1 Characteristics of study sites

Characteristics	Study sites			
	Dibatie	Aba Gerima	Guder	Andassa
Geographical coordinates	11°25′–11°55′N, 37°04′–37°39′E	10°01′–10°53′N, 36°04′–36°26′E)	10°57′–11°11′N, 36°40′–37°05′E	11°42′–11°92′N, 37°07′–37°65′E
Altitude (m a.s.l.)	1598; lowland	2090; midland	2719; highland	1730; midland
Temperature (°C)	18–29	17–31	15–24	14–25
Rainfall (mm)	1022	1343	2495	1434
Major livestock species	Cattle, sheep, goats, and donkeys	Cattle, sheep, goats, and donkeys	Cattle, sheep, donkeys, and horses	Cattle, goat, donkeys

Source (Bitew et al., 2010; Berihun et al., 2019).

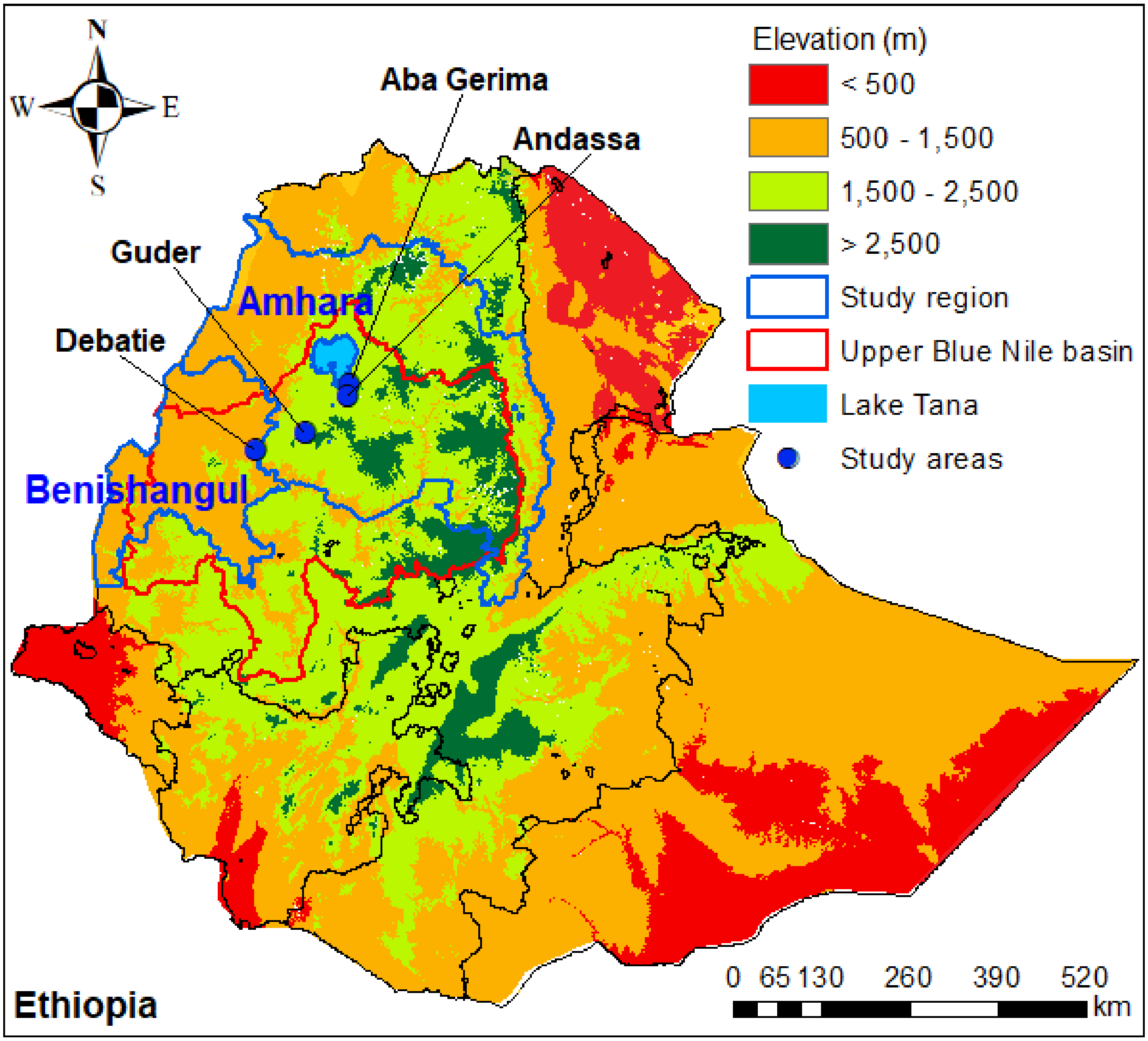

Figure 1.8 Location maps of the study areas

1.6 Organization of the thesis structure of the thesis

This thesis is organized into chapters (Figure 1.9). The first chapter (Chapter 1) presents the introductory sections (background, problem statement, objectives, and the study area). It explains the global dairy feeding and current status, the feed resource in Ethiopia and the challenges for feeding system proposing cattle feeding system and current research based on the existing literatures, field observation, and indicates the rationale of this study. Chapter 2 identify the available feed resource in three different agro-ecologies (lowland, midland, and highland) and analyses the nutritive values based on in vitro laboratory. Chapter 3 evaluate the mitigation of anti-nutritional factors using polyethylene glycol (PEG) for fodder species on in vitro assay laboratory technique. Chapter 4 Optimal diet formulation to enhance smallholder dairy production in dry season using goal programming approach. Chapter 5 evaluate the effect of balanced diet composed of feeding improved forage and treated tef straw on milk yield, nitrogen balance and methane emission (In vivo based). The last chapter (Chapter 6) presents the general conclusions and recommendations based on the key findings from the four main chapters (chapters 2, 3, 4 and 5).

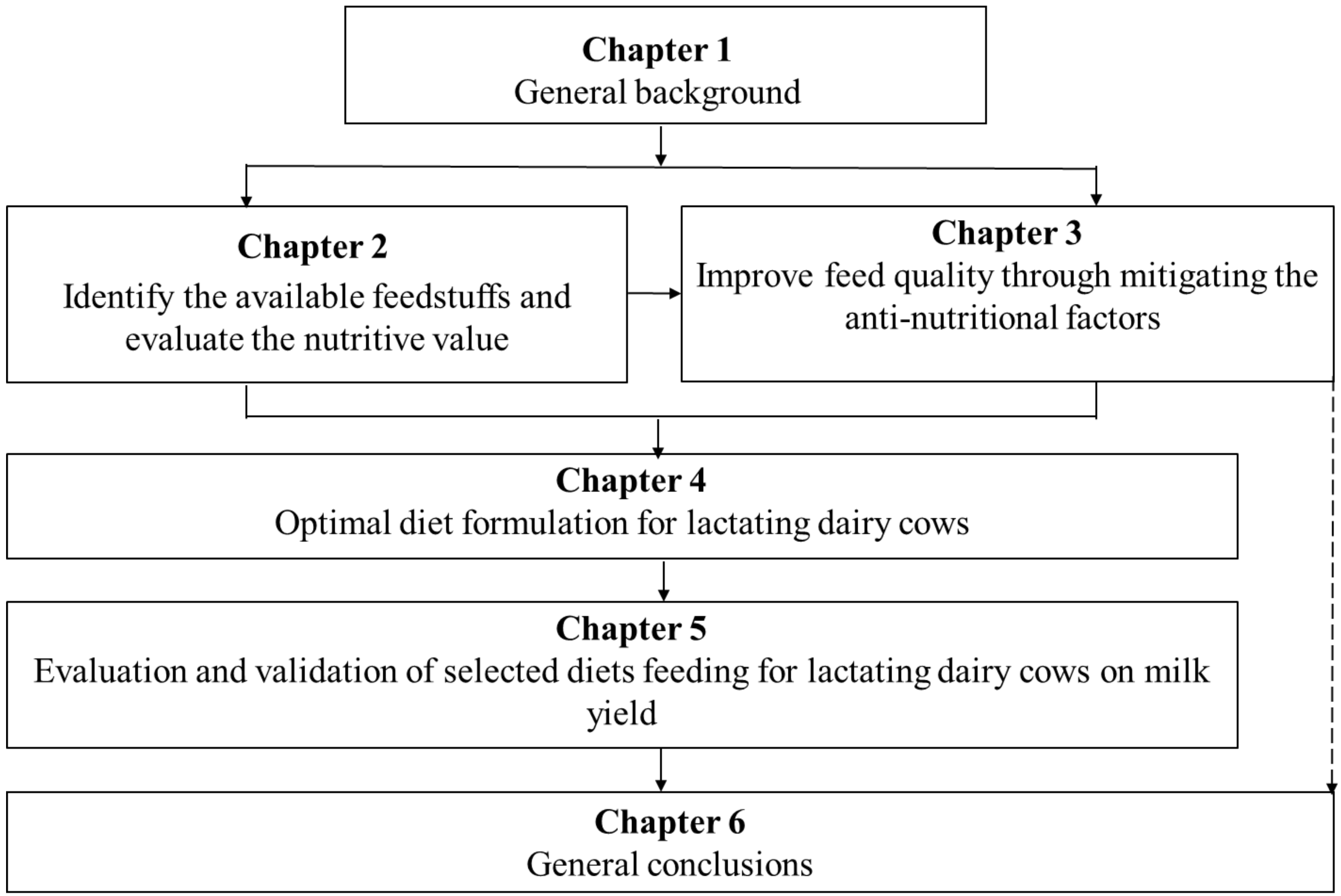

Figure 1.9 Organization structure of the thesis

Chapter 2:

Identify the available feedstuffs and evaluate nutritive values

2.1 Introduction

Livestock play an important role in Ethiopia's Economy (Harinder *et al.*, 2018). The prime constraint to livestock production in most developing countries is feed shortage (Bekele et al., 2017). Feed scarcity is one of the major technical constraints in livestock production and thus it challenges the economic contribution of the livestock sub-sector (Talore, 2015). In many developing countries of the tropics, such as Ethiopia, there is severe shortage of quality forages for livestock production (Habib et al., 2016). In addition, with the present trend of rising feedstuff prices and global inflation, livestock production is increasingly constrained by feed scarcity and the high cost of feeds (Balehegn et al., 2019). Therefore, the reduction of feed costs by using the locally available feed resource such as forage resources, crop residues, agro-industrial by-products and other non-conventional feed resources, and improvement of productivity are important in obtaining higher profits in livestock production (Wanapat et al., 2018). Moreover, the adoption and use of exotic feed resource and improved feed technologies remained limited calling for exploring existing feed resources (Gemiyo et al., 2013).

The Ethiopian livestock master plan have been undertaking significant measures to alleviate the situations, by improving local feeds (Shapiro et al., 2015). In this regard, participatory identification and evaluation of local feed resources is essential (Talore, 2015). It is therefore imperative to examine for cheaper feed resources that can improve intake and digestibility of low quality forages (Negesse et al., 2009). However, their chemical composition is variable due to variations in soil type, topography and other environmental factors that affect the forage yield and quality (Gemiyo *et al.*, 2013). The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion (Getachew et al., 2004). Information on nutritional characterization of locally available feed resources at country level is inadequate and where available the values are variably documented (Zinash and Seyoum, 1998). There is great diversity, variability and nutritional values of feeds in the northwestern part of Ethiopia in particular have not yet been investigated and their feeding value is largely unknown

(Gemiyo *et al.*, 2013).

Moreover, ruminant livestock production in the tropics is limited due to insufficient nitrogen (N) from low-quality forages (Selemani et al., 2013). Alternatively, fodder trees and shrubs offer considerable potential nutrient N to complement the low feeding value of crop residues and natural pastures in which the levels of crude protein (CP) are below the minimal requirement (i.e., 80 g/kg dry matter) for optimal rumen microbial function (Melesse et al., 2017). In addition, fodder trees and shrubs are important components of ruminant livestock diets in diverse farming systems in tropical regions (Babayemi and Bamikole, 2006; Derero and Kitaw, 2018). Recently, a high focus of the rehabilitation plan for the closure areas has been the on planting of various fodder trees and shrubs species, particularly those native to the tropical environment (Mulatu and Kassa, 2001; Tefera and Sterk, 2010). In this case, farmers are encouraged to enter the closure areas to harvest the fodder tree and shrubs and carry them to their animals (Mekoya et al., 2008). Consequently, the use of indigenous fodder species as a supplemental livestock feed is becoming increasingly important (Gwaze et al., 2009). This practice benefits smallholder farmers in developing countries, who typically cannot afford to purchase concentrates and thus depend almost entirely on fodder species for supplemental feeding of their animals (Olafadehan and Okunade, 2018). For the reasons stated above and because of the plants multipurpose nature, indigenous fodder trees and shrubs (IFTS) are now receiving increased research attention in tropical regions (Murgueitio et al., 2011), particularly Ethiopia (Balehegn et al., 2014; Derero and Kitaw, 2018). The subsequent use of fodder species selected on the bases of farmers' knowledge and scientific findings would be environmentally sound, socially acceptable, and economically sustainable for increasing ruminant livestock production (Khanal and Subba, 2001).

Thus, the identification and nutritional evaluation of potential quality feed resource is crucial for proposing strategic feeding supplementation for ruminant livestock (Santos et al., 2017). To this end, we initiated the current study to address the following specific objectives: i) to identify the locally available feedstuffs; ii) to evaluate the nutritive value and fermentation characteristics of feedstuffs; iii) to investigate the potential indigenous fodder tree and shrubs as supplementary feed for ruminant animals.

2.2 Materials and methods

The experiment was conducted in three sites of northwestern Ethiopia (For the detail of the study sites, please see section 1.6).

2.2.1 Assessment of available feedstuffs

Survey was conducted to assess the current livestock feed available in the smallholder livestock production systems. Farmers' perception on sustainability for livestock feed source also assessed. Random sampling of households was taken and conducted using 270 households to assess the feed resources, current livestock feed available and seasonality in the smallholder livestock production systems. The seasonal availability of feed resources was assessed based on farmers' judgment and scores given for a particular feed type in each month throughout the year. Availability of feed over the year was scored on a scale of 0–10, where 10 = excess feed available, 5 = adequate feed available and 0 = no feed available. Two major cropping seasons were identified in the three sites selected for the study. The two seasons are known as wet (Kiremt) and dry (Bega) seasons. Wet season covers the months from June to October while dry season covers the months from December to the end of May. Wet season is the main rainy season where major field crops are grown while the dry season where crop harvesting, collection, land preparation and irrigation activities are carried out.

2.2.2 Identification of and farmers' preferences for indigenous fodder trees and shrubs

The indigenous fodder trees and shrubs (IFTS) were identified based on community knowledge gathered through questionnaire surveys, group discussions, and one-on-one interviews across the study sites. In addition, field inventories were performed to identify potential fodder species at each site. Criteria for preferred fodder species were proposed in response to farmers' responses regarding important qualities of fodders of choice; the criteria were 1) feeding value (palatability and ability to support their animals); 2) fodder availability year-round (both rainy and dry seasons); 3) local abundance; 4) biomass production; and 5) multipurpose use (Figure 1). Herders were also asked to rank each fodder species according to their preference (1, least favored; 4, most favored).

A total of 129 indigenous plant trees and shrubs were collected in both wet and dry seasons (Appendix Table 1), and their seasonal availability was recorded for two consecutive years. A relative abundance vegetation survey was conducted to evaluate species distribution patterns, availability, and abundance. Quadrat sizes, determined by using the minimal area method, were 10 × 10 m for trees and 5 × 5 m for shrubs; a total of 105 quadrats were assessed across the three study sites. The total numbers of indigenous trees and shrubs plant species in the sampling quadrats were recorded to determine the relative abundance of each species, which was calculated according to Tadesse et al. (2019). Specimens of all plant species were transported to the Botany Department of Bahir Dar University for identification at the species level; remaining unknown species went to the National Herbarium at Addis Ababa University for identification. Any specimens still unidentified after evaluation at the National Herbarium were labeled as 'species unknown' (Appendix Table 1).

To screen the plant species as potential IFTS (Figure 2.1), we used four categories of fodder palatability for ruminant livestock: unpalatable, low palatability, moderate palatability, and high palatability (Hussain and Durrani, 2009) (Appendix Table 1). The different palatability of the fodder species was confirmed through knowledge gathered from local herders and field observation. Furthermore, to select the top 5 IFTS as potential fodder species at each site, we considered the following parameters: 1) farmers' preference-ranking score; 2) chemical composition and 3) anti-nutritional content (Figure 2.1).

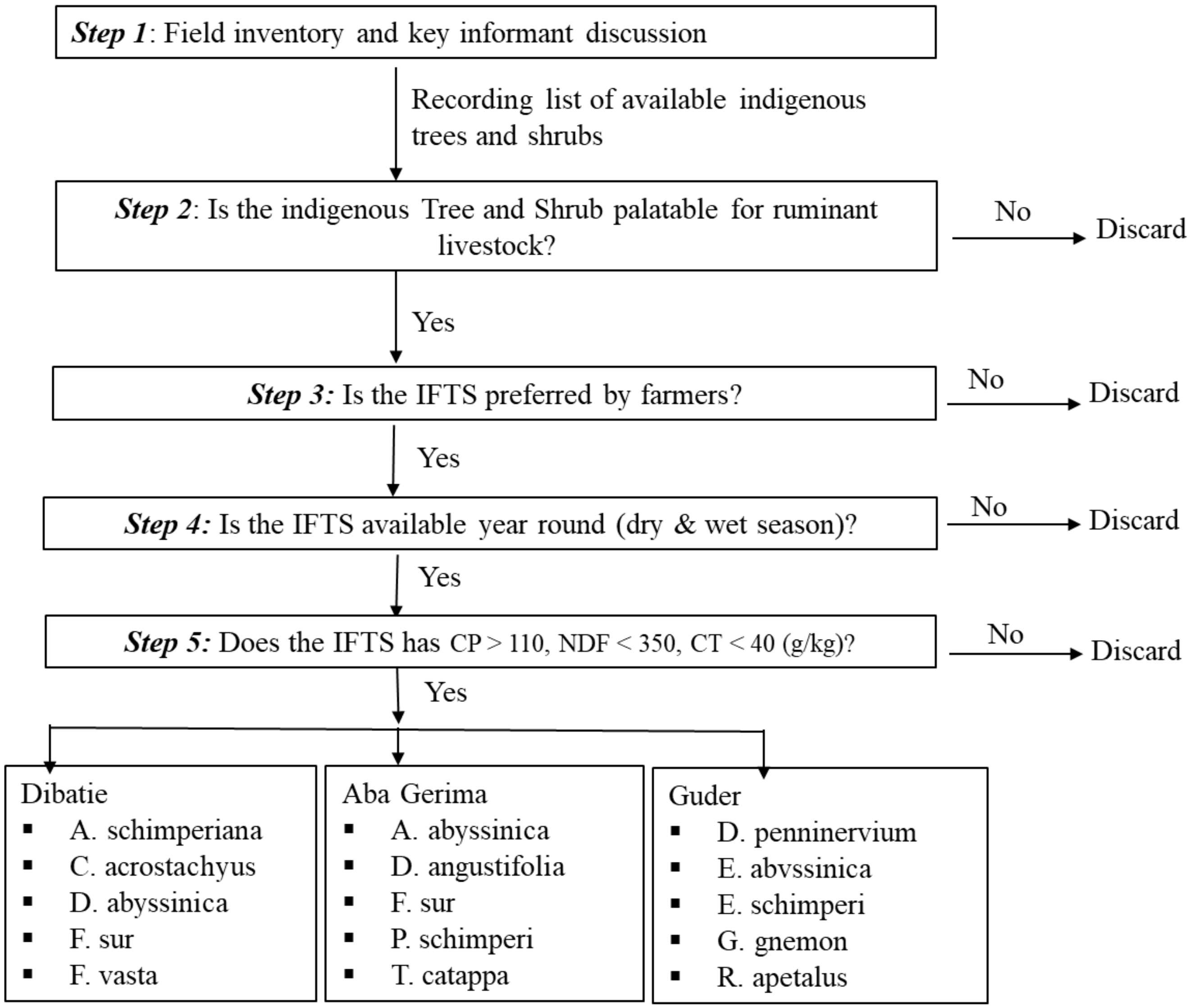

Figure 2.1 The methodological framework for selection and evaluation of indigenous fodder trees and shrubs (IFTS). CP, crude protein; NDF, neutral detergent factor; CT, condensed tannin.

2.2.3 Sample collection and procedures

From 32 identified feed resources, 207 samples were collected and air dried in the shade to minimize changes in tannin content then dried at 65^{o}C for 24 h in a forced air oven to constant weight and ground in a hammer mill to pass through a 1-mm sieve and stored in plastic bags for subsequent determination of chemical components, secondary compounds and in vitro fermentation characteristics. For fodder species, leaves and twinges from 3-5 randomly selected tree and shrubs were collected to form representative samples.

2.2.4 Analysis of chemical composition and mineral profile

Standard methods (AOAC, 1990) were used to determine dry matter content was determined by drying feeds at 135°C for 2 h (DM, # 930.15) and ash was determined by burning dried samples in a muffle furnace set at 550°C for 3 h (# 924.03). The nitrogen content of the feed was analyzed by using a CN coder analyzer (Shimadizu, Kyoto, Japan), and CP was calculated as N content × 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Van Soest et al. (1991). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents, which comprised the cell wall components in these samples, were analyzed according to Van Soest *et al.* (1991). Gross energy (GE) of the feed was determined by using an adiabatic bomb calorimeter (model CA-4AJ, Shimadzu) (Santos et al., 2017). To determine the CT content, the sample extract was treated with butanol–HCl in the presence of ferric ammonium sulfate, and CT was expressed in leucocyanidin equivalents as absorbance at 550 nm in a spectrophotometer (Shimadzu) (Porter et al., 1985).

The contents of minerals were analyzed by Inductive Coupled Plasma Mass Spectrometer (ICP MS) after digestion of the feed samples with a mixture of nitric acids according to the procedures described by Nardi et al. (2009). For mineral concertation analysis detail procedures, about 0.2 g of dried feed sample was added to the Quartz tube digested by microwave with 5 ml of concentrated nitric acid (HNO_3; 70% concentration; 1.42 g/cm^3) and cover (Wako Pure Chemical Corporation). The inside of the container is filled with Milli-Q water in order to remove the acid adsorbing to the inner wall, therefore discarded as acidic waste liquid. The 4 mL of Milli-Q water and 1 mL of hydrogen peroxide solution was added to the Teflon container, and place the quartz insert on it. After discarded, the container filled with Milli-Q water. The sample solution transferred from the quartz insert to 15 mL conical tubes. When the amount of sample solution is less than 5 mL, it was adjusted to 5 mL with 1% diluted nitric acid (1% diluted nitric acid is made from 5 mL of nitric acid and 495 mL of Milli-Q water). The digested samples were centrifuge at temperature 20°C, rotation speed 4800 rpm, time 10 minute. The digestion process has been done by High performance microwave digestion system (Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Santa Clara, CA USA). The program involved 760 W, power at 120ºC for 5 min, 760 W at 150ºC for 15 min. and 0 W for 30 min to cool down polypropylene volumetric flasks (Corning NY Mexico). After the digestion, the samples kept in a deep freeze at 4ºC until the analysis. The dilution of samples was prepared 1/10,000 solutions for typical samples. For analysis, standard for calibration curve was prepared. The diluted solution using the vortex machine check more than 5 times (Voltex, Delta Mixer Se-04). The standard was prepared as 1, 10, 100 and 500 ppm using standards using the internal standards XSTC-331 and XSTC-8. The macro (Ca, P, Mg, Na and S) and micro (Fe, Zn, Mn, Mo, Co and Cu) minerals were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; Agilent technologies, Japan) (Masson et al., 2010). All chemical composition and incubations were completed at the laboratory of Animal Nutrition, Faculty of Environmental and Life Sciences, Shimane University, Japan.

2.2.5 In vitro digestibility and fermentation characteristics

Rumen fluid for evaluation of in vitro digestibility was obtained from two healthy mature female Japanese Corriedale sheep fitted with permanent 46-mm rumen cannulas that were fed a daily ration of 800 g timothy hay and 200 g concentrates, which was divided into two equal meals at 8:00 and 16:00 h. The sheep were supplemented with minerals and had free access to water. Rumen fluid was obtained in the morning at 3 h after feeding, flushed with CO2, filtered through three layers of cheesecloth, and mixed (1:4, v/v) with an anaerobic mineral buffer solution as described by Makkar et al. (2000). An in vitro mineral buffer media for gas testing was prepared according to (Menke, 1988). Gas production was recorded before incubation (0 h) and after 3, 6, 12, 24, 48, 72, and 96 h of incubation. The in vitro organic matter digestibility (IVOMD, g/kg DM) and metabolizable energy (ME, MJ/kg DM) were estimated on the basis of 24-h gas production (ml) and CP content (g/kg

DM) by using equations 1 and 2 (Menke, 1988):

IVOMD (%) = 14.88 + 0.8893×IVGP24 + 0.045×CP (1)

ME (MJ/kg DM) = 2.20 + 0.136×IVGP24 + 0.057×CP (% DM) (2)

where IVGP24 is the 24-h gas volume (ml/0.2 g DM), and CP and ash are each given as % DM of the leaves of the browse species.

The gas production characteristics of each browse species were estimated by fitting the mean gas volumes to the following exponential equation (3) (Ørskov and McDonald, 1979):

$GP = a + b (1 - e^{-ct})$ (3)

where GP is the gas production at time t, a is the gas production from the immediately soluble fraction, b is the gas production from the insoluble but degradable fraction, a + b is the potential gas production, and c is the rate constant of gas production (h^{-1}).

incubation (0-h) gas production value was subtracted from each of those after incubation.

The in vitro gas production data were fitted by using the 'Neway' curve-fitting program (Macaulay Land Use Research Institute 2004 cited in Ichinohe and Fujihara (2009)) to estimate the rumen degradation parameters according to the model of McDonald (1981).

2.2.6 Rumen degradability, volatile fatty acid and methane production

The rumen effective dry matter degradability (EDMD) of feed sample was estimated using equation (4) proposed by (Kamalak et al., 2005):

EDMD = 24.7 + 0.358(a + b) – 60.3c (R^2 = 98.3; RSD = 0.780) (4)

Where EDMD is effective dry matter degradability, a is the gas production from the quickly soluble fraction, b is the gas production from the insoluble fraction, (a + b) is the total potential gas production, c is the gas production rate and RSD is relative standard deviation. The in vitro gas production data were fitted by using the Neway curve-fitting program (Ichinohe and Fujihara (2008) to estimate the rumen degradation parameters according to McDonald's model (McDonald, 1981).

For volatile fatty acid analysis, at the end of the 96-h incubation of the gas production, 5 mL of the supernatant in the syringe was collected, centrifuged at 25 000 × *g* for 15 min at 4°C, and the supernatant transferred to a 15 mL micro-centrifuge tube (capacity) for storage at –20°C until VFA analysis. The frozen samples were defrosted using gauze to remove foreign materials and centrifuge the filtrated liquid with 10000 RPM, 4°C, 30min. 0.5ml of supernatant is mixed with 20mmol/L crotonic acid (internal standard). Concentration of crotonic acid (internal standard) is 10mmol/L, which is the same as the concentration of external standard. The external standard solution comprised a mixture of acetic, propionic, n-butyric, isobutyric, and crotonic acids and was treated in the same manner as the sample. The samples were injected into GC machine and VFA were determined by gas chromatography (GC 14A, Shimazu Corp., Kyoto, Japan). The VFA concentrations were decided by comparing the proportion of peak area for target VFA to that for crotonic acid between the samples and the external standard solution.

The methane production was estimated using the equation;

CH_4= 0.5 acetate – 0.25 propionate + 0.5 (Moss et al., 2000)   (5)

2.2.7 Statistical analyses

The FEAST excel macro program (www.ilri.org/feast) was used to analyses quantitative data while qualitative data obtained through focused discussion were summarized. Narrative responses collected during the group discussions were examined and reported in a qualitative manner. Means, standard deviations and percentages were used to describe variables observed among farmers. The chemical composition, mineral and *in vitro* digestibility data for the various feedstuffs were pooled for the location and analyzed by using general linear models (GLM) procedure (SAS, 2001).

Due to the differences in the types of IFTS available at each site, means were calculated, and all data were analyzed by using a general linear model procedure (SAS, 2001) with a completely randomized design within site. The effect of IFTS species type on nutrient composition was analyzed using the model:

$$Y_{ij} = \mu + IFTS_i + e_{ij}, \quad (6)$$

where Y_{ij} is the dependent variable of chemical composition parameters, μ is the overall mean, $IFTS_i$ is the fixed effect of IFTS species (1–15 for each site), and e_{ij} is the random error. When the F-test was significant ($P < 0.05$), Tukey's test was used to compare significant differences ($P < 0.05$) among the nutritive values of IFTS species. Pearson correlation coefficients between chemical composition and farmers' preference ranking of IFTS were determined by using SPSS (SPSS, 2016). A regression analysis was performed to test the complementarity between farmers' preference ranking scores and nutritive values of IFTS, such as CP (Figure 2.1) (Mekoya *et al.*, 2008). The CP content was defined as the fodder plant nutritional quality parameter to relate the farmers' ranking score to nutritional composition (Nunes et al., 2016). The preference ranking scores were treated as quantities measured on a continuous scale (Kuntashula and Mafongoya, 2005). Cross tabulations were used to identify the proportion of respondents for each IFTS ranking score.

2.3 Results

2.3.1 Livestock feed resources and seasonal availability

The result indicates that a variety of different feed types were identified as categorized into natural pasture, crop residues, green fodder, improved forage, agro industrial byproduct and non-conventional feed resources (Figure 2.2). The dominant crops were tef (*Eragrostis tef*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and potato (*Solanumtuberosum*) at the Guder site, whereas tef, finger millet (*Eleusine coracana*) and maize (*Zeamays*) are the major crops at the Aba Gerima and Dibatie sites. Groundnut (*Arachis hypogaea L.*) in Dibatie and Telba (*Guizotia abyssinica*) at Aba Gerima also cultivated as cash crops. The crop residue from those dominant crops are used for livestock feed in the study locations usually at dry season when the green fodder availability is scarcity. The use of agro-industrial products (concentrates, wheat bran, Noug Seed cake) is occasionally practiced in smallholder feeding systems specifically in Aba Gerima since it is located near the Bahir Dar City that has accessibility to use. The production of improved and cultivated forage crops is relatively high in Aba Gerima followed by Guder. Among the improved forages;

Napier grass (*Pennisetum purpureum*), Dasho grass (*Pennisetum pedicellatum*), *Sesbania sesban* and Tree Lucern (*Chamaecytisus palmensis*) have been established in the backyard forage production. The main non-conventional feeds were local brewery (Atella) and distillery (Brintie) byproduct in which cheap feed resources even though they were not readily and regularly available at all the times in the households.

The availability of feeds is largely depending of the seasonality across different months of the year (Figure 2.3). Better feed supply is available during the autumn season (October to December) in which crop harvesting time for crop aftermath and crop residue followed by the main rainy season (July to September) in which more grazing pastures are available. Availability of grazing pastures largely relies on rainfall and it is adequately available from the onset to the end of the main rainy season. The severe shortage of livestock feed is in dry season (February to May) that represents a period of critical feed shortage. During this period, crop residues have a great role for the contribution of livestock feed sources. Availability of crop aftermath grazing follows harvesting periods of crops grown and it is short lived from November to January.

2.3.2 Chemical composition of the feedstuffs

The chemical composition values various among the feed types (Table 2.1). The CP contents of the feeds have highly significant ($P < 0.001$) variation; for example, the lowest for crop residues (*Zea mays* (28±1.3 g/ kg DM) and Teff Straw (43.5g/kg)) to the highest for Agro-industrial byproducts (Soya bean Cake (276.3 g/kg) and Sesame Seed Cake (246.8 g/kg) and improved forages (*Sesbania Sesban*, 232.2 g/kg). Most of the feed resources did not differ significantly ($P > 0.05$) in NDF, ADL, Ash and GE although the range of mean was wide. For instance, the ash content ranged from 15.8 to 220.29 g/kg DM while the NDF values of ranged between 216.8 g /kg (weed under maize) DM to 812.2 g/kg DM (herbage). The energy value of cereal straws ranges between 13.03 MJ/kg (*Eleusine coracana L.*) to 27.17 MJ/kg (*Vernonia amygdalina*) while pulse straws have fairly higher energy value (*Vicia faba L.*, 25.09 MJ/kg).

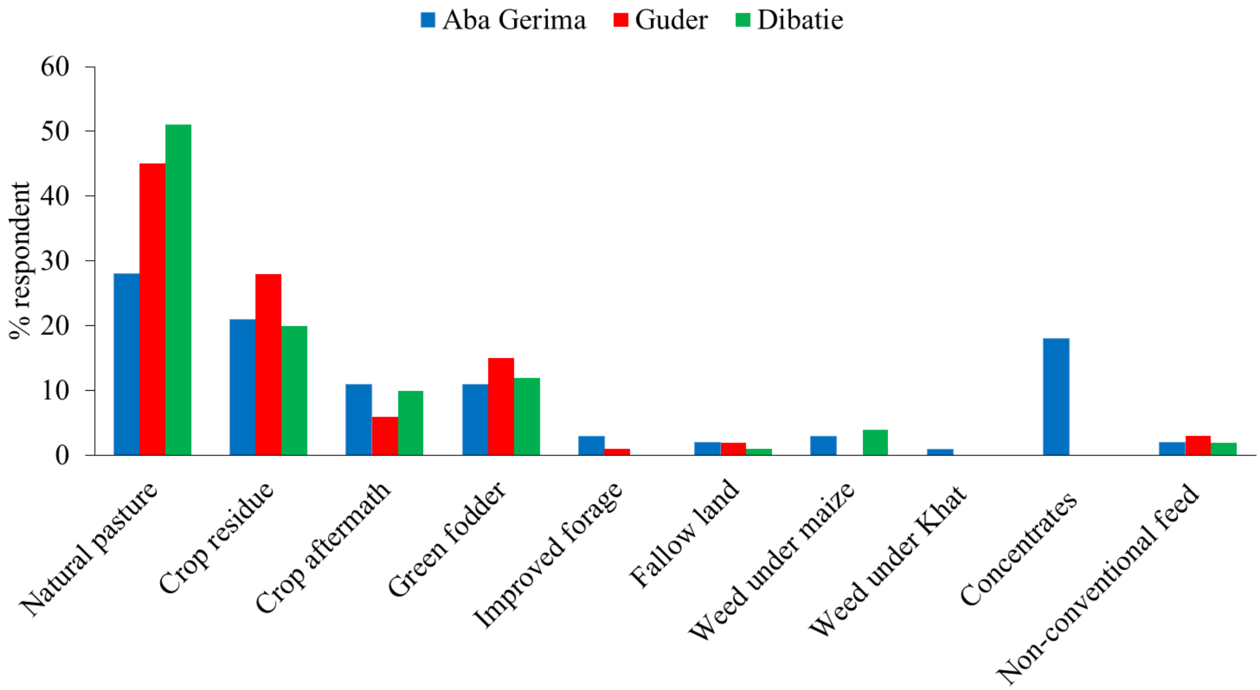

Figure 2.2 Major livestock feed resources across three sites.

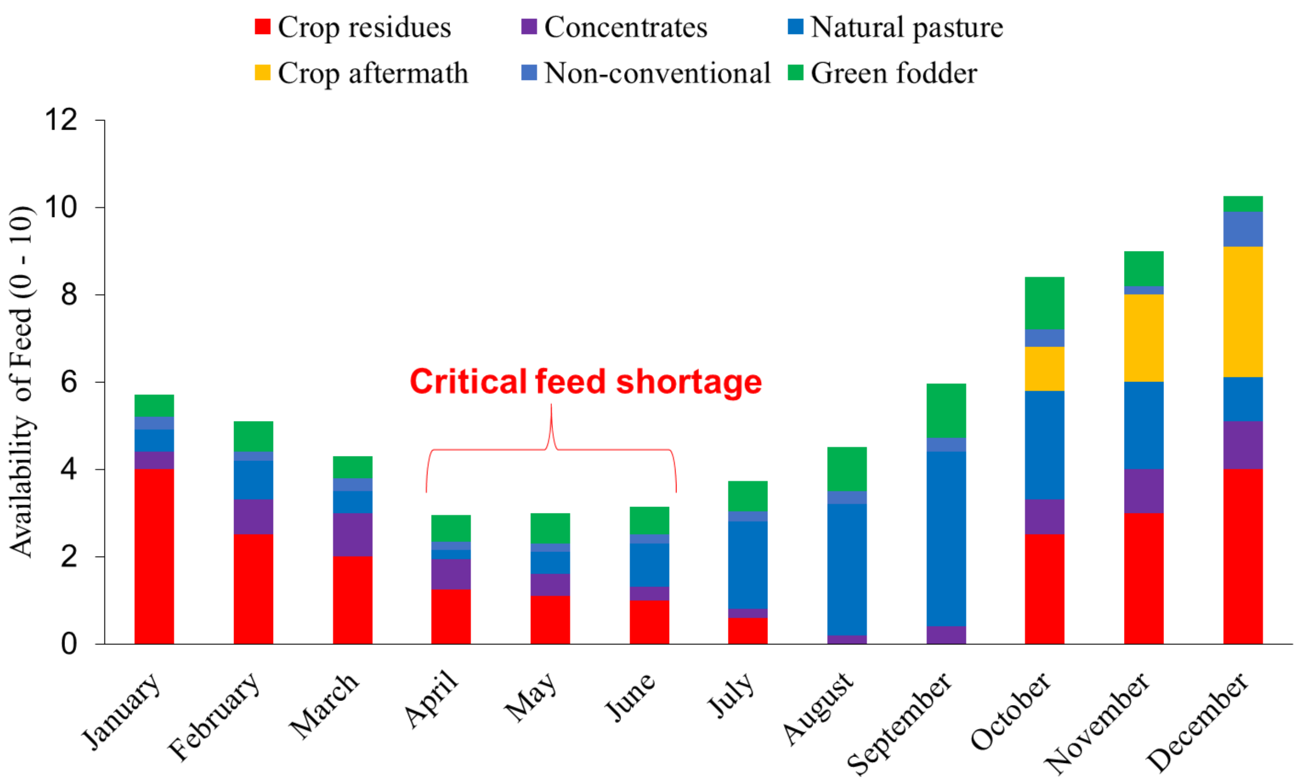

Figure 2.3 Seasonal availability of the feed resources

2.3.3 Mineral profile of the feedstuffs

The concentration of macro minerals has highly significant variation among the feedstuffs ($p < 0.001$) except for sodium (Table 2.2). The highest concentration of Ca (6.55 g/kg) in herbage lowest is in Cotton seed cake (0.79 g/kg). The micro minerals (Fe, Mn, Zn) has high concentration in herbage, agro industrial byproduct than crop residues even though they are not significant while the reverse was noted for Cu, Co and Ni. Cobalt and Copper quantity were recorded consistently low in almost all the collected feedstuffs. Distribution of iron was found to be unique in the sense that it exceeded the requirement in all the feedstuffs, being fed to livestock; even straw samples were quite rich in Fe (484 mg/kg).

2.3.4 In vitro digestibility and fermentation characteristics of the feedstuffs

The range of IVOMD was from 48–81% g/kg DM while for ME from 2.81-8.96 MJ/Kg even though they have no significant (Table 2.1 and Figure 2.4). Tef straw has better digestibility compared with wheat (45%), and oats (48%) straws. Pulses have medium to high digestibility ranging from 34-56%. Even though green fodder has high CP but it has low gas production. Anti-nutritional factors of green fodder (e.g Tannin conc. was 37.6 (11.1 to 141.3) g/kg DM. The lowest VFA production was with natural pasture and crop residues (mmol VFA/g dry matter) while higher for the agro industrial byproduct such as cotton seed cake among the feed types.

Table 2.1 Chemical compositions of the feedstuffs.

Feed Type	CP	NDF	ADF	ADL	Ash	GE	IVOMD	ME	NEm	NEl	TVA
Natural Pasture											
Hay	66.58	694.7.9	398.46	128.17	80.23	14.05	51.32	8.04	6.01	5.31	41.45
Herbage	69.4	812.12	319.23	87.27	98.13	15.06	60.71	8.02	6.02	5.63	47.21
Crop Residues											
Eragrostis tef	43.25	720.8.1	394.47	59.39	69.15	17.07	48.17	7.03	4.12	4.31	57.93
Hordeum vulgare	41.16	699.75	393.22	111.75	47.05	17.05	41.73	6.53	4.24	4.12	51.34
Triticum aestivum	39.29	730.15	498.41	86.35	83.31	17.08	48.17	7.03	4.12	4.31	49.79
Eleusine coracana	56.13	651.95	381.31	39.11	51.17	13.03	55.23	8.31	5.34	5.01	85.12
Zea mays	28.13	799.82	513.52	270.44	49.10	18.02	35.76	5.81	6.53	3.01	81.03
Vicia faba	74.39	581.61	508.61	164.46	64.11	25.09	54.13	6.41	7.23	4.23	89.72
Cicer arietinum	34.19	536.56	331.33	103.29	62.12	17.11	39.83	7.43	6.67	4.12	90.95
Arachis hypogea.	58.25	705.72	568.46	143.23	59.17	17.08	46.35	9.51	6.03	5.02	87.98
Phaseolus vulgaris	63.27	391.38	328.25	83.14	174.21	14.04	52.43	9.60	6.66	6.33	117.00
Guizotia abyssinica	259.84	245.23	198.18	104.23	95.14	18.03	57.12	8.16	7.36	5.37	98.00
Avena abyssinica	65.25	457.21	242.27	52.25	220.29	14.06	61.43	7.66	6.06	6.33	76.00
Improved forages											
Pennisetum purpureum	127.88	423.12	323.52	55.30	148.64	13.14	54.12	7.26	4.23	3.42	91.40
Brachiaria hybrids	163.29	446.56	203.35	100.66	164.89	15.08	58.28	8.03	5.55	4.14	89.69
Pennisetum pedicellatum	73.13	649.59	316.23	40.03	118.92	15.07	68.64	9.08	7.21	6.54	110.15
Chloris gayana	71.26	649.49	332.72	129.47	151.86	16.09	70.17	10.07	7.33	6.61	89.98

***Green fodder*[1]**											
Sesbania sesban	232.81	364.35	241.63	232.65	77.39	19.04	62.24	8.32	6.45	5.52	79.72
Ficus sur	108.82	447.45	312.74	173.93	184.59	13.72	73.41	7.82	5.41	6.27	92.15
Acacia abyssinica	217.02	423.55	325.24	165.96	88.95	17.22	56.24	8.67	6.15	6.21	84.98
Ficus thonningii	106.49	484.38	369.84	188.31	123.54	12.03	79.36	6.28	4.02	4.02	
Vernonia amygdalina	135.74	316.44	292.25	143.61	84.64	27.17	58.24	6.7	7.15	6.21	94.80
***Weed under crop*[2]**											
Weed	176.17	216.58	316.34	239.51	134.43	17.08	55.41	8.42	6.25	5.12	98.4
Concentrates											
Noug Seed Cake	181.22	367.59	218.48	58.22	97.13	21.03	60.27	8.36	5.13	4.03	100.90
Wheat bran	149.87	394.75	105.29	31.15	32.07	17.04	53.15	7. 25	5.41	4.13	85.82
cottonseed oil cake	232.69	361.87	308.49	152.37	42.10	18.08	65.53	6. 45	6.45	4.41	113.99
Soya bean Cake	276.44	389.89	162.26	63.10	72.17	18.01	73.87	7. 24	5.41	5.11	145.07
Sesame oil cake	246.89	379.80	276.434	103.63	75.37	18.04	81.46	8.37	6.23	5.47	112.81
Formulated Concentrate	152.52	366.48	116.32	36.69	132.64	15.07	76.31	9.15	6.15	5.12	110.52
Non-conventional feeds											
Atela[3]	161.54	400.31	112.34	44.13	15.08	14.07	59.34	8.24	6.24	5.58	88.70
Brint[4]	170.67	301.54	130.33	125.56	44.04	18.09	63.37	9.13	6.34	5.22	91.20
P value	*0.002*	*0.556*	*0.001*	*0.246*	*0.173*	*0.257*	0.023	0.123	0.089	0.082	0.041

CP; crude protein; NDF, neutral detergent fiber; ADF, neutral detergent fiber; ADL, neutral detergent fiber; GE, gross energy; IVODM, in vitro Organic Matter Digestibility; ME, Metabolisable Energy; NEm, net energy for maintenance; NEl, net energy for lactation; [1]Green fodder, selected trees and shrubs based on their availability; [2]Weed under maize consists of grasses with an interspersion of legumes and other forbs (non-grass herbaceous plants); [3]Atela, local brewery by-product; [4]Brint, distillery by-products; SEM, standard error of the means.

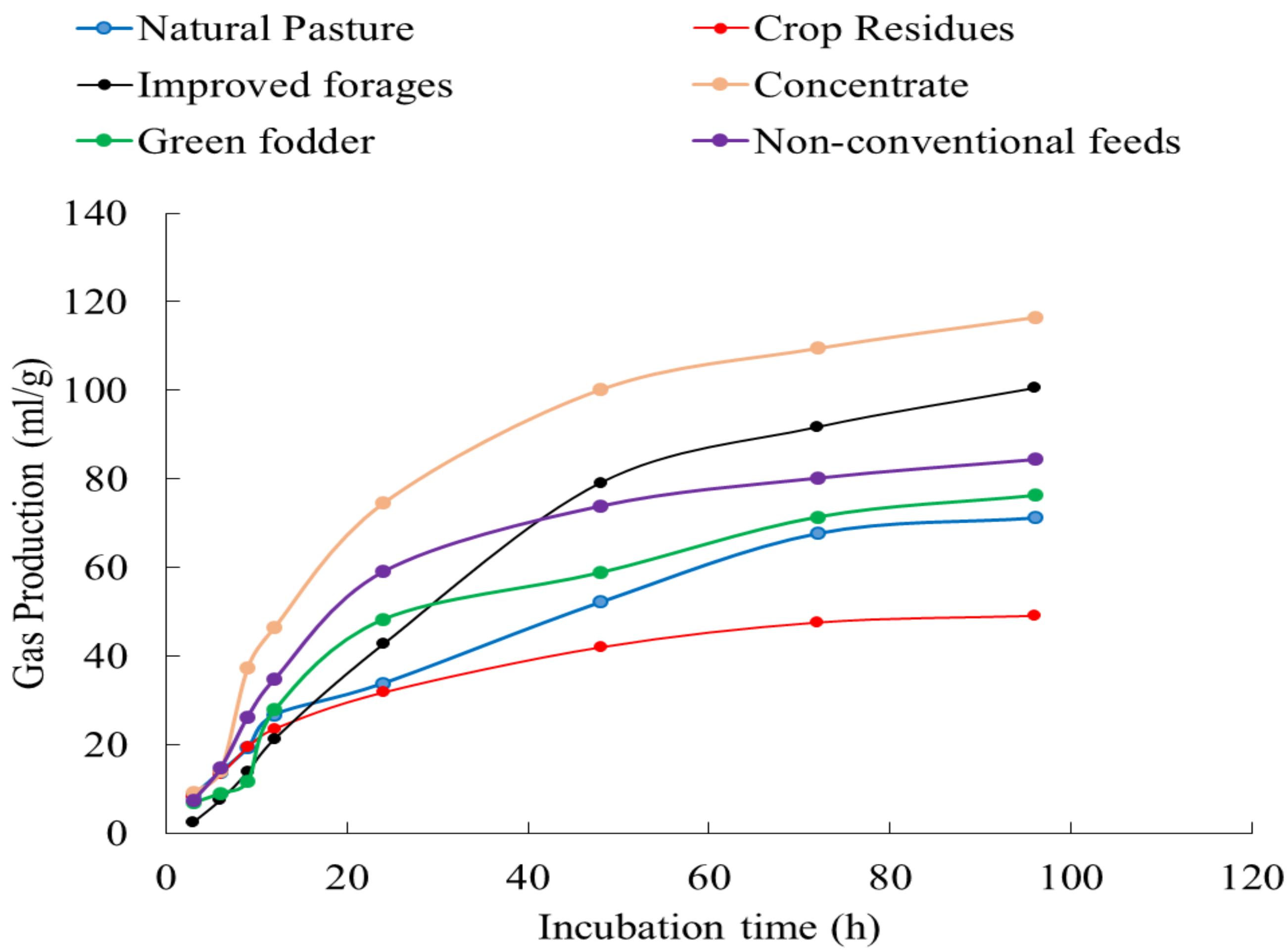

Figure 2.4 In vitro digestibility of the feed types

Table 2.2 Mineral content of feedstuffs

	Macro-mineral (g/ kg DM)						Micro-mineral (mg/kg DM)					
Feed types	Ca	Mg	P	K	S	Na	Fe	Mn	Zn	Se	Cu	Co
Hay	2.89	8.41	3.51	19.97	3.01	0.84	3268.91	939.20	274.40	0.01	27.11	0.81
Herbage	4.60	10.86	7.28	44.35	5.50	0.46	11630.77	628.42	298.48	0.01	23.91	7.43
Eragrostis tef	1.36	3.13	3.19	20.18	3.97	0.46	2504.13	292.55	200.11	0.01	3.79	1.06
Hordeum vulgare	2.11	2.18	1.34	16.40	2.69	0.22	547.28	76.63	157.26	10.52	32.43	4.42
Triticum aestivum	1.40	2.24	1.90	39.62	3.60	0.71	1043.72	250.29	214.77	0.01	1.85	0.01
Eleusine coracana	3.79	5.32	4.58	45.89	6.28	0.53	2636.67	1365.2	270.87	0.01	9.60	0.01
Zea mays	2.00	6.31	1.16	41.58	1.44	0.53	678.61	201.93	205.97	0.01	1.44	0.01
Vicia faba	2.59	4.70	1.68	32.41	3.01	2.39	2171.63	146.01	206.15	0.01	19.78	0.17
Cicer arietinum	4.38	2.71	0.77	24.50	1.72	0.10	861.03	37.96	178.30	7.92	31.98	2.69
Arachis hypogea.	2.53	6.17	1.88	29.86	1.93	0.91	1038.28	67.79	173.88	0.01	0.01	0.01
Phaseolus vulgaris	3.95	19.86	1.26	33.96	4.35	0.11	27599.00	382.27	211.61	3.78	69.04	24.29
Guizotia abyssinica	2.95	11.12	11.04	28.80	6.17	0.82	777.65	224.81	657.90	6.48	97.75	6.72
Avena abyssinica	2.03	4.67	5.31	17.04	2.57	0.34	11103.27	385.39	170.81	4.64	38.99	9.34
Pennisetum purpureum	2.52	8.21	4.68	46.79	2.74	0.26	1851.83	165.22	205.71	1.59	15.64	0.95
Brachiaria hybrids	3.34	11.49	3.92	49.41	4.15	0.25	11792.39	494.17	250.08	4.78	48.25	7.96
Pennisetum pedicellatum	2.90	8.20	2.10	37.17	3.04	0.23	5961.10	204.51	254.34	6.30	72.81	5.27
Chloris gayana	3.37	5.17	5.05	37.46	5.62	0.34	2808.48	350.10	392.41	3.77	58.57	2.98
Sesbania sesban	5.01	3.76	3.75	22.00	3.58	1.03	766.18	209.47	162.70	0.01	9.28	0.01
Ficus sur	8.73	13.51	4.41	29.85	2.56	0.23	673.87	79.30	602.82	1.85	12.42	0.28

Acacia abyssinica	5.57	6.98	6.00	38.39	4.88	0.13	813.81	72.38	206.73	1.56	16.54	0.55
Ficus thonningii	8.21	8.99	3.92	32.37	2.45	0.21	743.18	68.63	170.93	1.17	17.49	0.46
Vernonia amygdalina	4.69	9.37	4.73	34.28	4.75	0.24	1148.14	384.15	214.47	0.01	47.53	0.01
Weed under maize[8]	5.15	12.59	9.28	79.37	12.17	0.36	1095.70	209.59	286.91	0.01	35.20	0.01
Noug Seed Cake	3.00	12.83	13.35	27.08	7.76	0.17	1122.54	177.04	234.19	0.01	34.02	0.01
Wheat bran	1.12	10.19	22.48	29.63	4.21	0.28	147.89	347.43	245.20	0.01	22.44	0.01
Cottonseed oil cake	0.79	8.90	12.40	27.97	5.80	0.27	188.57	13.50	343.15	0.01	11.36	0.01
Soya bean Cake	1.79	8.01	10.98	31.91	6.45	0.23	2963.91	122.28	324.55	0.01	34.44	0.01
Sesame oil cake	5.36	14.90	22.40	32.39	10.44	0.21	1207.18	114.93	288.49	0.01	66.24	0.01
Formulated Concentrate	4.71	6.64	6.02	23.82	4.52	5.07	1939.95	223.83	545.29	0.01	18.26	4.69
Atela	0.91	3.21	4.19	5.46	4.13	0.48	1336.98	82.53	213.22	0.01	5.34	1.70
Brint	1.55	3.96	5.22	8.49	3.12	0.82	2940.14	160.83	237.28	0.01	23.05	6.75
P value	0.003	0.012	0.004	0.033	0.003	0.409	0.789	0.223	0.753	0.024	0.084	0.035

Ca, calcium; Mg, magnesium; P, phosphorus; K, potassium; S, Sulphur; Na, sodium; Fe, iron; Mn, manganese; Zn, zinc; Se, selenium; Cu, copper; Co, cobalt.

2.3.5 Identification and farmers' preferences regarding indigenous fodder species

Overall, 129 indigenous trees and shrubs were identified across the three study sites (Appendix Table 1). According to the farmers' interviews, 22 of the indigenous trees and shrubs were unpalatable to ruminant livestock and 40 were poorly or rarely palatable, so they excluded from further chemical analysis. From the 67 fodder species that were moderately or highly palatable, 37 potential indigenous fodder species (15 species from each site) were selected based on farmers' preference-ranking order and their use as feed for ruminant livestock (Appendix Table 2). During the final stage of the selection procedure (Step 5 in Figure 2.1), which was based on farmers' preference-ranking scores and plant nutritive requirements (CP > 110 g/kg, NDF < 350 g/kg, and CT < 40 g/kg), the top 5 IFTS at each site *A. schimperiana, C. acrostachyus, D. abyssinica, F. sur, and F. vasta for Dibatie; A. abyssinica, D. angustifolia, F. sur, P. schimperi, and T. catappa for Aba Gerima; and D. penninervium, E. abvssinica, E. schimperi, G. gnemon,* and *R. apetalus* for Guder were selected as supplementary feeds for ruminant livestock. In addition, the key informants stated that species of ruminant livestock preferred to eat different parts of these fodder species. The farmers' preference was based primarily on a plant's palatability and its availability. The farmers' preference-ranking scores among the various fodder species ranged from 1.21 to 3.56 (Dibatie), 1.27 to 3.68 (Aba Gerima), and 1.4 to 3.8 (Guder) (Table 2.3).

2.3.6 Relative abundance of selected indigenous fodder trees and shrubs

The distribution of the selected potential fodder species revealed variations in abundance even within a site Table 2.3). The most abundant species were *C. macrostachyus* (16.0%), *O. abyssinica* (11.5%) and *C. spinarum* (15.0%) in Dibatie; *Calpurnia aurea* (42.3%), *Caparis tomentosa* (23.1%), and C. africana (11.5%) in Aba Gerima; and Maesa lanceolata (7.6%), Discopodium penninervium (6.3%), and Yushania alpina (5.8%) in Guder (Table 2.3). For most of the selected IFTS, relative abundance corresponded with the farmers' preference, except for Ziziphus spina-christi in Dibatie, which had the highest preference score (3.5) but a low relative abundance (4.9%), and C. aurea in Aba Gerima, which had the lowest ranking score (1.2) but highest relative abundance (42.3%).

Table 2.3 Ranking score and relative abundance of indigenous fodder trees and shrubs of most preferred by farmers at each study site.

Dibatie			**Aba Gerima**			**Guder**		
IFTS	**RS**	**RA**	**IFTS**	**RS**	**RA**	**IFTS**	**RS**	**RA**
Albizia schimperiana	2.4	3.8	*Acacia abyssinica*	2.7	3.8	*Buddleja polystachya*	3.4	5.0
Carissa spinarum	3.3	15.0	*Calpurnia aurea*	1.2	42.3	*Discopodium penninervium*	3.5	6.3
Clutia lanceolata	1.5	10.0	*Caparis tomentosa*	1.5	23.1	*Dombeya torrida*	3.2	4.3
Cordea africana	2.5	7.5	*Cordea africana*	3.2	11.5	*Embelia schimperi*	3.8	5.4
Croton macrostachyus	1.3	16.0	*Dodonaea angustifolia*	2.3	7.7	*Ensete ventricosum*	2.9	4.2
Diospyros abyssinica	2.2	6.50	*Ficus sur*	2.4	6.2	*Ervthrina abvssinica*	2.8	5.4
Ensete ventricosum	1.2	8.5	*Ficus sycomorus*	3.4	8.5	*Ficus sur*	2.3	3.7
Ficus sur	2.7	8.8	*Ficus thonningii*	3.2	4.6	*Gnetum gnemon*	3.8	1.7
Ficus thonningii	3.1	3.9	*Ficus vasta*	3.6	8.5	*Maesa lanceolata*	2.6	7.6
Ficus vasta	2.1	7.2	*Grewia villosa*	1.8	11.5	*Polyscias fulva*	1.4	3.8
Grewia villosa	1.9	5.0	*Millettia ferruginea*	2.7	6.2	*Rubus apetalus*	2.1	4.6
Oxytenanthera abyssinica	1.4	11.5	*Mimusops kummel*	2.2	4.6	*Rytigynia neglecta*	1.9	2.3
Ximenia caffra	1.7	1.7	*Premna schimperi*	2.5	3.6	*Urera hypselodendron*	3.6	1.3
Xylopia aethiopica	2.97	10.0	*Terminalia catappa*	3.1	8.5	*Vernonia amygdalina*	3.2	3.3
Ziziphus spina-christi	3.56	4.93	*Vernonia amygdalina*	3.0	5.4	*Yushania alpina*	1.6	5.8

IFTS, indigenous fodder trees and shrubs; RA, relative abundance (%); Ranking score.

2.3.7 Correlation and complementarity of farmers' preference with nutritive value

Complementarities between the farmers' preference ranking score and nutritive values (specifically CP) of the 37 selected IFTS are shown in (Appendix Figure 1). Farmers in Dibatie and Guder used their preferences and acquired knowledge to effectively discriminate IFTS species (except for Ziziphus spina-christi in Dibatie) that had high CP from those with low CP. In contrast, in Aba Gerima, farmers' preference ranking was unrelated to CP and could not be used to discriminate individual IFTS (Appendix Figure 1). The combined regression equations for the relationship of farmers ranking score (Y) and CP (X) was: Y= 0.0169x+0.314 (R^2 = 0.58) (Figure 2.5). Farmers' preference-ranking scores were positively correlated with CP, OMD, ME, and TVFA (Table 2.4).

Table 2.4 Rank correlations of farmers' preference score with nutritive value of fodder species

Site	CP	NDF	ADF	ADL	Ash	CT	GE	OMD	EDDM	ME	TVFA
Dibatie	0.618**	–0.292*	–0.121	–0.160	0.053	–0.142	0.142	0.303*	0.238	0.254*	0.272*
Aba Gerima	0.161	–0.270	–0.164	–0.117	0.157	-0.127	0.164	0.174	0.123	0.163	0.145
Guder	0.386*	–0.339*	–0.175	–0.260	0.128	–0.155	0.137	0.318*	0.396*	0.285*	0.603**
Overall	0.61**	–0.392*	–0.121	–0.160	0.053	–0.142	0.142	0.303*	0.238	0.254*	0.272*

Abbreviations are defined in Table 2.1. Means followed by the same letter in the column do not differ (P > 0.05) statistically.

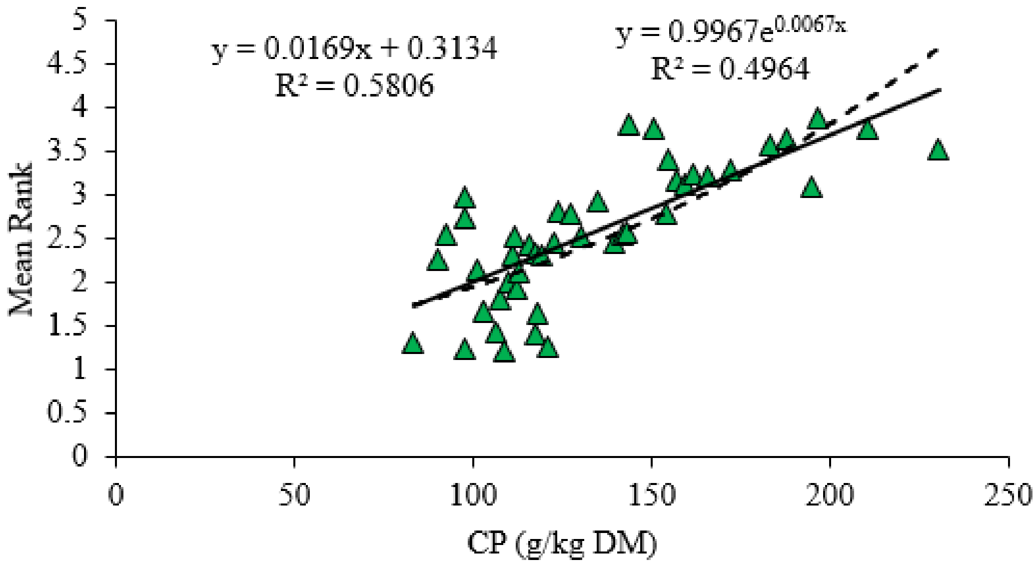

Figure 2.5 A linear and quadratic regression line depicting the relationship of farmers ranking score and crude protein.

2.4 Discussion

2.4.1 Diversification of feed resources and seasonal availability

The diversity of the feed resource base is wide across the agro-ecologies in the study sites. The type of feed resource in this study was in agreement with previous findings (Duguma et al., 2017) who reported that natural pasture was the main feed resource that smallholder dairy farmers used. Livestock in most regions of Ethiopia predominantly derive their feed from grazing on natural pastures (Duguma and Janssens, 2016). (Assefa et al., 2014) also reported that natural pastures which provide more than 90% of the livestock feed are generally very poorly managed. Crop residues are one of the major feed resources for livestock the study sites. The dominant crops in this study are in line with mentioned by Ebabu et al. (2020). In agreement with this study, Bogale et al. (2008b) also reported that crop residues were the main feed source for livestock during the dry period in 81.4% of the cases from early January to April when pasture from grazing area decrease to provide reasonable quantity of feed for animal (Mengistu et al., 2017a). Although, crop residues are the sole feed resource in the dry season, most of the farmers provide straw to their animals without any physical or chemical treatment such as chopping and application of urea. In this study, concentrate and agro-industrial by-products were mainly specific to Aba Gerima site since it is located near the regional city, this is supported by Mengistu *et al.* (2017a) that agro-industrial by-products were used by urban and peri urban livestock production system. The improved forages reported in this study is in agreement with Mutimura et al. (2015) that included Napier grass as the major feed resource in Uganda dairy production system and Talore (2015) that Napier (*Pennisetum purpureum*), *Brachiari*a brizantha and labab (*Lablab purpureus L. Sweet*) were some of the improved forages widely cultivated in the districts of the southern Ethiopia. Even though, the cultivated forages have been introduced very recently, although their adoption is limited due to land shortage (Gizaw et al., 2017). This study also revealed potentially valuable non-conventional feed resources such as brewery and distillery by products appreciable quantities year round which can be integrated into the ration formulation system (Negesse *et al.*, 2009).

Frequently livestock are exposed to seasonal feed shortages both in quantity and quality, especially during the dry season (Nardoneet al., 2010). In this study, the severe shortage of livestock feed was in dry season. During these critical feed shortage periods, conserved (hay and crop residues) and purchased feed were used preferentially for feeding lactating cows and oxen used for cropland preparation (Jimma et al., 2016). Nyaata et al. (2000) also reported that February and September were reported by the majority of farmers to be the most critical months in terms of fodder shortage while April, May, June and November have least fodder problems. In this study, the annual availability of feed resources in natural pasture correlated positively with rainfall pattern and increased from June to October in the rainy season but declined as the dry season approaches (Melesse et al., 2013). Similarly, (Fernandez-Rivera et al., 2005) reported that seasonal fluctuation in quantity supply has been associated with low rainfall and poor soil fertility in the Sahel. The major livestock production constraints in the study were in line with (Abioye and Adegoke, 2016) that reported inadequate quantity and quality feed throughout the year ranked next to shortage of water.

2.4.2 Nutritive value of available feedstuffs

The CP content of the feedstuffs of except natural pasture and crop residues was above the critical value (70 g/kg of CP) for ruminant animals for optimum activity of rumen microorganisms (NRC, 2001). The poor nutritional quality of natural pasture has been deteriorated due to recurrent droughts, continuous over-grazing and lack of range improvement interventions (Mengistu and Asfaw, 2016). In particular, the lowest CP content of Zea mays (28.13 g/kg DM) in this study was in agreement with the recovered from smallholder dairy feed in Rwanda (Mutimura *et al.*, 2015); this low value might be attributed to age of the plants that were harvested by farmers at maturity level. The report of Tegegne and Assefa (2010) supported the finding of this study that crop residues being extremely mature products, there is a need for quality improvement. As a result, the poor quality of natural pasture and crop residue feed need to supplement them with concentrate (Amole and Ayantunde, 2016). In contrast, the minimum requirements for lactation (120 g CP/ kg DM) and growth (113 g CP /kg DM) in cattle (ARC, 1984) suggesting that the agroindustry byproducts, green fodder and non–conventional feedstuffs were almost satisfying the recommended value. The NDF value of the feedstuffs in this study is comparable with report of most tropical grasses (Tessema and Baars, 2004) except for the natural pasture (hay, 694±7.9 g/kg; herbage, 812±12.5 g/kg) and few crop residues (Eragrostis tef, 720±8.1 g/kg; Hordeum vulgare, 699±7.5 g/kg; Triticum aestivum 730±11.5 g/kg; Eleusine coracana, 651±9.5; Zea mays, 799±8.2 g/kg and Arachis hypogea, 705±7.2 g/kg). On contrary, the low fiber content of Sesbania sesban might have contributed to the increasing IVDMD that can be supplement for low quality feed; Tessema and Baars (2004) also reported that supplementing sesbania sesban increased efficiency of microbial N supply to low quality basal diet. Generally, the wide variation in chemical composition of the different feeds offers users flexibility in formulating rations according to the productive performance of the dairy cow. These feeds showed less values for the extent and degradation rates in the rumen that inhibits voluntary feed intake and microbial activity, resulting in poor digestibility (Bruno-Soares et al., 2000).

2.4.3 Minerals concentration in relation to dairy cow requirements

The concentration of calcium, magnesium, potassium and phosphorus was sufficiently high to meet the requirements of dairy animals, while sodium concentration was low. In agreement with this study, Khan et al. (2009) reported that pasture grasses/ forages have sufficient levels of K, Ca, Mg, Mn, Fe and Zn to meet the requirements of ruminants. The Ca contents were very high compared to the recommended requirement of 4.3 g/kg in DM for cattle, whereas the P content was almost above the minimum requirement of 1.7 g/kg in DM for grazing ruminants whereas in the mid altitude the Ca concentration in the analyzed feed was below the requirement of dairy animals (NRC, 2001). Almost similar result was obtained in other parts of the world where Ca contents of the pasture ranged from 1.8 to 9.8 g/kg ((Aregheore, 2007); Reshi et al., 2013). In this study, the Ca concentrations of natural pasture fulfill Ca requirement of dairy cows whereas the agro-industrial byproducts, hays or crop residues are low in calcium. On the other hand, the agro-industrial byproducts contain more phosphorus than calcium (Adugna, 2008). This implies cows might lead to calcium-deficiency. Feeding a calcium-deficient diet may delay uterine involution. Moreover, the phosphorus intakes along with low calcium intakes also depress fertility (Funston, 2007). While dietary Ca concentrations of 2-6 g/kg, with higher requirements for lactation have been variously recommended for cattle (NRC, 1978). In the current study, the Mg concentrations of natural pasture were fulfil the requirement of dairy cows (NRC, 2001). The variation of Mg concentration in forage might be due to variation in forage species, climatic factors, soil types, seasons and rain fall pattern (Endale et al., 2015). In contrast to this study, (Aregheore, 2007)reported 13.2 g/kg K concentration for tropical forages. Most the feed examined in this study were marginal to sufficient in P. From the present study, it is concluded that big variation in nutrient supply and imbalances in the control diet/ home-mixed concentrates resulting in an apparently variation in economic viabilities across the production subsystems (Assaminew, 2014). The current study shows that the concentration of Sodium (Na) in natural pasture were below the requirement of dairy animals. To compensate this deficiency, some more obvious alternative feed supplement rich in Na would be salt that can be added to the diet with an advised amount of 20 g/day

(Neckermann and Kechero, 2017). Thus, traditional common salt supplementation practice of farmers for the animals should be encouraged (Yadessa, 2015).

The Fe concentration of natural pasture in this study were above the requirement of dairy animals. The concentration of Zn in the analyzed natural pasture were above the requirement of dairy animals. McDowell et al (1978) considered 30 mg/kg to be a critical level of dietary Zn, although the ARC (1980) has suggested that concentrations of 12-20 mg/kg are adequate for growing cattle. The concentrations of Cu in natural pasture in all locations were below the requirement of dairy cattle (10mg) as recommended by (NRC, 2001). In this study Cu concertation was a marginal to deficient supply for the dietary requirement of cattle lies in the range 8-14 mg/kg (NRC, 1984). This situation may be even further complicated by high levels of dietary Fe which can be elevated by soil ingestion during grazing. Any feeding system based on those feeds should take into account minerals that are lacking and hence should go for additional supplementation to alleviate the deficiency (Tsegahun et al., 2006).

2.4.4 In vitro digestibility, degradability and fermentation characteristics of feedstuffs

In this study the digestibility of natural pasture and crop residues is low and varies with the crop type from which the residue is produced whereas their fiber content was high enough to limit feed intake. Thus, the crop residues require some degree of supplementation or treatment to support optimum animal performance (Bogale et al., 2008a). This might be due to the nutritional quality of crop residues is influenced by several factors such as morphological fractions of the residue and varietal difference of the crop (Feyissa et al., 2007). A feed containing more than 700 g/kg DM digestibility is defined as high quality (Tessema and Baars, 2004) suggesting that concentrate and improved forages in this study were above the minimum recommendation for DM digestibility. Similarly, Assefa et al. (2016) reported that the oilseed cakes such as noug cake can be used as sources of rapidly degradable N source while cottonseed cake can be used as sources of escape N. The ME values of the feedstuffs were within the ranges reported by (Menke and Steingass, 1988), where the ME values of various European feeds ranged from 4.5 to 15 MJ kg−1 DM. The IVOMD values for soybean meal were higher than those reported by (Mabjeesh et al., 2000) which could be due to differences in the chemical composition of the feeds or in methods of removing attached microbial biomass.(Getachew *et al.*, 2004). The result of some crude residues in agreement with previous studies that Faba bean straw has 46.9 g/kg organic matter digestibility (Alkhtib et al., 2016).

The in vitro OMD values for some of the fodder species that we evaluated were within ranges reported for other multipurpose tree species in northern Ethiopia (Melaku et al., 2010). The ME values of *D. penninervium, M. lanceolate, U. hypselodendron, F. sur,* and *V. amygdalina* are higher than those in other selected IFTS that we studied and may be due to the high digestibility of their cell wall carbohydrates, as indicated by their very low ADL contents. Furthermore, the total VFA contents that we obtained are in line with other reports involving tropical fodder species, and the high average value (124.7 mmol/L) in our study indicates better substrate utilization by ruminal microbiota for the support of ruminant livestock (Mengistu *et al.*, 2017b).

2.4.5 The correlation between farmers' preferences and the nutritive value of fodder species

In our study, farmers' preferences regarding fodders were good indicators of the relative quality of IFTS for ruminant livestock. This result is in agreement with those of (Roothaert and Franzel, 2001) in Kenya and (Mekoya *et al.,* 2008) in Ethiopia, who likewise reported that farmers' appreciation of fodder species reflected their nutritive value. By extension, the complementarity pairwise comparisons in our study showed that farmers effectively discriminated between IFTS species with low protein content as compared with high content. This finding is in line with (Mekoya *et al.*, 2008) and (Talore, 2015), who reported significant complementarity between farmers' assessment of fodder quality and laboratory data. The strong relationship between farmers' preferences and the nutritive value (particularly for CP, OMD, ME, and VFA) of IFTS (except in Aba Gerima) confirms that farmers, through their accumulated experiential knowledge, effectively select good-quality fodder species for supplementing their ruminant livestock.

2.5 Conclusions

Natural pasture and crop residue are poor quality due to their low nutritive value (low protein and high fiber components) hence their digestibility is low to fulfil the maintenance requirement of the animal. On the other hand, agro-industrial byproducts and green fodders have high CP value (> 100 g/kg DM) with better digestibility can be used as feed supplementation, particularly during the dry season. Traditional brewery and distillery by-products (Atella and Birint) had medium NDF, medium level of CP, and moderate level of OM digestibility, ME, SCFA concentration implies that they can also use for supplementation. Most of the feedstuffs rich in Ca, Mg, K, P and Fe content but poor in Na, Co and Cu. Therefore, it is necessary to supplement these minerals in the diet by providing mineral mixture having better bio-available mineral salts. In addition, from the inventory of fodder species, 107 IFTS species were identified among three study sites, of which 37 species were considered as good-quality fodder in light of farmers' preferences and CP values exceeding 80 g/kg. Moreover, using nutritive values and considering farmers' preference ranking, we selected the top 14 IFTS species as potential supplemental ruminant livestock feeds in these areas. Extension bodies, policymakers, and farmers should be aware of the importance of these IFTS, and they should be included in the feeding formulation as supplemental diet for ruminant livestock feed. Our study revealed a large reserve of IFTS species that could be used to supplement poor-quality roughage in ruminant livestock feed for tropical regions. Future research is needed to evaluate animal performance, agronomy practices, and biomass production associated with these selected species, as well as mechanisms for preventing toxicity due to consumption of P. africana. Moreover, to use the huge resource of fodder species identified the mitigating mechanism of anti-nutritional factors should be studied.

Chapter 3:

Improve feed quality through mitigating anti-nutritional factors

This chapter is largely adopted from published and submitted Manuscript as:

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3.1 Introduction

Tropical browse species are well adapted to harsh environments which are resistant to variations in temperature and rainfall (Dentinho et al., 2018) and produce considerable good-quality foliage (Mengistu *et al.*, 2017b). Most tropical browse species contain reasonably high crude protein (CP) and low fiber contents (Rubanza et al., 2005; Gemeda and Hassen, 2015) and they can be considered as supplemental feeds for poor-quality roughage for ruminants animals (Osuga et al., 2008). However, the utilization of browse species as supplemental livestock feeds is limited by their high contents of polyphenolic compounds (Rubanza *et al.*, 2005). Moreover, *in vivo* (Ørskov et al., 1978; Salem et al., 2006) and in vitro (Salem et al., 2005) studies suggest that, due to the tannin effect, the rumen-degradable nitrogen supply from browse species is often insufficient to meet the requirements of rumen microbes and support acceptable animal performance (Salem et al., 2007). In addition, when present in high concentrations in livestock diets, tannins reduce intake and digestibility (Schofield et al., 2001) and exert toxic effects on the intestinal mucosa (Reed, 1995; Frutos et al., 2004). Furthermore, a recent report showed that even at low concentrations, supplementation with an extract rich in condensed tannin (CT) markedly decreased in vivo nutrient digestibility in sheep (Gerlach et al., 2018).

The anti-nutritional effects of polyphenolic compounds in browse species can be mitigated through biochemical treatment like polyethylene glycol (PEG) prior to feeding, which enhances the nutrient availability from tannin-rich feeds (Makkar, 2003; Brown and Ng'ambi, 2017; Besharati and Ghezeljeh, 2017). PEG is a polymer that binds irreversibly to tannins, thus preventing tannin–protein complexation (Dentinho *et al.* (2018). Consequently, PEG has been included as an additive in high-tannin feeds to improve voluntary feed intake, digestibility, and animal performance (Brown and Ng'ambi, 2017). Even though the results were inconsistent; previous studies reported that the addition of PEG to browse species contain tannin increases in vitro digestibility and gas production (Dentinho *et al.*, 2018).

However, only scant research has addressed the extent to which PEG reduces the anti-nutritional effects of fodder plants which expected to vary depending on the season and species, on the in vitro digestibility, degradability, metabolizable energy (ME), volatile fatty acid (VFA) and methane (CH_4) production (Basha et al., 2013). In addition, the tannin-mitigating effects of PEG treatment depend on the nature of the tannin and the feed type (Hernández et al., 2015), because different sources of tannin act differently (Gerlach *et al.*, 2018), and on the interaction of both PEG and tannins with other nutrients (Yisehak et al., 2014). So, knowing how season and species interact to influence the effectiveness of PEG treatment for decreasing the anti-nutritional effects of browse species is very important in terms of their efficient utilization as supplemental livestock feeds (Parissi et al., 2018), because the species availability differs markedly between the two seasons of Ethiopia (Gebrekirstos et al., 2008). Moreover, from Chapter 2, we observed that fodder species have high CP content but low in vitro gas production (Figure 2.4). This might be due the effect of anti-nutritional factors that hinder the potential of the digestibility and degradability of fodder species. To this end, we evaluated the influence of PEG treatment on the anti-nutritional effects of polyphenols in browse species harvested during different seasons on in vitro digestibility, rumen degradation, metabolizable energy, volatile fatty acid production and methane emission.

3.2 Materials and methods

3.2.1 Fodder species sample collection and preparation

From the list of important fodder species in Chapter 2, ten species having CP> 112 g/kg where used for this study and the samples of browse species leaves and twigs were collected from three sites namely: Guder, Aba Gerima and Dibatie in north western part of Ethiopia. The browse species chosen were those used predominantly as livestock feed, according to reports from herdsmen and knowledgeable community members (personal communication). Sampled species comprised *of A. nilotica*, *C. adonsonia*, *D. torrida*, *E. capensis*, *E. ventricosum*, *E. brucei*, *M. lanceolate*, *S. sesban*, *S. kunthianum*, and *T. laxiflora*. Mature plants samples were harvested during the wet season (September through

October 2017) and dry season (February through March 2018) (Asfaw et al., 2018). For each species, leaves and twinges from 3-5 randomly selected tree and shrubs were collected to form representative samples. Samples were air dried in the shade to minimize changes in tannin content then dried at 65°c for 24 h in a forced air oven to constant weight and ground in a hammer mill to pass through a 1-mm sieve and stored in plastic bags for subsequent determination of chemical components, secondary compounds and in vitro fermentation characteristics. All chemical composition and incubations were completed at the laboratory of Animal Nutrition, Faculty of Environmental and Life Sciences, Shimane University, Japan.

3.2.2 Extraction of polyphenols

For the chemical composition analysis see section 2.2.4. Extraction of total phenolic content (TEP) followed a previously described procedure (Getachew et al., 2000; Makkar, 2000). Total extractable tannin (TET) was estimated gravimetrically as the phenolic compounds remaining after the binding of tannins by using polyvinyl polypyrrolidone (Makkar, 2003). Concentrations of TEP and TET were expressed in tannic acid equivalents. Absorbance at 725 nm was measured spectrophotometrically (UV 1601, Shimadzu Co., Ltd., Kyoto, Japan). To determine the condensed tannin (CT) and the extract was treated with butanol–HCl in the presence of ferric ammonium sulfate and CT was expressed in leucocyanidin equivalents as:

(A550 nm × 78.26)

Weight of sample (g/kg DM)

where A550 nm is the absorbance at 550 nm (Porter et al., 1985). The soluble condensed tannin (SCT) was calculated as the difference between TTP and CT (Giridhar et al., 2018).

3.2.3 In vitro digestibility and tannin bioassay

Approximately 200 mg of each feed sample was placed in 50 ml syringes with or without 1 g of PEG (MW 6000, lot PTQ 2819, Wako Pure Chemicals Industries, Ltd., Osaka, Japan) (Kondo et al., 2018); duplicate syringes were prepared for each test feed. For controls, we also prepared syringes that contained test feed only. For detail in vitro digestibility and fermentation characteristics analysis procedure see section 2.2.5.

3.2.4 Rumen degradability of browse species

The rumen effective dry matter degradability (EDMD) of each browse species was estimated using equation (4) proposed by (Kamalak *et al.*, 2005):

$$EDMD = 24.7 + 0.358(a + b) – 60.3c \ (R^2 = 98.3; RSD = 0.780) \quad (4)$$

Where EDMD is effective dry matter degradability, a is the gas production from the quickly soluble fraction, b is the gas production from the insoluble fraction, (a + b) is the total potential gas production, c is the gas production rate and RSD is relative standard deviation.

3.2.5 Volatile fatty acid and methane production analysis

The volatile fatty acid and methane production were analysis at the end of the 96-h incubation of the gas production. For the detail procedure, see section 2.2.6.

3.2.6 Statistical analysis

The chemical composition data for the various browse species were analyzed by using general linear models (GLM) procedure (SAS, 2001) with a completely randomized design in a 10 species × 2 seasons (dry and wet) factorial arrangement:

$$Y_{ij} = \mu + B_i + S_j + (BS)_{ij} + \varepsilon_{ij} \quad (6)$$

Where Y_{ij} is the response variable (CP, ADF, NDF, TEP, TET, CT, and SCT), μ is the population mean, B*i* is the browse species effect (i = 1–10), S_j is the season effect (i = 1, 2), $(BS)_{ij}$ is the interaction between season and browse species effect, and ε_{il} is the residual error. To determine the effect of season, species, the addition of PEG, and their interaction on GP, IVOMD, ME, and VFA were arranged in a factorial design of 10 Browse species × 2 PEG states (with and without) × 2 seasons. The following model was used:

$$Y_{ijk} = \mu + B_i + P_j + S_k + (BP)_{ij} + (BS)_{ik} + (PS)_{jk} + (BPS)_{ijk} + \varepsilon_{ijk} \quad (7)$$

where Y_{ijk} is the observation; μ is the population mean; B_i is the browse species effect (j = 1–10); P_j is the PEG effect (j = 1, 2); S_k is the season effect (k = 1, 2); $(BP)_{ij}$ is the interaction between browse species and season; $(BS)_{ik}$ is the interaction between browse species and season; $(PS)_{jk}$ the interaction between PEG and season; $(SBP)_{ijk}$ is the interaction between browse species, PEG and season, and ε_{ijk} is the residual error. Statistical significance was defined as $p < 0.05$. Multiple comparisons among means were performed by using Duncan's multiple range test (Kim, 2014). The related correlation coefficients analyses between chemical composition and in vitro fermentation parameters were performed by using Pearson's correlation test (Sedgwick, 2012).

3.3 Results

3.3.1 Seasonal variation in chemical composition and anti-nutritional factors

Chemical composition varied significantly ($p < 0.01$) among browse species across seasons (Table 3.1). For example, CP values ranged from 112 g/kg DM (*S. kunthianum*) in the dry season to 274.1 g/kg DM (*E. brucei*) during the wet season. The lowest NDF (169 g/kg) and ADF (109.8 g/kg) recorded in *S. sesban* in wet season while the highest NDF (596 g/kg DM) in *E. capensis* and ADF (457.7) in *T. laxiflora* during the dry season. The phenolic content of samples also differed significantly ($p < 0.001$) among browse species. Specifically, total extractable phenol (TEP) ranged from 26.3 (*C. adonsonia)* to 250.3 (*T. laxiflora)* g/kg, total extractable tannin (TET) from 22.8 (*E. brucei*) to 210.9 g/kg (*T. laxiflora*), and condensed tannin (CT) from 11.1 (*C. adonsonia*) to 141.3 g/kg (*T. laxiflora)*. The interaction of season and species exerted a significant ($p < 0.05$) effect on all chemical composition parameters. For instance, the TET content of *T. laxiflora* increased from dry season (62.3 g/kg) to wet season (210.9 g/kg).

3.3.2 Effect of polyethylene glycerol addition on in vitro gas production and fermentation characteristics

The inclusion of PEG in the fermentation of the browse species significantly ($p < 0.001$) increased the amount of gas produced at 24 h, especially the largest increment was recorded for *E. Ventricosum* (114.6 %) in wet season and *A. nilotica* (177.4%) in the dry season (Table 3.2). In addition, season, browse species, and their interaction significantly (p <

0.001) affected in vitro gas production both with and without PEG. The highest gas production from samples harvested during the dry season, which had lower amounts of phenolic compounds and tannins than those from the wet season (Figure 3.1) indicate the anti-nutritional effects of polyphenols on nutrient availability. Furthermore, PEG-associated increases in IVOMD and ME differed between species and season. For example, *A. nilotica* had the greatest increases in IVOMD (from 38.1% to 79.1%) and ME (from 5.5 to 12.4 MJ/kg DM) during the dry season while *D. torrida* increase in IVOMD (31.1 to 54.0 %) and ME (from 3.2 to 5.5 MJ/kg DM) in wet season (Table 3.2).

3.3.3 Effect of polyethylene glycerol on effective dry matter degradability and other characteristics of degradability

Species, season, and PEG treatment all significantly ($p < 0.001$) affected EDMD and degradability characteristics except the rate of degradation (Table 3.3). The PEG had significant ($p = 0.041$) effects which varied by browse species on the degradability of both the slowly and rapidly degradable fractions as well as on EDMD. The combined soluble and rapidly degradable fraction (a+b) was largest ($p < 0.001$) in *E. brucei* during the wet season and in *E. ventricosum* during the dry season (Table 3.3). Addition of PEG has significant ($p < 0.05$) effect on the rate of degradation (c value) of the browse species in which after PEG treatment the highest vales were observed in E. *capensis* (0.11 ml/h) and *T. laxiflora* (0.12 ml/h) in wet season and *M. lanceolate* (0.09 h^{-1}) in dry season. Responses in EDMD due to the addition of PEG ($p < 0.01$) were further influenced by season, species, and the interaction of PEG and season ($p<0.05$). The largest increment in EDMD in the presence of PEG was observed in *D. torrida* collected during the dry season (56.8%) and in wet season (37.2 %).

Table 3.1 Seasonal chemical composition of browse species (means; g/kg DM)

	CP		NDF		ADF		TEP		TET		CT		SCT	
Scientific name	W	D	W	D	W	D	W	D	W	D	W	D	W	D
A. nilotica	151^{cd}	139^{cd}	330^{c}	385^{bc}	276.5^{bc}	371.2^{b}	84.8^{bc}	71.7^{c}	66.7^{a}	47.7^{b}	42.8^{c}	53.1^{a}	24.9^{bc}	19.6^{a}
C. adonsonia	211^{ab}	164^{cd}	412^{a}	530^{ab}	257.0^{bc}	365.8^{b}	72.3^{bc}	26.3^{f}	22.8^{f}	31.2^{e}	15.6^{f}	11.1^{d}	15.6^{e}	11.7^{c}
D. torrida	148^{cd}	190^{c}	232	430^{b}	207.8^{c}	261.9^{c}	92.9^{b}	52.4^{b}	43.5^{c}	82.1^{c}	54.3^{c}	30.7^{c}	27.8^{bc}	22.9^{a}
E. capensis	161^{cd}	148^{cd}	435^{a}	596^{a}	327.6^{ab}	431.0^{a}	67.2^{c}	61.6^{d}	49.9 ^{bc}	38.6^{e}	26.6^{d}	24.5^{cd}	8.5^{f}	9.6^{c}
E. ventricosum	125^{d}	123^{d}	367^{b}	373^{bc}	244.2^{bc}	306.5^{bc}	90.2^{b}	80.8^{c}	42.5^{c}	75.4^{c}	44.8^{c}	28.6^{c}	30.6^{b}	14.0^{bc}
E. brucei	274^{a}	263^{a}	343^{bc}	556^{a}	331.1^{ab}	311.5^{bc}	48.8^{d}	27.1^{f}	22.5^{f}	35.8	27.0^{d}	19.7^{d}	8.8^{f}	2.9^{d}
M. lanceolata	224^{b}	179^{c}	296^{d}	501^{ab}	365.0^{a}	288.0^{c}	98.4^{b}	91.1^{a}	55.2^{b}	73.1^{c}	86.3^{b}	45.8^{ab}	13.2^{f}	9.4^{c}
S. sesban	232^{ab}	241^{ab}	364^{bc}	169^{d}	109.8^{d}	241.3^{c}	41.1^{d}	38.2^{e}	32.9^{d}	35.4^{e}	17.0^{e}	14.0^{d}	18.4^{f}	18.9^{bc}
S. kunthianum	127^{d}	112^{d}	448^{a}	445^{b}	380.1^{a}	389.3^{b}	74.4^{bc}	37.1^{e}	31.7^{d}	49.9^{d}	29.3^{d}	22.0^{cd}	20.7^{cd}	9.7^{c}
T. laxiflora	133^{d}	142^{cd}	295	480^{bc}	298.6^{a}	457.7^{a}	250.3^{a}	86.4^{b}	210.9^{a}	62.3^{a}	141.3^{a}	57.1^{ab}	69.6^{a}	15.2^{bc}
P values	CP		NDF		ADF		TEP		TET		CT		SCT	
Species	0.0007		0.0389		0.0036		0.002		0.0006		0.0089		0.0023	
Season	0.001		0.0419		0.0107		0.001		0.0067		0.0456		0.0567	
Species ×season	0.0002		0.0009		0.001		0.0002		0.0370		0.0456		0.0567	
SEM	9.0		23.9		14.7		8.6		6.5		5.0		2.5	

ADF, acid detergent fiber; CP, crude protein; CT, condensed tannin; D, dry season, NDF, neutral detergent fiber; SCT, soluble condensed tannin; SEM, standard error of mean; TEP, total extractable phenol; TET, total extractable tannin; W, wet season Within each column corresponding to species, different superscripts indicate significant differences.

Table 3.2 In vitro organic gas production, in vitro organic matter digestibility and metabolizable energy of browse species in the presence (+) or absence (–) of PEG.

	24-h GP			**IVOMD**			**ME**		
	(–)	(+)	% Incr.	(–)	(+)	% Incr.	(–)	(+)	% Incr.
Wet season									
A. nilotica	63.1^{a}	94.6^{a}	49.2^{cd}	38.0^{a}	61.2^{a}	60.2^{b}	6.8	10.4	52.8^{b}
C. adonsonia	22^{cd}	42.2^{c}	91.8^{b}	35.6^{c}	53.7^{c}	50.8^{c}	3.8	4.9	28.9^{cd}
D. torrida	27^{cd}	48.0^{bc}	77.8^{c}	31.1^{c}	54.0^{c}	73.6^{a}	3.2	5.5	71.9^{a}
E. capensis	15.5^{d}	26.3^{e}	69.7^{cd}	29.8^{d}	44.9^{cd}	50.7^{c}	3.0	4.7	56.7^{b}
E. ventricosum	19.2^{d}	41.2^{c}	114.6^{a}	33.1^{c}	53.2^{c}	60.7^{b}	3.5	4.8	37.1^{c}
E. brucei	26.4^{c}	28.0^{de}	16.1^{f}	39.5^{c}	41.9^{d}	18.1^{e}	4.4	5.2	18.2^{e}
M. lanceolata	19.4^{cd}	33.8^{cd}	74.2^{c}	23.2^{d}	37.2^{e}	60.3^{b}	3.5	4.1	17.1^{e}
S. sesban	20.1^{cd}	34.7^{cd}	72.6^{c}	32.7^{c}	43.2^{d}	32.1^{d}	4.9	6.2	26.5^{cd}
S. kunthianum	41.8^{b}	65.8^{b}	57.4^{cd}	45.4^{b}	64.3^{b}	41.6^{cd}	3.8	5.2	36.8^{c}
T. laxiflora	22.9^{cd}	36.5	59.4^{cd}	27.5^{d}	40.7^{cd}	48.0^{cd}	2.6	3.1	19.2^{e}
Dry season									
A. nilotica	26.1^{e}	72.4^{bc}	177.4^{a}	38.1^{de}	79.1^{bc}	108.1^{a}	5.5^{cd}	12.6^{bc}	109.5^{a}
C. adonsonia	42.1^{cd}	56.7^{de}	34.4^{de}	52.3^{cd}	65.3^{cd}	24.8^{de}	7.4^{bc}	9.9^{cd}	24.9^{ef}
D. torrida	77.6^{a}	101.0^{a}	30.1^{de}	83.9^{a}	104.3^{a}	24.8^{de}	12.6^{a}	15.4^{a}	24.9^{ef}
E. capensis	36.3^{cd}	84.1^{ab}	131.7^{b}	47.1^{cd}	89.7^{ab}	90.1^{ab}	7.4^{bc}	13.9^{ab}	91.5^{b}
E. ventricosum	24.2^{d}	51.1^{cd}	111.4^{bc}	18.3^{f}	24.8^{f}	33.1^{cd}	2.7^{f}	3.1^{f}	33.9^{de}
E. brucei	63.1^{b}	74.6^{bc}	18.4^{f}	71.1^{b}	81.2^{bc}	14.4^{f}	10.9^{b}	12.5^{bc}	14.4^{f}
M. lanceolata	21.0^{e}	48.3^{de}	130.2^{b}	19.5^{f}	33.8^{de}	75.9^{b}	2.8^{f}	5.1^{ef}	77.8^{c}
S. sesban	43.9^{cd}	60.9^{cd}	38.4^{de}	53.4^{cd}	69.7^{cd}	28.5^{de}	8.8^{bc}	10.9^{bc}	28.3^{ef}
S. kunthianum	28.5^{d}	65.3^{cd}	128.2^{b}	31.7^{de}	55.1^{cde}	75.0^{b}	4.2^{d}	8.3^{cd}	77.2^{c}
T. laxiflora	31.7^{de}	61.8^{cd}	94.3^{c}	25.6^{df}	34.2^{ef}	35.2^{cd}	3.0^{e}	5.1^{ef}	35.3^{ef}
SEM	2.12		4.61	2.87		2.13	1.01		3.35

P value	24-h GP	IVOMD	ME
Species	0.0001	0.001	<0.0001
PEG	<0.0001	0.0015	0.0021
Season	<0.0001	0.0026	<0.0001
Species × PEG	0.0292	0.6121	0.1551
Species× season	0.0002	0.001	0.0002
PEG × season	0.0041	0.2738	0.0893
Species × season × PEG	0.0241	0.0321	0.0893

Incr., increase; GP, gas production (ml/g); IVOMD, In vitro organic matter digestibility; ME, metabolizable energy (MJ/kg DM); SEM, standard error of mean. Data are given as mean values. Within each column, different superscripts indicate differences.

Table 3.3 Effective dry matter degradability and other degradability characteristics of browse species.

Species	Wet season						Dry season					
	a+b (ml)		c (ml/h)		EDMD (%)		a+b (ml)		c (ml/hr)		EDMD (%)	
	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)
A. nilotica	24.7[cd]	36.7[cd]	0.03	0.06	31.7[bc]	35.2[cd]	88.6[ab]	103.1[b]	0.05	0.08	53.9[ab]	57.90[b]
C. adonsonia	24.7[cd]	29.4[de]	0.03	0.06	41.2[bc]	53.8[cd]	95.6[a]	107.7[b]	0.08	0.09	53.9[ab]	59.56[ab]
D. torrida	9.5[f]	21.5[def]	0.03	0.08	36.3[a]	49.8[a]	38.2[de]	96.7 [bc]	0.02	0.06	37.3[cd]	58.09[ab]
E. capensis	38.3[a]	45.3[f]	0.09	0.11	20.4[de]	23.9[de]	9.1[h]	24.0[f]	0.03	0.06	26.0[ef]	31.67[d]
E. ventricosum	26.4[cd]	38.4	0.04	0.05	31.6[bc]	35.2[cd]	93.2[a]	117.1[a]	0.02	0.06	56.4[a]	63.03[a]
E. brucei	26.9[cd]	50.8[b]	0.04	0.07	32.6[bc]	41.4[b]	26.4[f]	50.8[e]	0.02	0.04	32.6[cd]	40.39[cd]
M. lanceolata	16.5[de]	23.5[de]	0.04	0.07	28.3[bcd]	31.2[cd]	85.5[ab]	106.2[b]	0.06	0.09	51.8[ab]	57.51[b]
S. sesban	35.7 [bc]	47.3[bc]	0.03	0.05	35.2[b]	38.6[cd]	41.2[d]	50.4[e]	0.04	0.07	36.1[cd]	40.22[cd]
S. kunthianum	17.1 [de]	29.1[de]	0.04	0.05	28.4[de]	32.5[cd]	79.6[ab]	90.7 [cd]	0.03	0.05	51.7[ab]	54.27[ab]
T. laxiflora	3.8[g]	14.3[f]	0.09	0.12	20.4[e]	28.7[de]	39.8[de]	91.8[cd]	0.04	0.07	36.8[cd]	55.57[ab]
SEM	1.24		0.02		2.40		4.31		0.01		3.22	
P value	a+b		c		EDMD							
Species	<0.0001		0.2314		<0.0001							
Season	<0.0001		0.1218		<0.0001							
PEG	0.0118		0.0400		0.0001							
Species × PEG	0.0410		0.9996		0.3676							
PEG × season	0.1315		0.2108		0.0572							

–, without PEG; +, with PEG; EDMD, effective dry matter degradability (%); Relative error, 14.7; a, the rapidly degraded fraction (ml); b, slowly degraded fraction (ml); c, rate of degradation of the b fraction (h-1); SEM: standard error of the mean. Data are given as mean values. Within each column, different superscripts indicate differences.

3.3.4 Effect of polyethylene glycerol on volatile fatty acid and methane production

The total and individual VFA contents produced differed significantly ($p \leq 0.05$) between browse species (Table 3.4), and the species × PEG interaction had a significant ($p = 0.023$) effect on both VFA and CH_4 production. For example, *E. ventricosum* had the largest increase (23%) in acetic acid during the wet season, whereas PEG-treated *M. lanceolata* showed a 49% increase in this VFA during the dry season. *C. adonsonia* produced the largest volumes of propionate and butyric acid. In addition, the interaction of season and PEG influenced acetate, total VFA, and CH_4 production ($p = 0.041$). For instance, with addition of PEG, *M. lanceolate* showed marked seasonal variation in acetate (90.7–139.9 mmol/L), total VFA (133.1–204.4 mmol/L) and CH_4 (40.1-63.4 mmol/L).

3.3.5 Correlation between chemical composition and in vitro fermentation

Crude protein had positive correlations with GP (maximum r value = 0.706 for wet season without PEG, $p < 0.01$), IVOMD ($r = 0.649$, $p < 0.01$), ME ($r = 0.634$, $p < 0.01$) and VFA ($r = 0.314$, $p < 0.01$) (Table 3.5). In contrast, polyphenols (TEP, TET, CT, and SCT) were negatively correlated ($p < 0.01$) with in vitro fermentation products (GP, IVOMD, ME, and VFA) during both seasons and PEG states; these outcomes indicate the anti-nutritional effects of the browse species on their in vitro digestibility. Furthermore, the fiber component NDF was negatively correlated with in vitro fermentation products for total VFA production ($r = –0.407$, $p < 0.01$).

Table 3.4 Total and individual volatile fatty acid (mmol/L) concentrations and methane production (mmol/L) without (-) PEG and wit (+) PEG.

Species	Acetic		Propionic		Butyric		Total VFA		CH_4	
	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)
Wet season										
A. nilotica	96.6*	110.6*	27.3	32.3	10.4	10.7	136.9	156.9	46.7*	52.6*
C. adonsonia	53.2	65.2	32.1	36.7	18.6	18.9	104.8	122.3	27.9	32.9
D. torrida	52.8	71.0	17.3	23.4	6.8	8.3	78.1	104.4	25.5	33.8
E. capensis	93.3*	106.0*	23.4	33.4	9.0	9.5	127.5	151.2	45.3	49.4*
E. ventricosum	115.9***	138.9***	32.7	36.2*	12.3	19.3	163.7**	197.6**	55.9*	70.1**
E. brucei	71.9	81.9	24.5	44.5*	8.9	9.4	106.4	137.6	34.3	34.5
M. lanceolata	81.9*	94.9	36.3	42.2*	6.5	6.8	126.9	146.9	35.1	40.3
S. sesban	89.7*	111.7**	28.0	30.3	5.4	11.0	125.5	156.0*	40.6	53.8*
S. kunthianum	73.9	89.2	24.4	34.0	7.6	8.2	107.7	133.9	34.7	40.2
T. laxiflora	76.8	97.7*	18.3	21.4	8.4	10.0	108.8	136.7	38.0	48.5*
Dry season										
A. nilotica	106.6**	124.5**	31.3	35.4	10.7	10.4	151.1*	172.2*	50.8*	58.6*
C. adonsonia	103.2**	119.8**	34.8	40.4*	8.0	9.1	150.3*	170.7*	48.4*	54.4*
D. torrida	76.4	95.0*	24.2	29.4	6.4	6.2	108.1	132.3	35.4	43.3
E. capensis	93.3*	99.9*	23.4	30.4	9.0	9.3	127.5	141.6	45.3	47.0
E. ventricosum	42.6	52.2	15.7	17.0	5.1	5.9	65.0	77.0	19.9	24.8
E. brucei	78.2	81.2	26.1	32.1	8.9	9.3	114.4	124.4	37.0	37.2
M. lanceolata	90.7*	139.9***	34.0*	50.3**	6.5	12.0	133.1*	204.4**	40.1	63.4*
S. sesban	94.7*	107.2**	29.0	31.3	3.9	5.1	130.5	146.7	42.1	48.3
S. kunthianum	73.9	82.1	24.4	28.3	7.6	7.9	108.1	121.1	34.7	37.9
T. laxiflora	98.2***	130.0***	30.0	39.6*	9.7	11.0	159.4*	182.7**	56.5*	60.6*
SEM	3.45		1.17		0.53		4.32		1.65	
P value	Acetic		Propionic		Butyric		Total VFA		CH_4	
Species	0.0023		0.0434		0.123		0.0434		0.213	
Season	0.016		0.0601		0.0701		0.060		0.0531	
PEG	0.0041		0.031		0.0601		0.0401		0.0321	
Species×PEG	0.0234		0.128		0.745		0.352		0.0982	
Season × PEG	0.0153		0.128		0.745		0.028		0.0412	

–, without PEG; +, with PEG; CH_4, methane; SEM, standard error of the mean. Data are provided as means. Within each column, different superscripts indicate differences.

Table 3.5 Correlation coefficients (r) between chemical composition and in vitro fermentation parameters.

	GP		**IVOMD**		**ME**		**Total VFA**	
	(–)	**(+)**	**(–)**	**(+)**	**(–)**	**(+)**	**(–)**	**(+)**
Wet season								
CP	0.706**	0.454**	0.649**	0.419**	0.634**	0.423**	0.314*	0.261
NDF	–0.221	–0.021	–0.185	–0.299	–0.122	–0.132	–0.207	–0.106
TEP	–0.571***	–0.353*	–0.511***	–0.511***	–0.407**	–0.237	–0.438**	–0.238*
TET	–0.584***	–0.513***	–0.345*	–0.345*	–0.345*	–0.252	–0.452**	–0.252
CT	–0.635***	–0.534**	–0.575***	–0.475**	–0.415**	–0.386*	–0.405**	–0.495***
SCT	–0.625*	–0.455*	–0.428*	–0.432*	–0.401*	–0.328*	–0.439*	–0.229
Dry season								
CP	0.483**	0.354**	–0.443**	0.391**	0.482**	0.423**	0.242	0.225
NDF	–0.282	–0.108	–0.173	–0.243	–0.184	–0.173	–0.407**	–0.312*
TEP	–0.544***	–0.321*	–0.501***	–0.441***	–0.307*	–0.307*	–0.388*	–0.438**
TET	–0.513***	–0.376***	–0.398*	–0.325*	–0.345*	–0.312*	–0.432**	–0.452**
CT	–0.654*	–0.554*	–0.385*	–0.375*	–0.425**	–0.156	–0.487**	–0.425**
SCT	–0.335*	–0.395*	–0.328*	–0.345*	–0.328*	–0.228*	–0.449**	–0.419**

–, without PEG; +, with PEG; CP, crude protein; CT, condensed tannin; GP, Gas Production, IVOMD, organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber; SCT, soluble condensed tannin; TEP, total extractable phenol; TET, total extractable tannin; VFA, volatile fatty acids. Data are provided as means. Within each column, different superscripts indicate differences.

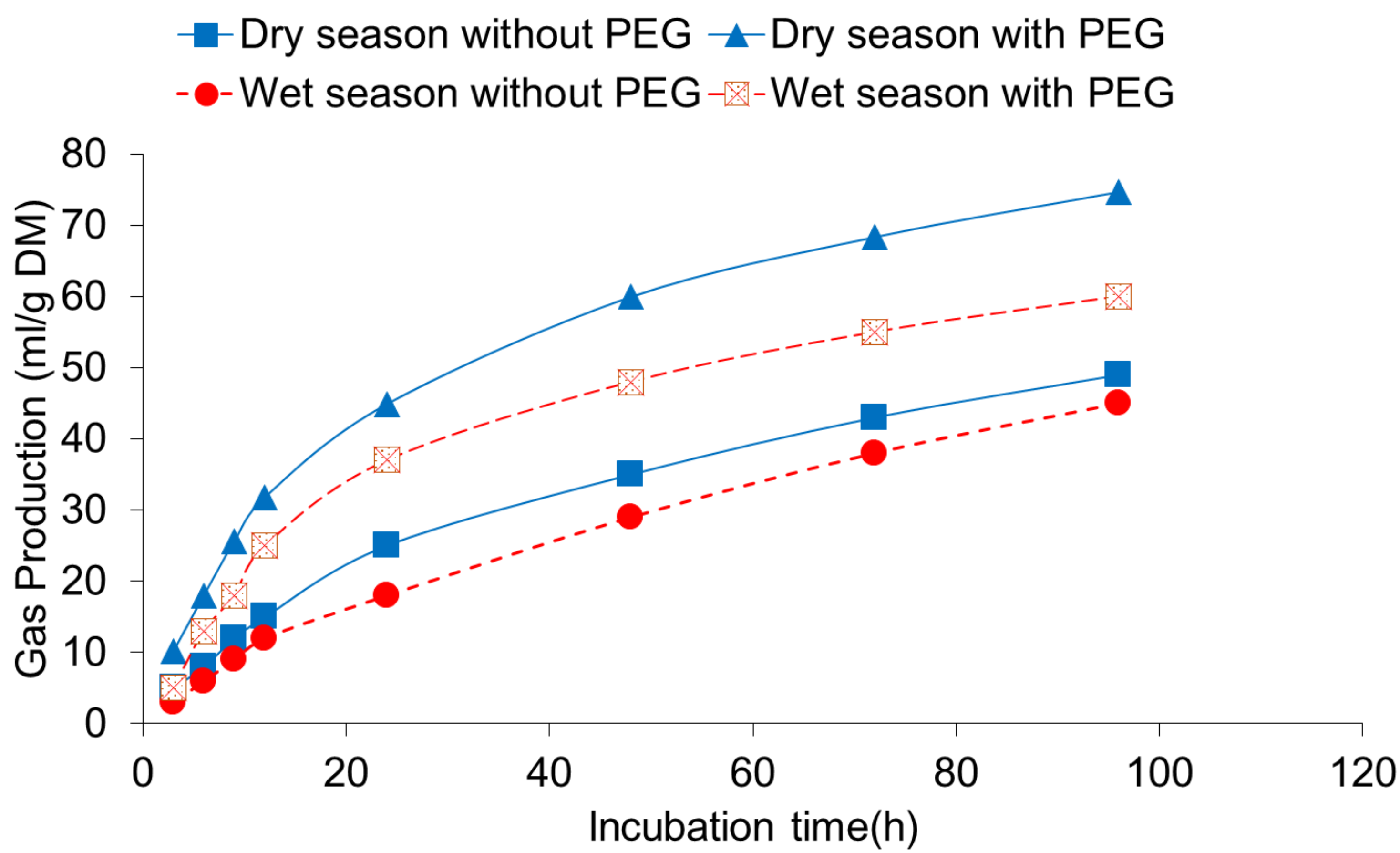

Figure 3.1 Seasonal variation in the in vitro gas production of browse species relative to the addition of PEG. Wet Season without PEG addition (●), wet season with PEG addition (×), dry season without PEG addition (■) and dry season with PEG addition **(▲).**

3.4 Discussion

3.4.1 Effect of seasonal variation on chemical compositions and polyphenol contents

The chemical composition (e.g., CP, NDF, TEP, TET, CT) of the browse forages were within the ranges previously reported for species from Kenya (Osuga *et al.*, 2008; Rubanza *et al.*, 2005), fodder trees and shrubs in Algerian arid and semi-arid areas (Bouazza et al., 2012), tropical browse plants in south Africa (Gemeda and Hassen, 2015), and in the mid-Rift Valley of Ethiopia (Shenkute et al., 2012). In addition, the CP content of the browse species assessed in both seasons exceeded the minimum required for ruminal microbes to support metabolic functions of their host (Van Soest *et al.*, 1991) and were adequate to support meat and milk production in ruminant animals (Lamers and Khamzina, 2010). These results show that, considering their tannin content which is acceptable except for *A. nilotica, D. torrida, E. Brucei, M. lanceolate* and *T. laxiflora* that have more CT above the recommended level (20-40g/Kg) (Ramírez-Restrepo et al., 2004); these browse species can be used to supplement the CP of the low-quality roughage, such as natural pasture and crop residues, that is available during harsh conditions in arid regions (Gemiyo *et al.*, 2013). The marked differences in seasonal changes in the chemical composition of browse species and their patterns might reflect the climatic variation (Singh et al., 2005) and concentration of phenols and tannins is species dependent (Parissi *et al.*, 2018). Moreover, the high CP content recorded during the rainy season might be due to regrowth of leaves (Anele et al., 2008). In contrast, the slight increase in the CP content of *S. sesban* and T. *laxiflora* in the dry season might be due to their regrowth and capability to retain important nutrients under the moisture stress during this period (Arigbede et al., 2012). The differences observed in NDF and ADF in this study may be partly due to buildup to the accumulation of lignocellulose fiber structures of the plants as they aged in the dry season (Kandel et al., 2013). This species variation in fiber concentration may encourage animals to forage more from one type of browse than another, depending on the season (Gemeda and Hassen, 2015).

In the current study, the variation in polyphenol levels among and even within browse species during different seasons might be due to morphological differences within the same species and variation in climatic conditions that influence the accumulation of polyphenolic compound with plant phenology (Ammar et al., 2004) and water supply that affect tannin levels in plants (Abu-Zanat et al., 2003; Guerrero et al., 2012). Furthermore, we noted greater concentrations of tannins during the wet season than the dry season particularly for *A. nilotica* and *T. laxiflora.* In agreement with this result, a previous report indicated that an increased concentration of tannins was found during the summer season when water and probably light availability were higher while decline in water availability from May to June was sufficient to expose the plants to a severe drought, drastically reducing tannin production (Espírito-Santo et al., 1999)**.** In contrast, we also observed high TET concentration for *D. torrida, E. Ventricosum* and *M. lanceolate* species in dry season than in wet season. This might be due to the carbon is required for tannin production, and the carbon is more consumed for photosynthesis in the wet season than in dry season for growth (Top et al., 2017**).** On the other hand, increase in tannin production of those browse species under dry season could be a defense strategy in plants against dehydration, since these compounds apparently hinder the passage of water from the cells around the plant veins under drought conditions (Espírito-Santo *et al.*, 1999). The difference in tannin contents between the browse species in this study could be due to the climatic effect on foliage regrowth and plant nutrient accumulation (Elghandour et al., 2013)**.**

3.4.2 Effect of polyethylene glycerol on in vitro digestibility, degradability and fermentation characteristics of browse species

In this study, the addition of PEG increased the GP, IVOMD, ME, and degradability of the tannins contained in the browse species. These findings parallel those of previous studies. For example, Silanikove et al. (2001) noted that PEG was effective in reversing the nutritionally negative effect of tannins, and Salem *et al.* (2006) reported that the use of PEG increased the IVOMD and ME of various browse species. However, the results from our study are in contrast to those of Monforte-Briceño et al. (2005), who reported that the inclusion of PEG had no effect on IVOMD of some shrub species. Alternatively, the increase in GP after PEG addition that we noted confirms the fermentation depressing effect of tannins observed by Getachew et al. (2002) and (Rubanza *et al.*, 2005). *T. laxiflora* contains the largest amounts of tannins in wet season but the highest gas production increment after PEG addition was recorded in *E. Ventricosum* (114.6 %) in dry season and A. nilotica (177.4 %) in wet season with medium tannin content. Since gas production is a result of fermentation of carbohydrates in the rumen mainly, it seems that the *T. laxiflora* tannins suppressed the carbohydrate fermentation. This was also observed by Kondo et al., (2018) that the extent to which PEG affects gas production is determined by the negative effects of tannins and that would depend on the amount of the tannins in the species. Addition of PEG could overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters and IVOMD in tannin-rich feeds. However, effects were not uniform among species of trees and shrubs (Singh *et al.*, 2005). Current findings on the effect of PEG on improved in vitro digestibility are comparable to findings of (Rubanza *et al.*, 2005) who noted 46.6, 28.7 and 43.0% for *A. nilotica*, *A. tortilis* and *A. polyacantha* leaves, respectively but lower than 64% for *A. unedo* species Hajer et al. (2004).

The result also showed that PEG-associated responses in IVOMD and ME were influenced by species, PEG treatment, and their interaction (Schofield *et al.*, 2001). In the current study, *M. lanceolata* had the lowest potential gas production (a+b), probably due to its high NDF and ADF contents. The gas production and fermentation characteristics of browse forages of this study are within the ranges reported earlier for browse forage species from Kenya (Osuga *et al.*, 2008). The negative correlation between the tannins content and the degradability characteristics indicates the adverse effects of the tannins on the use of such browse species as animal feeds (Osuga *et al.*, 2008; Silanikove *et al.*, 2001).

3.4.3 Effect of polyethylene glycerol on volatile fatty acid and methane production

The increase in the total VFA that obtained after adding PEG in this study is in line with other reports involving tropical browse species and indicates improved substrate utilization by ruminal microbiota (Mengistu *et al.*, 2017b). The increased production of acetate, propionate and total VFA due to PEG inclusion demonstrates that the high tannin content of browse species is a major limiting factor to their ruminal digestion (Gemeda and Hassen, 2015). This could be also due to the negative correlation between VFA and phenolic compounds (TEP, TET, CT, and SCT) as our result indicates. The addition of PEG to browse species seems to be an effective approach to neutralize the tannins present, allowing for increased utilization of these browse species by ruminants (Salem *et al.*, 2007; Dentinho *et al.*, 2018). The increase of CH_4 production after PEG addition in some of the browse species in this study confirms the fermentation depressing effect of tannins as also observed in previous studies (Gemeda and Hassen, 2015; Rubanza *et al.*, 2005) because one of the anti-nutritional effect of tannins is considered to be through inhibition of bacteria by forming complexes with bacteria cell wall, membrane and extracellular enzymes (Patra and Saxena, 2009).

3.5 Conclusions

Most of the ten browse species we evaluated contained more than 112 g/kg of CP, thus implying that they can used as nitrogen sources for ruminant feed supplementation. Our study highlights the considerable differences in the levels of phenolic compounds in browse plants depending on their species and the season in which the plants were collected. The addition of PEG dramatically increased in vitro fermentation, digestibility, ME, and VFA production almost certainly by reversing the effects of secondary compounds. However, the extent to which the addition of PEG reduced the anti-nutritional effects of phenolic compounds and tannins varied by browse species and season. Therefore, attention should be given to select species that have high concentration of secondary compounds and has greater mitigating effect on their anti-nutritional functions, relative to the season of harvest. The phenolic compounds of TEP, TET, CT, and SCT showed negative correlation with GP, IVOMD, ME and VFA. Together, these findings confirm the fermentation-depressing effect of tannins. Therefore, based on their chemical composition, IVOMD, and CT concentration, the following investigated browse species likely would be beneficial as feed supplements for ruminant livestock in Ethiopia: *E. ventricosum*, *S. sesban*, *M. lanceolata*, *E. capensis*, and *A. nilotica*. Overall, PEG increased the in vitro digestibility and rumen fermentation characteristics of the tanniferous browse species vary in relation to season. Furthermore, these PEG treated fodder species should be incorporated in the diet formulation for ruminant animals and recommended for field application.

Chapter 4:

Optimal diet formulation for lactating dairy cows for dry season

4.1 Introduction

In developing countries, farmers have a wide range of locally available cheap ingredients but there is lack of knowledge and experience on balanced ration formulation to incorporate these potentially nutritious feedstuffs. Economic analysis indicated that formulating diets more frequently generally increased profitability, because any increase in feed cost was mitigated by an increase in profitability through improved milk yield (White and Capper, 2014). Poor quality feeds and feeding system could lead to reduce milk production and increase methane emission (Niu et al., 2018). In the predominant small scale, subsistence farming systems in Ethiopia, most of the farmers cannot give balanced diet supplementation for their animals (Ayalew et al., 2003). And so, the feed formulation can allow livestock producers to utilize economically and environmentally effective local feed resources (Oishi et al., 2011). Furthermore, based on the chemical composition of available feed resources and in accordance with the nutrient requirement of the animal, the software computes the least cost ration within the given constraints (Garg and Makkar, 2012).

Recently, interest has increased for linear programming model (LP) for diet formulation that can solve for a complicated set of nutrient requirements to give a relatively well-balanced ration (Alqaisi et al., 2019). Linear programming is one of the most commonly used methods followed by many commercial and non-commercial feed formulation programs (Rehman and Romero, 1984). The assumption in LP restricts objective function to be single and constraints to be fixed-right hand side (RHS), which means the reduction of goal programming model consists of constraints and sets of goals, which are prioritized sometimes (Kuntal et al., 2018). The purpose of goal programming (GP) is to find the solution, which satisfies the constraints, and come close to the stated goals of respective problem. Subsequently, the GP approach was used by (Moraes *et al.*, 2015) to model trade-offs between minimizing diet costs and minimizing methane emissions. Theoretically, ration manipulation is a method to potentially reduce methane emission for dairy cows without dramatically increasing diet costs (Qu et al., 2019).

In this study, the idea of incorporating maximizing milk yield is considered applicable for exploring the trade-offs between minimizing diet cost and methane emission for dairy production. Thus, the objectives of this study are to (1) To investigate the existing lactating dairy cow feeding practice in dry season on feed intake and milk yield, and 2) To develop optimal diet formation for maximize milk production and minimize methane emission.

4.2 Materials and methods

The study was carried out in 48 smallholder dairy farms located in the Aba Gerima and Guder sites in northwestern Ethiopia (see section 1.5 for detail site description).

4.2.1 Monitoring of the existing feeding practice and sample collection

A total of 60 multiparous dairy cows of mid-lactation stage with 2^{nd} and 3^{rd} parities were studied from 48 small holder dairy farms during the critical feed shortage period of the dry season for 5 months (January to May). Daily feed offers and refusal were recorded and weighted using hanging digital balance (50 kg capacity, 10g accuracy, made in china). The feed samples were collected and immediately brought to Bahir Dar University and prepared for further chemical analysis. Further information regarding the amount and types of feeds and fodder being offered to the animals, actual rate of daily feed intake and milk yield of individual animal were collected from individual farmer, using standard sampling procedure.

Daily milk yield was recorded while milk composition (fat, protein, and lactose) was determined by using a Lacto Scan milk analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria). The fat protein corrected milk yield (FPCM) was calculated according to (Nichols et al., 2019): FPCM = (0.337 + 0.116 × milk fat (%) + 0.06 × milk protein [%]) × kg of milk yield.

The body weight of the animals was measured using is heart girth (HG) in cm and calculated with the equation proposed by (Tebug et al., 2018) for indigenous Zebu:

BW = 4.81 HG–437.52 (r^2=0.85) where BW is the body weight (kg) and heart girth in cm.

4.2.2 Chemical composition, mineral profile and fermentation characteristic

The feed ingredients chemical analysis was conducted in Tottori and Shimane university Laboratories in Japan. The detail methodology describes in section 2.2.2. A summary of the mean nutritive value and mineral contents of each feed ingredients used for this study is shown in Table 4.1.

4.2.3 Selection of feed ingredients

In optimization of feed formulation, it is first necessary to define the sorts and quantities of the required feed ingredients, which naturally has to be adjusted to the feed formulation plan for a particular period. Multi-criteria approach was used to determine the potential feed ingredients and the priority for the feeds availability on the farm or cheap non-conventional feed resources before the farmer can purchase anything else. There were 21 feed ingredients selected from among the available feedstuffs based on their cost, availability and nutritive value (Table 4.1 and 4.2). They were categorized as follow based on (NRC, 1978) feed classification: 1) dry roughages (natural grass hay, improved grasses hays (*Pennisetum purpureum* and *Brachiaria* hybrids), straw (*Eragrostis tef, Hordeum vulgare, Triticum aestivum, Eleusine coracana and Zea mays*)); 2) fodder species treated with PEG to reduce the anti-nutritional factors (*Sesbania sesban*, *Ficus sur* and *Ficus thonningii*) 3) silage (teff straw treated with effective microbes, urea and molasses) 4) energy feeds (formulated concentrate, wheat bran) and 5) protein feeds supplements (formulated concentrate, noug seed cake, cottonseed oil cake, soya bean sake, sesame oil cake, atela and brint) and 6) mineral and vitamin supplements (ruminant premix) (Table 4.1 and 4.2). These feeds are those typically available to dairy producers in the northwestern Ethiopia in dry season (Tegegne and Assefa, 2010).

Table 4.1 Sorts of feed ingredients

Feed ingredients	Limit*	Cost ($/kg)
Hay	No limit	0.33
Eragrostis tef	No limit	0.12
Hordeum vulgare	No limit	0.18
Triticum aestivum	No limit	0.14
Eleusine coracana	No limit	0.13
Zea mays	No limit	0.08
Pennisetum purpureum	No limit	0.2
Brachiaria hybrids	No limit	0.21
Pennisetum pedicellatum	No limit	0.2
Sesbania sesban	0.25	0.15
Ficus sur	0.17	0.1
Ficus thonningii	0.15	0.1
Formulated concentrate	No limit	0.1
Noug Seed Cake	No limit	0.25
Wheat bran	No limit	0.2
cottonseed oil cake	No limit	0.26
Soya bean Cake	No limit	0.32
Sesame oil cake	No limit	0.22
Atela	0.20	0.15
Brint	0.18	0.17
Ruminant premix	0.02	6.33

*In kilogram per kilogram of diet DM.

Table 4.2 Nutrient content of ration ingredients and right hand side values for the ration formulation constraints

Feed ingredient*	CP (g)	NDF (g)	NEm (Mcal)	NEl (Mcal)	Ca (g)	Mg (g)	P (g)	K (g)	S (g)	Na (g)	Fe (mg)	Mn (mg)	Zn (mg)	Se (mg)	Cu (mg)	Co (mg)
Hay	66.6	694.7	6.0	5.3	2.9	8.4	3.5	20.0	3.0	0.8	3268.9	939.2	274.4	0	27.1	0.8
Eragrostis tef	43.3	720.8	4.1	4.3	1.4	3.1	3.2	20.2	4.0	0.5	2504.1	292.6	200.1	0	3.8	1.1
Hordeum vulgare	41.2	699.8	4.2	4.1	2.1	2.2	1.3	16.4	2.7	0.2	547.3	76.6	157.3	10.5	32.4	4.4
Triticum aestivum	39.3	730.2	4.1	4.3	1.4	2.2	1.9	39.6	3.6	0.7	1043.7	250.3	214.8	0	1.9	0.0
Eleusine coracana	56.1	652.0	5.3	5.0	3.8	5.3	4.6	45.9	6.3	0.5	2636.7	1365.2	270.9	0	9.6	0.0
Zea mays	28.1	799.8	6.5	3.0	2.0	6.3	1.2	41.6	1.4	0.5	678.6	201.9	206.0	0	1.4	0.0
Pennisetum purpureum	74.4	581.6	7.2	4.2	2.6	4.7	1.7	32.4	3.0	2.4	2171.6	146.0	206.2	0	19.8	0.2
Brachiaria hybrids	34.2	536.6	6.7	4.1	4.4	2.7	0.8	24.5	1.7	0.1	861.0	38.0	178.3	7.9	32.0	2.7
Pennisetum pedicellatum	58.3	705.7	6.0	5.0	2.5	6.2	1.9	29.9	1.9	0.9	1038.3	67.8	173.9	0	0.0	0.0
Sesbania sesban	127.9	423.1	4.2	3.4	2.5	8.2	4.7	46.8	2.7	0.3	1851.8	165.2	205.7	1.6	15.6	1.0
Ficus sur	163.3	446.6	5.6	4.1	3.3	11.5	3.9	49.4	4.2	0.3	11792.4	494.2	250.1	4.8	48.3	8.0
Ficus thonningii	73.1	649.6	7.2	6.5	2.9	8.2	2.1	37.2	3.0	0.2	5961.1	204.5	254.3	6.3	72.8	5.3
Formulated concentrate	71.3	649.5	7.3	6.6	3.4	5.2	5.1	37.5	5.6	0.3	2808.5	350.1	392.4	3.8	58.6	3.0
Noug Seed Cake	232.8	364.4	6.5	5.5	5.0	3.8	3.8	22.0	3.6	1.0	766.2	209.5	162.7	0	9.3	0.0
Wheat bran	108.8	447.5	5.4	6.3	8.7	13.5	4.4	29.9	2.6	0.2	673.9	79.3	602.8	1.9	12.4	0.3
cottonseed oil cake	217.0	423.6	6.2	6.2	5.6	7.0	6.0	38.4	4.9	0.1	813.8	72.4	206.7	1.6	16.5	0.6
Soya bean Cake	106.5	484.4	4.0	4.0	8.2	9.0	3.9	32.4	2.5	0.2	743.2	68.6	170.9	1.2	17.5	0.5
Sesame oil cake	135.7	316.4	7.2	6.2	4.7	9.4	4.7	34.3	4.8	0.2	1148.1	384.2	214.5	0	47.5	0.0
Atela	181.2	367.6	5.1	4.0	3.0	12.8	13.4	27.1	7.8	0.2	1122.5	177.0	234.2	0	34.0	0.0
Brint	149.9	394.8	5.4	4.1	1.1	10.2	22.5	29.6	4.2	0.3	147.9	347.4	245.2	0	22.4	0.0
Teff straw silage	232.7	361.9	6.5	4.4	0.8	8.9	12.4	28.0	5.8	0.3	188.6	13.5	343.2	0	11.4	0.0
Ruminant premix**																
RHS[b]	1121	2120	10.8	11.9	42	14.9	13.4	67.0	14.9	13.4	1860	1490	1470	60	370	276

CP, crude protein; NDF, natural detergent; NEm, net energy for maintenance; NEl, net energy for lactation; Ca, calcium; Mg, magnesium; P, phosphorus; K, potassium; S, Sulphur; Na, sodium; Fe, iron; Mn, manganese; Zn, zinc; Se, selenium; Cu, copper; Co, cobalt; RHS = right hand side; *All measures are on a unit per kg DM basis; **Ruminant premix containing (vitamin A, 99 750 IU/Kg; vitamin D3, 199 950 IU/kg; vitamin E, 800 mg/kg).

4.2.4 Nutrient requirement of lactating dairy cows

The rations formulated in this study are based on the nutrient requirement of lactating dairy cow according to (NRC, 2001) using the equations below:

Maintenance Energy = 0.080 Mcal NE / kg $W^{0.75}$ (1)

Maintenance Protein = 7.45 g CP/ kg $W^{0.75}$ (2)

Lactation Energy = (0.0929 * fat% + 0.0547 * protein% + 0.0395 * lactose %) Mcal NE/kg milk (3)

Lactation Protein = 83 g CP/kg milk (4)

The Net energy for lactation (NEL) is reported in megacalories (Mcal) and specifies the amount of energy a dairy cow needs for maintenance and lactations.

For grazing cows, the dry matter intake is derived from the relationship DMI = 0.025*BW + 0.1 M where BW = body weight (kg) and M = milk yield (kg). ME for maintenance is taken as 487 KJ/$M^{0.75}$ according to NRC (2001). The average weight of the cows was taken as 232 kg, producing milk with 4% fat at a daily dry matter intake (DMI) of 3% body weight plus the nutrient requirements for maintenance and milk production depending on fat content of the milk was computed accordingly NRC (2001).

For existing lactating dairy cows feeding practice, after baseline data collection, the ration of dairy cow was checked for balanced of energy, protein and minerals based on NRC (2001) feeding standard. The intake was also compared against the requirements on dry matter basis (NRC, 2001), so as to identify quantitative deficiency, sufficiency or excess.

4.2.5 Model structure, constraints and solving the linear programing model

The nutrient diet model formulation is a combination of different feed ingredients needed for a balance diet of the dairy cow. The model has to satisfy a set of constraints on nutritional levels, availability restrictions, special ingredients to be included, demand constraint, energy and budget constraints. The generic mathematical model which is applicable to each type of ration using the available ingredients.

Three linear programming models were used to individually minimize diet costs and CH_4 emissions from lactating dairy cows and maximized milk yield. The first model is the least cost diet model, and the second model is a modified version of the linear programming model proposed by Moraes et al. (2015) to minimize daily CH_4 emissions based on nutrient intakes. Both linear programming models were solved in the lpSolve package of the software R (Buttrey, 2005). The third is to estimate the milk yield based on NRC (2001).

The model was structured in 3 sequential parts are:

1) ***Least Cost Diet Model**:* the least cost diet model was formulated to minimize diet costs while delivering nutrients to sustain a given level of milk production. The first LP model was formulated to minimize diet costs while meeting the nutrient requirements for the lactating dairy cow. The objective function (Eq. (5)) and the constraints that determine the feasible region (Eq. (6–8)) are described below:

$$\min Z_1 1 = \sum_{j=1}^{n} c_j x_j, \qquad x_j \geq 0 \tag{5}$$

$$\sum_{j=1}^{n} a_j x_j \geq \mathrm{b}_i, \qquad \mathrm{i} = 1, \dots \dots, \mathrm{m} \tag{6}$$

$$l_i \leq \frac{\sum_{j=1}^{n} x_j \varphi_{ij}}{\sum_{j=1}^{n} x_j} \leq \mathrm{U}_i, \quad \mathrm{i} = \mathrm{m}+1, \dots \dots, \mathrm{m}+\mathrm{d} \tag{7}$$

$$\sum_{j=1}^{n} \mathrm{xj} \leq \mathrm{h}, \tag{8}$$

where Equation (5) is the objective function for which z_1 is the value representing dietary costs ($/cow/d) for the first linear programming model, *cj* is the cost of feed *j* ($/kg of DM), *xj* is the *j*th decision variable representing the amount of feed *j* (kg of DM), and *n* is the number of decision variables (number of feeds). Feeds available for diet formulations were from survey and monitoring result of the feed resource (Chapter 2; Table 2.1) that represent feeds used dairy farmers in the study sites and nutrient composition from laboratory analysis. Feed ingredients costs were collected locally in study sites (Table 4.1). Diets were formulated to sustain the milk production of the 48 dairies farms and to attain maximum milk yield (up to 4 kg/d/cow). Nutrient requirements of cows were used according to the NRC (2001) guidelines system. Equations (6 to 8) are constraint equations that determine the feasible region. Equation (5) sets the minimum nutrient requirement by using aij represents the quantity of nutrient i on feed j (MJ, g or mg/kg of DM) and bi as daily nutrient i (MJ, g or mg/cow/d) such as energy, protein, etc., requirements for a given level of an animal's performance; *m* is the number of nutrient requirements in the LP model. Equation (7) sets the nutrient proportion constraints and several feed limits; *d* is the number of the corresponding nutrient proportion constraints and feed limits in the LP model; *φij* is the proportion of nutrient *i* in feed *j* (kg/kg of DM), and *li* and u*i* are the lower and upper limits in kilograms per kilogram of DM, respectively, of the *i*th nutrient (in kg/kg of DM). Current NRC requirements do not include neutral detergent fiber (NDF) requirements for cows. Therefore, NDF was derived from Adams et al. (1985) and is a measure of the fiber requirements of the cow. Specifically, dietary NDF (kg/kg of DM) was set between 0.25 and 0.4 kg/kg of DM to avoid rumen dysfunction. The minimum and maximum proportions of CP in the diet were set as 0.16 and 0.2 kg/kg of DM, respectively. The requirement of protein was calculated from NRC (1989) using the minimal requirement of CP. The dietary forage percentage (kg/kg of DM) was set to be between 0.35 and 0.65. The inclusion of determined feeds in the diet were also limited to predetermined proportions (Table 4.1) to ensure the formulation of realistic diets that would be readily accepted by dairy farmers. Equation (8) was used to set the maximum DMI with *h* (kg of DMI/cow per day) using TMR denoting the calculated maximum DMI. Dry matter intake, calculated according to NRC (2001) as a function of BW and FCM, was set as the maximum DMI constraint. The net energy of lactation measures the total energy required by a dairy cow for maintenance and milk production and is specified in mega-calories (Mcal)., and the energy requirement for the lactating cow was calculated on a NE_l basis (Qu *et al.*, 2019). All mineral (i.e., Ca, Mg, P, K, S, Na, Fe, Mn, Zn, Se, Cu and Co) requirements are also specified in absolute units (g for macro and mg for micro minerals) and the requirements were calculated according to NRC (1989).

2) ***Prediction of Minimum Methane emission estimation Model:*** an equation was used to predict CH_4 emissions from lactating dairy cows. The minimum CH_4 model was formulated to minimize CH_4 emissions while delivering nutrients to sustain a given level of milk production. The best model (smallest deviance information criterion) fitted to predict CH_4 emissions used NDF and CP intakes as independent variables (Moraes *et al.*, 2015):

$$CH_4 = 4.59\ (0.68) + 1.46\ (0.16) \times NDF + 1.39\ (0.31) \times CP,$$

where CH_4 is the methane emission (MJ/cow/d), NDF is the NDF intake (kg of DM/cow/d), and CP is the CP intake (kg of DM/cow/d).

3) ***Estimation of allowable milk yield based NRC (2001)***:

i) Milk production based on CP = CP supply for lactation / CP Requirement for lactation where CP supply for Lactation = Total CP Supply - CP maintenance requirement. The Total CP supply = DMI (Kg/Cow/day) required* CP from the formulated ration.

ii) Milk production based on Net Energy = NE Supply for Lactation/ NE Requirement lactation where NE Supply for Lactation = DMI (Kg/Cow/day) required* Energy supply from the formulated ration.

iii) Therefore, there imbalance between the energy and protein based milk production was subtracted and became zero. That is OK, to check the fulfillment of the nutrient requirement.

4.3 Results

4.3.1 Dry matter intake and milk yield of lactating dairy cows under farmers feeding practice

In the existing feeding practice of the farmers in Aba Gerima used different variety of feed ingredients as compared to Guder site. This related to the availability of feed resources. The dairy cows in Aba Gerima has high DMI as compared to Guder (Table 4.3). This high total DMI of cows higher in Aba Gerima is related to the variety feeds consumed that can increase intake. The milk yield was higher (1.89 kg/d) for the cows feed in hay based supplemented with the different local brewery byproduct and wheat bran. In similar trend, the estimated methane emission also reduced for dairy cows feeding hay supplemented with other feed ingredient

There were clear variations among the farmers with respect to supplementation of the dairy cows at the beginning of the experiment. The variation was both in type and amount the supplemental feed. The supplemental feed mentioned includes sole wheat bran (12.7%), wheat bran with Atela (locally brewery) 43.3%, wheat bran and oil seed cake (20.7%) and combination of wheat bran Atella, Britie and oil seed cake (23.3%) at Aba Gerima. One of the major problems with dairy cattle supplementation in the area was absence of regular supplementation. The frequency of supplementation was depending on the availability of their feed ingredients. This might be due to the high CP and better digestibility of hay than straw based diet.

Table 4.3 Dry matter intake and milk yield of lactating dairy cow under existing farmers' management

Feed Ingredients	**Existing feeding practice diet**			
	Guder		***Aba Gerima***	
	Hay based	Straw base	Hay based	Straw base
Hay (kg/d)	3.17	-	3.74	-
Straw (kg/d)	-	4.01	-	5.51
Atella (kg/d)	-	0.41	0.74	0.6
Brintie (kg/d)	-	-	0.97	0.3
Wheat bran (kg/d)	-	-	1.65	1.3
Grazing	1.37	1.45	-	-
Common salt (kg/d)	0.029	0.03	0.02	0.022
Nutritive value of diet				
CP (g/kg)	69.72	58.3	61.2	57.1
NDF (g/kg)	756.6	442	667.6	621
NEm (Mcal/Kg DM)	2.01	2.54	1.84	2.47
NEl (Mcal/Kg DM)	1.68	2.32	1.71	2.15
Output				
DMI (kg DM/d DM)	4.23	5.02	6.04	6.42
Milk yield (L/day)	1.31	1.41	1.89	1.69
CH_4 (g/Kg DMI)	421.1	489.8	341.1	389.1
Cost/kg milk (USD)*	0.21	0.19	0.32	0.23

CP; crude protein; NDF, neutral detergent fiber; NEm, net energy maintenance; NEl, net energy lactation; DMI, dry matter intake; CH_4, methane. * No cost estimation for grazing.

4.3.2 Optimal formulated diet milk yield, cost and methane emission

The diet formulated by the least cost diet model showed the lowest for diet 3 ($3.16/cow/d) whereas the highest cost estimated for diets 11 ($4.37/cow/d). This might be related to the high cost of the feed ingredient in the diets such as wheat bran. The formulated diet 4 emitted the highest predicted CH_4 emission (450.1 g/kg DMI/cow) followed by diet 12. This could be due to the low digestibility of ingredients in the formulated diet; for example, in case of diet 4 the inclusion of natural pasture hay as a basal diet (Table 4.3). The minimum CH_4 estimation (260.1 g/kg DMI kg) is for the formulated a diet 3 at a cost of $3.16/cow/d and generated a predicted milk yield of 3.89 kg/cow/d. The reduction in CH_4 emissions was obtained by the manipulation of the nutrient composition of the diet and also by a reduction in DMI for the same level of milk production. In the least cost diet model, the hay, straw and improved forages were the main forage sources as basal diet. The least cost model reduced the selection of feeds rich in high fiber and increased the use of feeds rich in energy and protein, causing a reduction in CH_4 achieved by higher milk yield (Formulated diets 1-3).

Table 4.4 The formulated optimal diets cost, methane emission and milk yield

	Formulated Diets*											
Feed ingredients (kg/day)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Brachiaria hybrid hay (BhH)	5.23								1.23			
Napier grass hay (NGH)		6.23			3.23						1.34	
Treated teff silage (TTS)			7.23		4.43	7.23		3.20				
Natural pasture hay (NPH)				7.45			1.26	1.45			1.24	
Eleusine coracana straw							3.45			1.54		4.26
Wheat bran								1.03	1.35	1.28	2.01	
Zea mays Stover							1.65		5.78	4.50		1.23
Ficus thonningii leaves											0.72	
Ficus sur leaves									0.49			
Sesbania sesban leaves						0.67		0.27				
Formulated concentrate**	3.25	3.89	3.45	3.55	3.15	2.45	3.67	3.45	3.56	3.15	3.12	3.17
Ruminant premix	0.14	0.15	0.13	0.11	0.12	0.16	0.18	0.11	0.12	0.17	0.11	0.13
Output												
DMI (Kg/d/cow)	8.23	8.68	8.02	7.18	7.23	7.24	7.33	6.68	7.01	7.24	7.18	7.78
Diet cost ($/cow/d)	3.23	3.31	3.16	3.69	3.29	3.26	3.32	4.03	4.25	4.28	4.37	3.43
CH_4 (g/kg DMI/d)	340.4	350.1	260.1	450.1	368	379.5	379	395.6	332.3	367.3	382.4	426.6
Milk yield (kg/day)	4.23	3.83	3.79	2.03	3.07	2.35	3.23	3.03	2.67	3.43	3.63	3.73

Formulated Diets*, only top 12 formulated diets were selected from the list of options to present in this table; Formulated concentrate* is composed of Zea mays grain (4%), Guizotia abyssinica seed cake (49%), Triticum Aestivum (9%) and iodized salt (2%); DMI, dry matter intake (Kg/d/cow); Diet cost ($/cow/d); CH_4, methane (g/kg DMI/d); Milk yield (kg/day).

4.4 Discussion

4.4.1 Feed intake and milk yield under existing feeding practice

The high fed intake and milk yield of the cows in Aba Gerima site is due the variety of feed ingredients supplemented. This might be due the farmers has an access to purchase the wheat bran from Bahir Dar town. In general, feed intake in the farmers practice feeding is restricted the high fiber content of the diet that has impact on the capacity of the rumen (Zhao et al., 2015). Moreover, the diets have not fulfilled the maintenance and lactation requirement of the dairy cow. To fulfill the dairy cow requirements, a total dry matter intake supplied be at the rate of at least 3% of live weight. The assumption is that as per NRC (2001) the requirement of ME (kcal/kg) per kg milk production is 5.25 MJ.

The observed milk yield in both study sites were comparable with the finding of (Ketema, 2014) which indicated milk yield of 1.15 ± 0.386 L for local cattle in kersa malima distsrict in southern Ethiopia. And slightly lower than the finding of ((Yesihak, 2011)) reported 1.99 ± 0.77 kg/day for Ogaden breed at Haramaya University. What we observed in the ground of the dairy farmers practice use separate and different forms of feeding for the different concentrate ingredients.

4.4.2 Estimated methane emissions and milk yield from formulated diets

The high milk yield and the lower CH_4 emissions in the optimal formulated diet might be due the quality of the ingredients that improve the nutrient utilization efficiency of the dairy cow. More importantly, the high milk yield from the formatted diet also related to the ingredient composition that have high nutrient digestibility and feed intake (Asaduzzaman et al., 2018). In the present study, there was high difference in methane emission/DMI and estimated milk yield among the diets and this shows the variation in nutrient quality of the feed ingredients incorporated in the diet. The methane emission can be affected by the dietary composition of the cow consumed (Archimède et al., 2018).

4.4.3 Implication of formulated diet towards sustainable dairy production

A dairy cow producing 4.0 kg milk per day required 928.5 g/day of CP (NRC, 2001). This implies that the protein supply by the existing feeding practice is below the requirement for the potential milk yield. Whereas the formulated diets could be fulfilled the cow's protein requirement for optimum milk production at the maximum of 4.23 kg/day from formulated diet 1 (*Brachiaria* hybrid hay (BhH) based diet). The balanced diet formulation also enhance the utilization of poor quality feedstuffs can be improved by their incorporation in complete diet rather than feeding separately along with concentrate mixture to meet the maintenance, growth and lactation requirements of the dairy cow (Beigh et al., 2017). Therefore, the current lactating cows feeding practice should be supplemented with different quality feed ingredients to increase the milk production.

4.5 Conclusions

The existing feeding practice diet has no adequate nutrient for lactating dairy cow and produce low milk yield. This implies that the dairy cows should be supplemented for optimum milk production. The results of this study suggest that the inclusion of quality feed ingredients in the formulated diet could increase milk yield and reduce CH_4 emissions. Therefore, an opportunity for strategic feeding containing high quality ingredients reduce CH_4 emissions on the environment while maintaining milk yield as well as nutrient digestibility for lactating dairy cows in dryland environments. Thus, the optimal diet formulation result indicates that in combination of high quality feed ingredients it can be possible to fulfill the maintenance as well as the lactation requirement of lactating dairy cows. Based on the optimal feed formulation, Bracheria and Napier grass hay and treated teff straw diets were selected to improve milk yield of the indigenous lactating dairy cows. Further studies that aim to integrate feeds that have better nutritive values into the existing feeding system are required to evaluate feed intake, level of inclusion, and animal's responses for more efficient utilization of available feedstuffs for sustainable animal production. Moreover, the formulated diets should be tested and validated for the actual milk yield and methane emission.

Chapter 5:

Evaluation and validation of selected diets containing improved grasses hay and treated E*ragrostis tef* straw silage on milk yield, nitrogen utilization and methane emission

This chapter is largely adopted from submitted Manuscript as:

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5.1 Introduction

Limited availability of high-quality feed is the major stumbling block affecting dairy cattle production in tropical regions (Mediksa, 2017). Moreover, owing to an alarmingly rising human population, grazing lands being used for natural hay production for dry season are widely converted to croplands and plantations (Berihun *et al.*, 2019), thereby forcing cattle to graze on marginal and overgrazed lands with poor-quality forage (Kitaba and Tamir, 2007; Mekasha et al., 2014). This practice is further aggravated by the fact that the yield and nutritive value of tropical grasses decline sharply as the dry season approaches, leading to reduced feed intake and milk production in conventional grazing systems (Phelan et al., 2015; Ali *et al.*, 2019). In addition, compared with those elsewhere, dairy animals in developing countries produce more methane and excrete more nitrogen, primarily because of the feeding of low-quality roughage, which is unbalanced in nutrients (Garg et al., 2018). This situation is most severe in countries like Ethiopia which has a huge livestock population (CSA, 2017) and farmers typically feed dairy cows arbitrarily, without balancing the diet, thus decreasing potential milk production and increase methane emission (Mesfin and Kebede, 2011). Hence, proposing appropriate feeding regimen that enable to reduce methane emission and avoid environmental N pollution from dairy cattle production is still remain major concern (Nichols *et al.*, 2019; Ali *et al.*, 2019; Berhanu *et al.*, 2019). Moreover, the previous common feeding practice of dairy production is tropical region is separate feeding but in this study the total mixed ration (TMR) was used that allowed to incorporate the basal and supplemented feed ingredients for precision feeding (Uyeh et al., 2019).

One potential strategy for better feeding of dairy cows during periods of critical feed shortage particularly in the dry season is to use improved forage varieties and enhance the quality of available crop residues (Shapiro *et al.*, 2017). To this end, supporting the traditional feed resources with planted forages appears to be a plausible and sustainable solution (Berhanu *et al.*, 2019). This situation encourages the cultivation of suitable and adapted improved forages to the tropical regions such as Napier grass (*Pennisetum purpureum*) (Tessema and Baars, 2004; Mutimura et al., 2018) and *Brachiaria* hybrid (CIAT 36087) Mulato II grasses (Adnew et al., 2018) to fulfill the nutrient requirements of dairy cows for milk production (Nyambati et al., 2003). Napier grass is a fast-growing perennial grass widely grown for smallholder dairy production in tropical and subtropical regions (Teressa et al., 2017; Mutimura *et al.*, 2018). While *Brachiaria* hybrid grasses are the newest options for improving productivity in semi-intensive systems, and some of these cultivars are high-yielding, nutritious, and ecofriendly (Creemers and Aranguiz, 2019). As a result of these benefits, *Brachiaria* hybrid grasses have recently garnered considerable interest in Africa and have factored into several ongoing initiatives to support the emerging livestock industry in tropical regions, particularly to prepare hay for dry season (Ghimire et al., 2015; Wassie et al., 2018). However, despite the potential of these improved forages in various tropical regions, including Ethiopia (Adinew et al., 2019), information regarding their contribution to the performance of lactating dairy cows and methane emission is sparse.

As another option, crop residues inherently poor in nutritive values represent a large roughage feed resource for ruminant animals, especially during the dry season in tropical regions (Valbuena *et al.*, 2012). However, most smallholder farmers currently feed untreated crop residues, consequently diminishing dairy cows production performance (Kashongwe et al., 2014). Therefore, in the current study, we assessed whether treating teff straw with effective microbes, molasses, and urea would enhance the nutrient utilization of this available poor-quality roughage feed and performance of lactating dairy cows (Gulilat and Walelign, 2017; Saylor *et al.*, 2018).

In this study, from the formulated diet in chapter 4, the top 3 with high estimated milk yield (Diet 1, Bracheria grass; diet 2- Napier grass hay and diet 3-treated teff straw) and natural pasture hay (diet 4 - as a control) tested and validated. We hypothesized that using improved forage grasses hay and treated teff straw silage would increase the performance of dairy cows and reduce methane emission. Therefore, the objective of this study was to evaluate the effects of feeding improved grasses hay and treated teff straw silage as a basal diet on milk yield, nutrient intake and digestibility, dietary nitrogen utilization efficiency, plasma metabolites, and methane emission. Hence, for similar situation in Ethiopia, in which the feed priority in livestock master plan is focusing on introducing and promotion of improved forages to enhance dairy production (Shapiro *et al.*, 2017); this study proposed alternative improved forages hays basal TMR feeding regimen for critical feed-shortage periods in dry season. The findings will also contribute to inform policy makers and development practitioners regarding future feeding strategies for dairy production.

5.2 Materials and methods

5.2.1 Experimental location, cows and design

The study was conducted at the Andassa Livestock Research Center of the Amhara Region Agricultural Research Institute, Ethiopia (for detail site description see section 1.5).

We obtained eight Fogera dairy cows in their second lactation stage with 120 ± 18 days in milk, an average body weight of 238.3 kg, and 2.4 parity from Andassa Livestock Research Center dairy farm. The lactating dairy cows were placed in an individual pen in a well-ventilated barn with concrete floor and appropriate drainage slope. All cows were weighed and drenched with broad-spectrum anthelmintic before the start of the experiment. The experimental design was a replicated 4 x 4 Latin-square design (Kuehl, 2000). The experiment consisted of 4 treatments and 4 periods; the treatments were assigned randomly for dairy cows within each period. Each of the four periods had 2l days in duration, comprising 14 days of TMR dietary feed adaptation and 7 days of data collection. The experiment was conducted for a total of 12 weeks. All animal care and handling procedures were reviewed, and the experimental protocols were approved by the Andassa Livestock Research Center prior to conducting the experiment, and the animals were under constant observation of veterinarians.

5.2.2 Experimental dietary treatments and feed management

The four roughage basal dietary treatments supplemented with formulated concentrate in TMR based were: control (natural pasture hay (NPH)); treated teff straw silage (TTS); Napier grass hay (NGH), and brachiaria hybrid grass hay (BhH). The natural pasture hay was purchased from a private dairy farm and was composed predominantly of grass species (*Andropogon*, *Cynodon*, *Digitaria*, *Hyparrhenia*, and *Panicum* spp.) and legumes (*Trifolium quartinianum*, *T. polystachyum*, and *Indigofera atriceps*) as characterized by Denekew et al. (2005). The improved forages were planted according to the recommended agronomic practices for napier grass (accession number 1574) (Tessema and Baars, 2004) and *Brachiaria* grass (Accession number CIAT 36087) (Adnew *et al.*, 2018) in 2.65 ha of irrigated land at Andassa Livestock Research

Center. The forages were irrigated to support continuous growth for harvesting sufficient amounts of hay. The harvested forage was wilted from 2-3 days in the field before storage for the feeding trial. The hay was chopped into 2 to 5 cm length and mixing with the concentrate in the TMR. For chemical analysis, samples of each forage were taken and oven-dried to constant dry weight, ground to pass through a 2-mm screen and then stored in airtight plastic bags. For teff straw treated silage preparation; 1 L of effective microbes, 1 kg of molasses, 2.5 kg of urea, and 18 L of chlorine-free water were mixed and used to treat 50 kg of teff straw as described previously by Dejene et al. (2013). Treated teff straw silage was put into airtight plastic bags and well packed to avoid trapping air. To facilitate anaerobic fermentation, filled bags were kept at room temperature (25°C) for 21 days, as recommended for environmental conditions such as those in Ethiopian climate (Alemu et al., 2020). The formulated concentrate mixture was purchased from a private animal-feed factory company and comprised of maize (*Zea mays*) (40%), noug (*Guizotia abyssinica*) seed cake (49%), wheat bran (*Triticum Aestivum*) (8%), iodized salt (1%), and ruminant premix (2%). The ruminant premix (produced by INTRACO Ltd., Antwerp, Belgium) contained the following additives (per kg): Ca, 1310.5 g; Na, 192.2 g; Mg, 520.8 g; Fe, 5000 mg; Mn, 5000 mg; Zn, 10 000 mg; I, 150 mg; Se, 40 mg; Co, 15 mg; vitamin A, 999 750 IU; vitamin D3, 199 950 IU; vitamin E, 800 mg; butylated hydroxytoluene, 50 mg; and ethoxyquin, 55 mg).

Cows were fed a TMR consisting of 70% roughage and 30% on a DM basis and formulated to meet the maintenance and lactation nutrient requirements of dairy cows for targeted milk yield (4 kg/d) (NRC, 2001). For feed allowance, the total dry-matter intake (DM) of a mature lactating dairy cow was assumed to be equivalent to 3% of its body weight (NRC, 2001). The TMR diets were offered in the morning (8:00 h), at noon (12:00 h), and during the afternoon (16:00 h). Cows had individual and free access to drinking water throughout the entire experiment. The chemical composition of feed ingredients and the TMR diets are presented in Table 1. The acid detergent lignin (ADL)/neutral detergent fiber (NDF) ratio of the dietary treatments for NPH, TTS, NGH and BhH is 0.13, 0.17, 0.22, and 0.25, respectively.

Table 5.1 Chemical compositions of feed ingredients and the experimental total mixed ration diets.

Feed ingredient	DM	OM	CP	NDF	ADF	ADL	GE	ME	NEm	NE$_l$
Natural pasture hay	878.6	869.9	41.8	741.8	512.1	76.5	15.5	8.0	1.44	1.27
Treated teff straw silage	660.7	618.3	115.5	644.2	454.3	49.8	16.2	7.0	0.98	1.03
Napier grass hay	856.9	837.7	92.5	626.5	450.6	83.1	14.1	7.2	1.01	0.82
Brachiaria grass hay	822.7	817.9	135.9	564.2	362.6	44.6	15.8	8.0	1.33	0.99
Formulated concentrate	910.4	904.9	201.6	340.6	153.8	60.3	18.3	9.2	1.47	1.22
Treatment TMR diets (g/kg DM)										
NPH	901.2	892.4	122.9	541.2	333.0	68.4	16.9	8.5	1.45	1.25
TTS	690.1	604.4	158.1	492.4	304.0	55.0	17.3	8.1	1.23	1.13
NGH	863.6	839.3	147.8	483.6	302.2	71.7	16.2	8.2	1.24	1.02
BhH	866.5	827.9	168.	452.4	258.2	52.2	17.0	8.6	1.40	1.11

DM, dry matter (g/kg as fed); OM, organic matter (g/kg DM); CP, crude protein (g/kg); NDF, neutral detergent fiber (g/kg DM); ADF, acid detergent fraction (g/kg DM); ADL, acid detergent lignin (g/kg DM); GE, gross energy (MJ/kg DM); ME, metabolizable energy (MJ/kg DM); NEm, net energy for maintenance (Mcal/kg DM); NEl, net energy for lactation (Mcal/kg DM); NPH, natural pasture hay; TTS, treated teff straw (DM/As fed); NGH, Napier grass hay; BhH, Brachiaria hybrid grass hay.

5.2.3 Measurements and sample collection

TMR feed offered and orts were recorded daily at each feeding time over the whole experimental period. From day 15 to 21 of each experimental period, TMR offered and orts samples were collected and stored for laboratory analysis. Daily milk yields for all 8 cows were recorded at each milking time. Cows were hand milked twice daily (08:00 h and 16:00 h) throughout the experiment. During each period (15 to 21 days), milk samples were collected at milking time and pooled by treatment group and transported in an ice box for milk composition analysis. Another pooled milk sample for each period was stored immediately at –20°C for determination of milk urea nitrogen (MUN) content.

Spot urine samples from all cows were collected in plastic containers between days 15 and 21 of each experimental period and immediately stored at –20°C for determination of N and creatinine contents. A sample of 100 mL was collected for every cow and 8 mL of an aqueous solution of sulfuric acid 10% (v/v) added in which outflowing air was led to trap aerial ammonia and reduce urine pH to below 3, as described by Castro-Montoya et al. (2019). Fecal samples were collected on days 15 through 21 of each experimental period by direct sampling from the rectum of each cow in the morning and afternoon. A composite fecal samples from each cow in each period were maintained at -20°C until laboratory analysis.

Blood samples were collected on the 21th day of each experimental period after feeding of cows from the jugular veins of individual animals into 10-mL sodium heparin and potassium EDTA vacuum tubes. The blood samples were pooled over sampling time points according to cow and period; plasma was prepared by centrifuging blood at 1000 × g for 5 min at 23°C; the supernatant was transferred to identified plastic tubes and stored at –20ºC until laboratory analysis. Rumen fluid samples were collected from each cow two hour after the morning feeding on day 21th day of each experimental period by inserting a rumen-fluid collector through esophageal gavage with manual sucker (Steiner et al., 2014). The samples were temporarily placed on ice and then processed for ruminal ammonia nitrogen analysis. The body weight (BW) of each dairy cow was weighed at the beginning, middle, and end of each period for the whole experiment time by using a ground weight balance; the weight of cows was obtained by averaging weighing taken before feeding and after milking on two successive days.

5.2.4 Laboratory analyses and procedures

All feed and fecal samples were oven-dried at 60°C for 48 h. The dried samples were ground to pass through a 2-mm screen and stored in plastic bags for subsequent determination of chemical components. The DM and organic matter (OM) contents of the diets and feces were determined according to (AOAC, 1990). The CP concentration was estimated by multiplying the N concentrations by 6.25 and N concentrations were determined according to the Kjeldahl method (AOAC, 1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents of feed and feces were determined according to the procedures of Goering and Van Soest (1970). Metabolizable energy (ME) concertation (MJ/kg DM) was estimated on the basis of 24-h gas production (mL) from *in vitro* gas fermentation (Menke, 1988);

$$ME = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0029 \times CP^2 \quad (1)$$

Where GP is the 24-h gas production volume (mL/0.2 g DM) and CP is crude protein content ((%)of the feed. Estimated net energy maintenance (NEm) and net energy lactation (NE_l) were calculated according to the following equation (NRC, 2001):

$$NEm = ME\ (0.554 + 0.287 \times ME / GE) \quad (2)$$

$$NE_l = ME \times (0.4632 + 0.24 \times ME / GE) \quad (3)$$

Where the gross energy (GE) concentrations of feed was determined by using a bomb calorimeter (CA-4AJ, Shimadzu Corporation, Kyoto, Japan). For the TMR, these parameters were calculated according to the ratio composition of roughage and concentrate ingredients. Analysis of in vitro gas production for the feed samples was conducted at Shimane University (Japan) as the procedure described in detail in previous publication by Mekuriaw et al. (2019).

Feed intake was determined on days 16 through 21 of each period as the difference between the weight of the TMR feed offered to animals and that left unconsumed.

The nutrient intake (kg/d) = ([DM offered (kg/d) × % of nutrient in TMR] – [DM remaining (kg/d) × % of nutrient in orts]) (4)

The nutrient digestibility was calculated as (De Seram et al., 2019):.

([nutrient intake (kg/d) – fecal nutrient output (kg/d)] / nutrient intake) × 100% (5)

Milk fat, protein, and lactose contents were determined by using a LactoScan milk analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria). Fat and protein corrected milk production (kg/d) was calculated according to De Koster et al. (2019):

FPCM = (0.337 + 0.116 × milk fat [%] + 0.06 × milk protein [%]) × kg of milk yield (6)

The milk yield efficiency was calculated according to (Nichols *et al.*, 2019):

Efficiency = milk yield (kg) / DMI (kg) (7)

The milk urea nitrogen (MUN) concentrations were obtained through spectrophotometric enzymatic colorimetric methodology using commercially available kits (urease-GIDH method, λ = 525 nm; product code 410-55391 _ 418-55191, Fujifilm, Wako, Japan).

Urinary creatinine concentrations were determined by using commercial enzymatic colorimetric assay kits (sarcosine oxidase method, λ= 515 nm; product code 439-90901, Fujifilm, Wako, Japan) and UV-VIS spectrophotometry (DR6000, Hach, Dusseldorf, Germany). Total daily urine volume was estimated by dividing daily urinary creatinine excretions by the observed creatinine concentration of spot urine samples, assuming a daily creatinine excretion of 0.197 ± 0.047 mmol/kg BW (Jardstedt et al., 2017). Urinary N was analyzed according to the Kjeldahl method (AOAC, 2000) by using the same equipment for feed, feces, and milk samples. Then, daily urinary N excretion was calculated by multiplying urinary N by urine volume.

Nitrogen excreted in milk was calculated by using the equation developed by Silva et al. (2018):

Milk N (g/d) = milk CP concentration (g/kg) × milk yield (kg/d) / 6.38 (8)

Milk nitrogen efficiency (MNE) was calculated based the following equation (Nichols *et al.*, 2019)

MNE = (N milk / N intake) × 100% (9)

The N excreted in feces was determined as (Silva et al., 2018):

Fecal N (g/d) = CP in feces (g/kg) × DM fecal excretion (kg/d) / 6.25 (10)

Fecal output was estimated by using chromium oxide as an external indicator according to Kimura and Miller (1957). N balance was obtained by subtracting the values for N in urine, feces, and milk from the total N intake in grams (Silva *et al.*, 2018).

N retention = N intake − N fecal − N urine − N milk (11)

Plasma underwent determination of glucose, non-esterified fatty acid (NEFA), β-hydroxybutyrate (BHBAA), and plasma urea nitrogen (PUN) contents. These parameters were measured by using commercial enzymatic colorimetric assays (glucose: mutarotase-GOD method, product code 439-90901, λ = 455 nm; NEFA: ACS-ACOD method, product code 279-75401, λ = 550 nm; BHBAA, cyclic enzyme method, product code 279-75401, λ = 405 nm; and BUN: urease-GIDH method, product code 410-55391 _ 418-55191, λ = 340 nm; all from Fujifilm) and a UV–VIS spectrophotometer (DR6000, Hach). Immediately after collection, rumen fluid was tested for pH by using a handheld portable pH meter and then stored at −20°C until analysis of ruminal ammonia nitrogen concentration. For ruminal fluid samples that were preserved with 1% H_2SO_4, the rumen fluid collected was centrifuged at 2000×g for 15 minute and the resulting supernatant was analyzed for ruminal ammonia nitrogen concentration as described previously (Smith and Murphy, 1993).

5.2.5 Estimation of enteric methane emission

The enteric methane emissions of lactating dairy cows were calculated according to a recommended intercontinental equation Mutian et al. (2018):

CH_4 production (g/day per cow) = 124 (±10.44) + 13.3 (±0.32) × DM intake (DMI, kg/day) (12)

This estimation equation was chosen because of its low root mean square prediction error (RMSPE = 17.5) compared with those of other equations in the same publication. Correspondingly, Appuhamy et al. (2016) also evaluated the performance of more than 40 empirical models in predicting enteric CH_4 emissions and suggested that using DM intake alone can be sufficient for satisfactory enteric methane emission prediction.

5.2.6 Statistical analysis

Data regarding feed intake, nutrient digestibility, nitrogen balance, plasma metabolites, milk yield, and milk composition were analyzed through analysis of variance (ANOVA) using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The model is:

$$Yijk = \mu + Ti + Pj + Ck + (Ti \times Pj) + \varepsilon ijk, \qquad (13)$$

Where Y_{ijk} represents the observation on cow k given treatment i at period j; μ is the intercept;

T_i represents the fixed effect of the i^{th} diet treatment, i = 1 to 4;

P_j represents the fixed effect of the j^{th} period, j = 1 to 4;

C_k represents the random effect of the k^{th} cow, k = 1 to 8;

$TPij$ is the interaction between the i^{th} treatment and j^{th} period, and

ε_{ijk} is the random residual error.

The model contained treatment and period as fixed effects and cow as a random effect. Differences among the means were considered significant at the $p \leq 0.05$ level according to Tukey's test. To assess the relationships among plasma urea concentration, milk urea concentration, and ruminal ammonia N; as well between CH_4 and GE intake (GEI), and milk yield, regressions were fitted by using individual cow data within all periods and dietary treatments.

5.3 Results

5.3.1 Feed intake and nutrient digestibility

The nutrient intake of the lactating Fogera dairy cows fed the different roughage based TMR diets, and their digestibility, are presented in Table 2. Compared with those given NPH, cows consumed TTS, NGH, and BhH diets had higher ($p = 0.012$) DMI by 1.50, 1.89, and 2.63 kg/d, respectively. Cows fed with BhH had the highest DM, OM and CP intake followed by NGH and TTS ($p = 0.011$). Likewise, cows fed with BhH, NGH, and TTS had higher ($p < 0.05$) DM, OM, and N diet digestibility than those fed with NPH. Cows fed with BhH had increased average daily gain (ADG) ($p = 0.02$) followed by NGH and TTS, as compared to NPH. The digestibility of N increased ($p < 0.01$) by 27.79%, 21.7%, and 39.5% from NPH to the TTS, NGH, and BhH diets, respectively. On the other hand, cows fed NPH had higher NDF ($p = 0.031$) and ADF ($p = 0.014$) intake whereas lower NDF ($p = 0.027$) and ADF ($p = 0.012$) digestibility than those fed the other TMR diets. Cows fed with BhH had the highest GE ($p = 0.005$) and ME ($p= 0.004$) intake followed by NGH and TTS.

Table 5.2 Feed intake and nutrient digestibility of lactating dairy cows fed natural pasture hay, treated teff straw, Napier grass hay, and *Brachiaria* hybrid grass hay.

Item	**Dietary treatment**				**SEM**	**P**
	NPH	**TTS**	**NGH**	**BhH**		
Intake (kg/d)						
DM	6.2^{c}	7.7^{b}	8.1^{b}	8.8^{a}	0.56	0.012
OM	5.6^{c}	6.6^{b}	7.2^{ab}	8.0^{a}	0.46	0.001
CP	0.8^{c}	1.0^{b}	1.0^{b}	1.1^{a}	0.15	0.011
NDF	4.3^{a}	3.9^{b}	2.6^{c}	2.8^{a}	0.29	0.031
ADF	2.5^{a}	2.6^{a}	2.0^{b}	1.3^{c}	0.08	0.014
GE (MJ/d)	104.9^{c}	133.0^{b}	131.2^{b}	150.6^{a}	9.4	0.005
ME (MJ/d)	53.4^{b}	39.6^{b}	42.0^{b}	76.0^{a}	8.30	0.004
DMI, g/kg $BW^{0.75}$	97.6^{b}	115.6^{b}	124.1^{b}	153.9^{a}	0.01	0.003
ADG (g/day)	326.2^{b}	446.4^{b}	485.7^{b}	627.5^{a}	27.08	0.002
Nutrient digestibility (%)						
DM	64.2^{c}	79.6^{b}	80.6^{b}	87.7^{a}	4.95	0.031
OM	58.9^{c}	71.4^{b}	74.1^{ab}	79.4^{a}	4.35	0.021
N	59.7^{c}	76.2^{b}	72.7^{b}	83.3^{a}	4.94	0.033
NDF	48.1^{c}	52.9^{b}	54.2^{a}	56.5^{a}	1.77	0.027
ADF	47.7^{c}	49.1^{b}	50.5^{b}	53.4^{a}	1.22	0.012

NPH, natural pasture hay; TTS, treated teff straw; NGH, napier grass hay; BhH, *brachiaria* hybrid grass hay; SEM, standard error of mean; DM, dry matter (g/kg as fed); OM, organic matter (g/kg DM); CP, crude protein (g/kg); NDF, neutral detergent fiber (g/kg DM); ADF, acid detergent fiber (g/kg DM); GE, gross energy (MJ/kg DM); ME, metabolizable energy (MJ/kg DM); DMI (g/kg $BW^{0.75}$), dry matter intake in metabolic body weight; ADG, average daily gain; DM, dry matter; N, nitrogen; $^{a–c}$ Means within a row with no common superscripts differ ($p < 0.05$).

5.3.2 Nitrogen balance and utilization efficiency

Nitrogen intake increased by 61.8%, 57.6%, and 97.4% with the TTS, NGH, and BhH diets, respectively, compared with the NPH diet (p = 0.013; Table 3). Nitrogen retention showed a similar trend and was greater (p = 0.025) by 174.0%, 160.2%, and 264.2% for cows fed TTS, NGH, and BhH, respectively, than those fed NPH diet. The milk nitrogen of cows fed TTS, NGH, and BhH was increased (p = 0.023) by 6.8, 9.9, and 15.1 g/d, respectively, over that for those fed the NPH diet. The urinary N: fecal N ratios decreased (p = 0.015) by 10.5%, 34.6%, and 38.2% from NPH to TTS, NGH, and BhH, respectively. Urinary N: N intake ratios showed a similar trend and were decreased (p = 0.045) by 29.5%, 44.0%, and 53.0% NPH for TTS, NGH, and BhH, respectively, compared with those with the NPH diet.

Table 5.3 Nitrogen utilization and balance of lactating dairy cows fed natural pasture hay, treated teff straw, Napier grass hay, and *Brachiaria* hybrid grass hay.

Item	**Dietary treatment**				**SEM**	***P***
	NPH	**TTS**	**NGH**	**BhH**		
Nitrogen balance (g/d)						
N intake, g/d	121.0^{c}	195.6^{b}	190.6^{b}	238.7^{a}	24.1	0.013
Fecal N	42.8^{a}	30.0^{c}	33.1^{b}	35.1^{b}	4.5	0.012
Urinary N	25.7^{b}	29.3^{a}	22.7^{c}	23.8^{c}	1.9	0.041
Milk N	12.9^{c}	19.6^{b}	22.8^{ab}	28.0^{a}	2.3	0.023
N retention	39.7^{d}	92.3^{b}	87.6^{c}	125.1^{a}	17.5	0.025
Nitrogen utilization efficiency (g/g)						
Fecal N / N intake	0.35^{a}	0.28^{b}	0.30^{b}	0.27^{b}	0.02	0.031
Urinary N / N intake	0.21^{a}	0.15^{a}	0.12^{b}	0.10^{b}	0.02	0.045
Urinary N / fecal N	0.60^{a}	0.54^{b}	0.39^{c}	0.37^{c}	0.06	0.015
Milk N / N intake	0.11	0.10	0.12	0.12	0.01	0.367
N retention / N intake	0.33^{c}	0.47^{b}	0.46^{b}	0.51^{a}	0.05	0.01

NPH, natural pasture hay; TTS, treated teff straw; NGH, napier grass hay; BhH, *brachiaria* hybrid grass hay; SEM, standard error of mean; $^{a–d}$ Means within a row with no common superscripts differ (p < 0.05).

5.3.3 Plasma metabolites and ruminal fermentation characteristics

Cows fed TTS had the highest ($p = 0.015$) plasma urea nitrogen concentration among the dietary treatments (Table 4). In contrast, the lowest ($p = 0.014$) plasma glucose content was recorded for TTS than other diets. Remarkably, cows fed NPH had higher ($p \leq 0.05$) NEFA and BHBA than those fed other diets. Ruminal ammonia N was higher ($P = 0.013$) in cows fed NGH than in those fed the other diets. Rumen pH was not affected by dietary treatments ($p \geq 0.05$). Ruminal ammonia N was positively correlated with plasma urea nitrogen ($R^2 = 0.53$; Figure 1a).

Table 5.4 Plasma concentrations (mmol/L) of metabolites, and ruminal fermentation characteristics in lactating dairy cows fed natural pasture hay, treated teff straw, Napier grass hay, and Brachiaria hybrid grass hay.

Item	**Dietary treatment**				**SEM**	**P**
	NPH	**TTS**	**NGH**	**BhH**		
Plasma urea nitrogen	3.0^{b}	3.6^{a}	2.7^{b}	2.6^{b}	0.23	0.015
Plasma glucose	2.9^{b}	2.6^{c}	2.7^{b}	3.8^{a}	0.40	0.014
NEFA	0.5^{a}	0.4^{b}	0.3^{b}	0.3^{b}	0.39	0.017
BHBA	0.2^{a}	0.05^{b}	0.05^{b}	0.04^{b}	0.06	0.045
Ruminal ammonia N	2.3^{c}	2.7^{b}	3.3^{a}	3.0^{b}	0.21	0.013
Rumen pH	6.6	6.5	6.9	6.7	0.33	0.364

NPH, natural pasture hay; TTS, treated teff straw; NGH, Napier grass hay; BhH, *Brachiaria* hybrid grass hay; SEM, standard error of mean; NEFA, non-esterified fatty acids; BHBA, β hydroxybutyrate; N, nitrogen; a–c Means within a row with no common superscripts differ ($p < 0.05$).

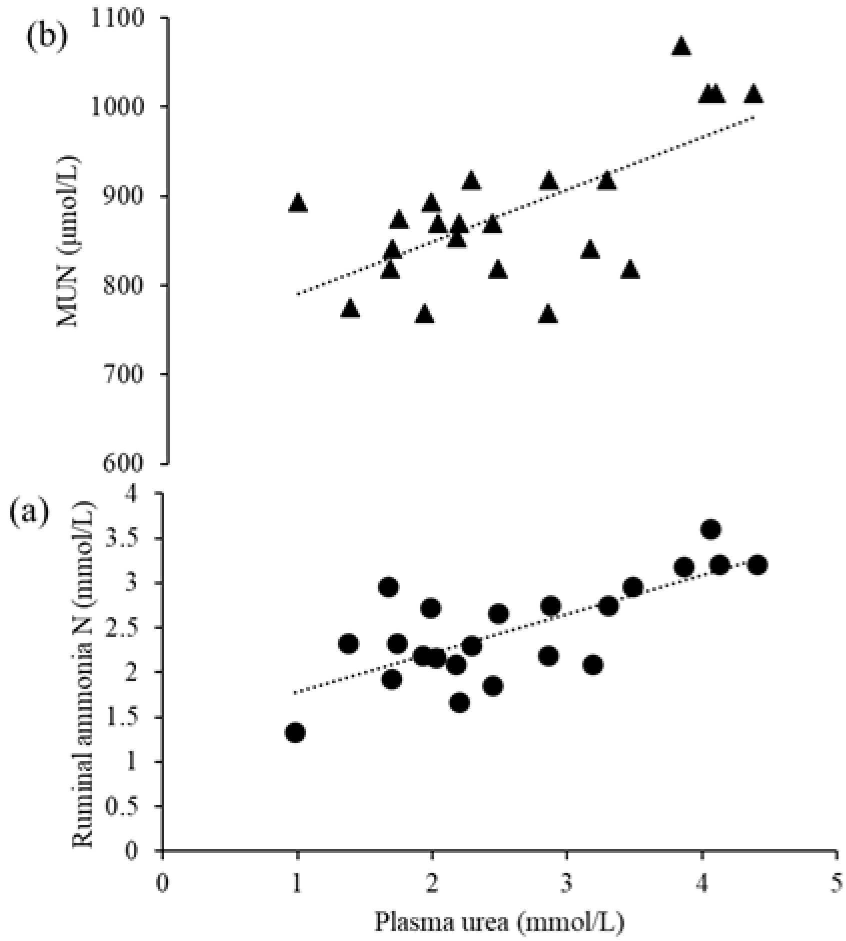

Figure 5.1 (a) Plasma urea nitrogen relative to ruminal ammonia N (ruminal ammonia N = 0.438 × plasma urea nitrogen + 1.3416; R2 = 0.53); (b) plasma urea nitrogen relative to milk urea nitrogen (MUN = 57.507 × plasma urea nitrogen + 734.86; R2 = 0.45).

5.3.4 Actual milk yield and validation of estimated milk composition

Compared with that in cows fed NPH, daily milk yield was increased (p = 0.013) by 31.98%, 52.97%, and 71.60% in cows fed TTS, NGH, and BhH, respectively (Table 5). Fat and protein corrected milk showed a similar increasing ($p < 0.016$) trend among the dietary treatments (Table 5). Although dietary treatment had no effect on the concentrations of milk fat ($p = 0.158$) and protein ($p = 0.063$), TTS and NGH increased (p = 0.039) milk lactose, and MUN was greater (p = 0.041) in cows fed TTS compared with other diets. Cows fed NGH has higher milk nitrogen concertation (MUN) compared with other diets. The MUN was positively correlated with plasma urea (R^2 = 0.45) (Figure 1b). The TTS, NGH and BhH diets has increased (p = 0.032) the efficiency of milk production of lactating dairy cows compared with NPH, by 6.13%, 25.94%, and 23.55%, respectively. For the validation of the estimated milk yield using the formulating diet in Chapter 4, there is positive relationship between actual and estimated milk yield with formulated diet (R^2 = 0.61) (Figure 5.2). However, the NRC (2001) equation estimation more milk yield than the actual. Thus, further research needed considering the coefficients (e.g CP = 83 g/kg milk).

Table 5.5 Milk yield, composition, and urea content of lactation dairy cows fed natural pasture hay, treated teff straw, Napier grass hay, and Brachiaria hybrid grass hay.

Item	**Dietary treatment**				**SEM**	***P***
	NPH	**TTS**	**NGH**	**BhH**		
Yield						
Milk (kg/d)	1.8^{c}	2.3^{b}	2.8^{b}	3.3^{a}	0.33	0.013
FPCM (kg/d)^{d}	2.0^{c}	2.7^{b}	2.9^{b}	3.4^{a}	0.38	0.016
Composition (%)						
Fat	5.5	6.4	5.7	5.4	0.13	0.158
Protein	2.8	2.8	2.9	2.9	0.16	0.063
Lactose	4.2^{c}	4.8^{a}	4.6^{ab}	4.2^{b}	0.23	0.039
MUN (µmol/L)	858.6^{b}	975.6^{a}	837.2^{b}	879.5^{b}	37.8	0.041
Efficiency	0.28^{b}	0.29^{b}	0.35^{a}	0.34^{a}	0.02	0.032

BhH, *Brachiaria* hybrid grass hay; FPCM, Fat- and protein-corrected milk; MUN, milk urea nitrogen; NGH, Napier grass hay; NPH, natural pasture hay; SEM, standard error of mean; TTS, effective microbe–urea–molasses-treated teff straw; $^{a–c}$ Means within a row with no common superscripts differ ($P < 0.05$).

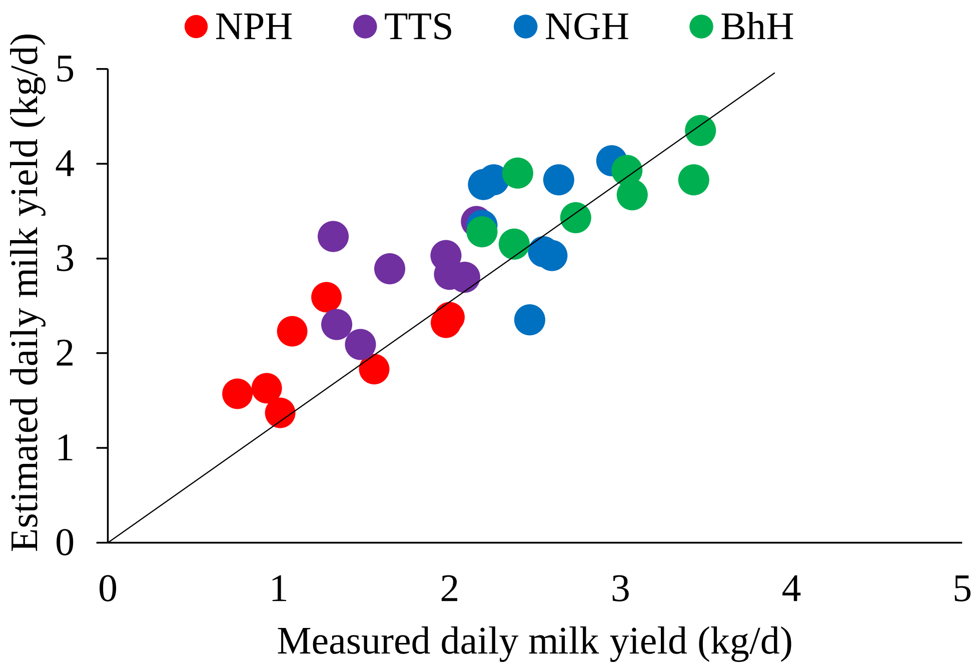

Figure 5.2 Relationship between measured and estimated milk yield with formulated diets

5.3.5 Estimated enteric methane emission

Estimated methane production increased with increasing DMI by 19.95, 25.14, and 34.98 g/d/cow for TTS, NGH, and BhH, respectively, compared with NPH (p = 0.011; Table 6). In comparison with that for the NPH diet, CH_4 / DM intake decreased (p = 0.014) by 11.67%, 14.0%, and 17.85% for TTS, NGH, and BhH, respectively. Similarly, cows fed TTS, NGH, or BhH produced less CH_4 per kg of total OM (p = 0.032) and GE (p = 0.025) intake than those fed the NPH diet. In reflecting the difference in DMI per milk yield, CH_4 per unit of milk yield (p = 0.015) and FPCM (kg) (p = 0.021) were lower for TTS, NGH, and BhH than NPH diet. The amount of CH_4 emitted per unit of DMI was negatively linearly correlated (R^2 = 0.76) with milk yield production across the dietary treatments (Figure 5.3). This finding similarly showed lower methane production with higher milk yield from cows fed BhH compared with other diets (p = *0.015*).

Table 5.6 Methane emission of lactating dairy cows fed TMR diets

Item	Dietary treatment				SEM	P
	NPH	TTS	NGH	BhH		
CH_4 (g/d)	206.5[d]	226.5[c]	231.7[b]	241.6[a]	7.66	0.011
CH_4 / $BW^{0.75}$ (g/kg)	3.0	3.2	3.3	3.4	0.08	0.214
CH_4 / feed intake or milk yield (g/kg)						
CH_4/DM intake	33.3[a]	29.4[b]	28.6[b]	27.3[b]	1.44	0.014
CH_4/OM intake	36.9[a]	32.6[b]	32.2[b]	30.7[b]	1.28	0.032
CH_4 /Milk yield	116.5[a]	96.9[b]	79.5[c]	71.1[c]	9.09	0.015
CH_4 /FPCM	84.1[a]	69.6[b]	65.2[b]	38.1[c]	8.85	0.021
CH_4/GE intake(MJ/MJ)*	0.10[a]	0.08[b]	0.08[b]	0.07[b]	0.01	0.025

NPH, natural pasture hay; TTS, treated teff straw; NGH, Napier grass hay; BhH, *Brachiaria* hybrid grass hay; SEM, standard error of mean; CH4, methane; BW0.75, metabolic body weight; DM, dry matter; OM, organic matter; GE, gross energy; FPCM, fat- and protein-corrected milk yield; [a-d] Means within a row with no common superscripts differ ($p < 0.05$).

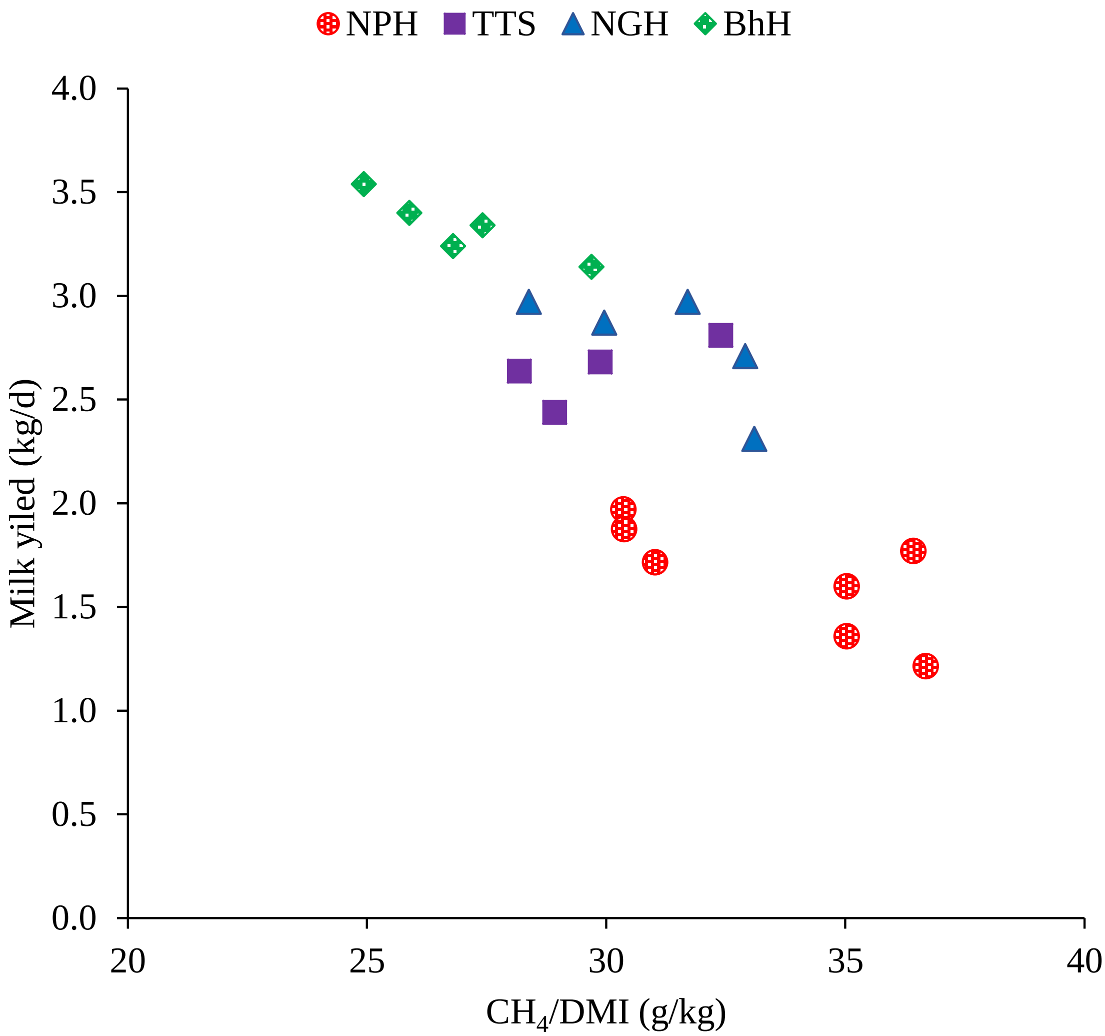

Figure 5.3 CH_4 emissions per dry matter intake (DMI) corresponding to daily milk yield. BhH, *Brachiaria* hybrid grass hay; TTS, treated teff straw; NGH, Napier grass hay; NPH, natural pasture hay.

5.4 Discussion

5.4.1 Feed intake and nutrient digestibility

Our findings support the use of improved forages (BhH and NGH) and TTS as a basal TMR diet to increase feed intake and digestibility of nutrients compared to that of NPH. Dry matter intake increased as dietary CP content increased because of the inclusion of TTS, NGH, or BhH as nitrogen sources in a basal diet. Similarly, Mutimura *et al.* (2018) reported that the differences in DMI of Napier grass and *Bracheria brizantha* fed as sole diets are related to the dietary composition of grasses. In this study, among the dietary treatments, improved grasses hay (BhH and NGP) followed by TTS based TMR diets have high potential of nutrient digestibility over NPH diet. This could be related to the better palatability and higher fermentable CP that NPH because CP of these four TMR diets have wide variation (Table 1). In supporting this, Moreira et al. (2004) reported that the CP content of a forage is closely correlated to its digestibility in which the higher CP contents of feed increased digestibility. However all the TMR diets in this study with the ratio of forage to concentrate (70:30), the CP and ME of actual TMR diets are sufficient for the nutrient requirement of lactating dairy cows for Indigenous dairy cows breed in tropical region (NRC, 2001). In our current study, NDF digestibility increased from NPH (48.1%) to BhH (56.5%) in TMR diets. These differences in digestibility of TMR diets likely altered diet retention time in the rumen and reflect differences in the lignin concentration of the forages (Christensen et al., 2015), given that the digestibility of a feed is influenced by the composition of the other feeds consumed with the TMR (Henderson et al., 2015). Similarly, the inclusion of improved forages in the TMR diet affects DMI through their influence on nutrient digestibility (Sánchez et al., 2006). Moreover, the higher intake of TTS as compared to NPH may reflect increased palatability due to the effective microbes, urea and molasses added to this TMR diet(Alemu *et al.*, 2020). Thus, our findings support the use of improved forages (BhH and NGH) and TTS as basal TMR diet to increase feed intake and digestibility of nutrients than NPH.

5.4.2 Nitrogen excretion and utilization efficiency

In this study, the dietary TMR treatments influenced nitrogen intake, excretion, balance, and utilization the efficiency for milk production (Table 5.3). Particularly striking is our finding that feeding TTS, NGH, or BhH hay redirected the N excretion pathway from urine to feces a change that can benefit the environment. Urinary N is more volatile and harmful to the environment than fecal N because the urinary urea N is inorganic N; microbial ureases rapidly hydrolyze urinary urea N to ammonium, which is then converted to ammonia, leading to N loss from the farm to the environment (Koenig and Beauchemin, 2018). In agreement with our result, previous studies also reported that dairy cows fed large quantities of high-quality fresh forage or hay shift their excretion of N from urine to feces and thus toward sustainable dairy production (Hymes-Fecht et al., 2013; Ghelichkhan *et al.*, 2018). The urinary N to fecal N ratio decreased from NPH to the TTS, NGH, and BhH diets; this indicates that using treated teff straw silage and improved forages as a basal diet in the TMR reduces urinary N excretion and indirectly mitigates ammonia emission in dairy cows for smart farming that is environmentally-friendly. Furthermore, N balance also varied among the dietary treatments we evaluated, in which the cows fed TTS, NGH and BhH retained increasingly more N than those fed NPH. This variation might be due to the difference in N intake, digestibility and excretion of the TMR diets fed for dairy cows. Therefore, the improved forages and treated tef straw based TMR diets we used as basal diets for dairy cows increased protein utilization efficiency and decreased the amount of N excreted to the environment per kg of milk produced. This indicates that inclusion of improved forages in the TMR diets of dairy cows can improve N efficiency (Ghelichkhan *et al.*, 2018).

5.4.3 Plasma metabolites and rumen fermentation characteristics

The plasma urea nitrogen concentrations that we obtained (2.6–3.5 mmol/L) are within the acceptable values for lactating dairy cows (2.6–7.0 mmol/L) (Morales et al., 2016). We attribute the lower plasma urea nitrogen concentrations recorded for the BhH and NGH diets to better utilization of nitrogen in the rumen; because the end-product of protein in the rumen is ammonia N, then ammonia N is absorbed by rumen epithelial cells and transferred to liver to form urea, which is released to blood in the form of blood urea (Kan and Meijer, 2007). This might be because the higher values of ruminal NH3-N in BhH and NGH diets were reflecting the higher plasma urea N in this study. The plasma glucose values in this study (2.55-3.77 mmol/L) is under the range of 2.5 - 4.2 mmol/L for lactation dairy cows and above the level (2.29 mmol/L) that cause hypoglycemia in early lactation cows (NRC, 2001). However, the highest plasma glucose concentration recorded for the BhH TMR diet in this study might be due to a positive energy status of the cow related to a higher DMI (NRC, 2001). With the exception of the higher value for cows fed the NPH diet, plasma concentrations of NEFA and BHBA in our study were within the ranges proposed for mid-lactation dairy cows (NRC, 2001; Ospina et al., 2013). The high NEFA concentration of the NPH diet (0.5 mmol/L) was likely due to low DMI. Previous reports of the negative correlation of DMI with NEFA and BHBA support our current results (Djoković et al., 2017). The NEFA concentration of cows fed NPH in our study exceeded the threshold (NEFA ≥ 0.5 mmol/L) that may be associated with milk loss, thus perhaps accounting for the low milk production of this treatment group (Djoković *et al.*, 2017). Furthermore, the maximal BHBA (0.22 mmol/L) of cows fed NPH in our study was below the threshold proposed by Gruber and Mansfeld (2019) (≥1.2 mmol/L).

We found here that the increase in ruminal ammonia nitrogen concentration was directly associated with the observed plasma concentration (Figure 1a). In agreement with our finding, Migliano et al. (2016) likewise reported a strong positive correlation between ruminal ammonia N and plasma urea nitrogen. The inclusion of improved forages in TMR diets might contribute for highly degradable protein sources that decreased ruminal ammonia N concentrations but ruminal ammonia N likely remained adequate for microbial growth (Prakash et al., 2013). The ruminal pH that we obtained is normal for lactating dairy cows fed roughage-based diets (Ghelichkhan *et al.*, 2018), perhaps because the dietary NDF concentrations of all of the dietary treatments were sufficient to maintain the optimal ruminal pH for cows fed high-forage diets

5.4.4 Milk yield and methane emission

The milk yield in the cows fed BhH was almost double that with the NPH diet. These findings indicate the promise of improved forages inclusion in the TMR diets such as *Brachiaria* hybrid cultivars, for increasing the milk production of indigenous dairy cows in tropical region.

In contrast, the lowest milk yield in this study was for the cows fed the NPH diet; this might be related to the low dietary CP in this TMR, as well as the decreased DMI. Moreover, Hussien et al. (2013) reported even lower milk yield (1.4 kg/d) for Fogera dairy cows fed natural grass hay a basal diet supplemented with concentrate in separate feeding. This variation might be due to the TMR feeding system used in this study that can increase the precision-feeding of dairy cows(Uyeh *et al.*, 2019). There is no TMR based lactating dairy cows feeding system for indigenous dairy cows in tropical regions to compare the milk composition result of this study. However, it is comparable with the same dairy breed reported by Hussien *et al.* (2013) that fed natural hay pasture and concentrate mix in separate feeding system. Indicative of inefficient N utilization (Doska et al., 2012), the MUN concentration was higher for TTS than with all other diets. This might have been related to the higher amounts of CP due to the inclusion of urea in TTS based TMR diet that led to increased MUN excretion (NRC, 2001).

Estimated enteric CH_4 emission is the by-product of ruminal fermentation via methanogenesis and is thus substantially affected by a range of factors, including dietary components (Dong et al., 2019). Thus, the methane production values variation in our study among the dietary treatments might be related to the proportion of totally digested nutrient such as the high NDF content of the NPH-based TMR diet (Berhanu *et al.*, 2019). Specifically, the fermentation of fibrous materials favors the formation of acetate and butyrate over the production of CH_4 (Dong *et al.*, 2019). In our study, methane emission per kg of OM intake was lower for the TTS, NGH, and BhH diets than for NPH, thereby indicating that highly digestible roughages in are promising means for reducing CH_4 emission. Likewise, cows fed NGH and BhH, followed by TTS, had significantly lower methane output per unit of daily milk yield than NPH diet, showing that the TMR diets containing improved forage species with high CP contents have great potential in mitigating methane emission for climate smart dairy production (Berhanu *et al.*, 2019). Moreover, the environmental cost of producing NGH and BhH forages should be examined before recommending using this strategy to mitigate CH4 emissions and urinary N excretion. In the present study, CH4 emissions from enteric fermentation were estimated using intercontinental equations because, as far as the authors' knowledge, there is no methane emission prediction equation developed for the Ethiopian dairy breed; this may be the first to report such methane emission data. Therefore, more studies are needed to measure the actual CH4 emissions of the Ethiopian dairy breed, considering the negative effects of CH4 production on global warming.

5.5 Conclusions

Dairy cows fed with treated teff straw (TTS), Napier grass hay (NGH) and *Brachiaria* hybrid grass hay (BhH) had better feed intake and nutrient utilization, as well as milk yields, than cows fed natural pasture hay (NPH). Feeding TTS, NGH, and BhH hay as a basal diet changed the N excretion pathway from urine to feces and indirectly mitigated ammonia emissions, thus potentially benefitting the environment. Furthermore, N utilization varied among dietary treatments; for example, the N retained in the bodies of cows fed TTS, NGH and BhH was higher than in cows fed the NPH diet. Moreover, cows fed TTS, NGH, and BhH produced significantly less estimated methane per daily milk yield and fat protein correct milk. The NRC (2001) equation estimate more milk yield than the actual yield and further research needed for precision validation. In summary, feeding TMR diets containing improved forages and treated teff straw silage as a basal diet to dairy cows increased nutrient digestibility, milk yield, and nitrogen utilization efficiency, as well as reduced nutrient excretion and CH4 emission to the environment, thereby potentially improving dairy production in tropical regions. Consequently, this study highlighted the possibility of increasing production and reducing GHG emissions in countries like Ethiopia, where precision-feeding is a limitation.

Chapter 6: General conclusions and recommendations

6.1 General Conclusions

This study showed that the availability of diversified feedstuffs for dairy cattle. Among these based on nutritional value evaluation, natural hay and crop residue are poor quality due to their low nutritive value and digestibility to fulfil the maintenance requirement of the lactating dairy cow. In contrast, agro-industrial byproducts, improved forages and green fodders have high CP value (> 100 g/kg DM) and better digestibility that can be used as feed supplementation. Moreover, traditional brewery and distillery by-products (Atella and Birint) had medium level of CP, and moderate level of OM digestibility, ME and VFA concentration implies that they can also use for supplementation. The mineral profile result indicates that most of the feedstuffs are rich in macro and micro elements except in sodium, copper and cobalt. Therefore, it is necessary to supplement for deficient minerals in the diet by providing mineral mixture such as salt for sodium. The potential feed ingredients for in vivo experiment (Chapter 5) and diet formulation (Chapter 4) were selected according to their availability, cost, chemical composition and digestibility.

Feed ingredient having high anti-nutritional factors such as green fodder species can be treated using PEG. Thus, the addition of PEG dramatically increased *in vitro* digestibility, metabilizable energy and volatile fatty acid production by reversing the effects of secondary compounds.

The existing farmers' feeding practice indicates that it has no adequate nutrient for lactating dairy cow. This implies that the dairy cows should be supplemented for optimum milk production. Alternatively, the optimal formulated diet indicates that in combination of high quality feed ingredients in the diet, it can be possible to fulfill the maintenance as well as the lactation requirement of dairy cows that can increase the milk production as well as reduction of methane emission. Thus, the optimize diet formulation indicates that in combination of high quality feed ingredients it can be possible to fulfill the maintenance as well as the lactation nutrient requirement that enhance productivity of dairy cows in tropical regions. In addition, the validation of the estimated milk yield using the formulating diet showed a positive relationship with the actual milk yield. However, the NRC (2001) equation estimate more milk yield than the actual yield and further research needed for precision validation.

The dairy feeding experiment results provided important results for smallholder dairy cattle farming systems in the north western Ethiopia, through inclusion of nutritionally upgraded roughage as basal diet which could be used to maximize the milk yield and nitrogen utilization efficiency (NUE). Then again, the data of enteric CH_4 emissions reduce per dry matter and GE intake as well as per unit of milk yield. Eventually, this research gives us a perspective to change the route from urine nitrogen (UN) to faecal nitrogen (FN) output considering UN is more violated to NH_3 emissions than FN, which not only accelerates global warming but also aggravates nitrification process of N. These aspects are useful to establish the scientific feeding standard which is attempted to minimize CH_4 emissions and improve NUE for sustainable dairy production systems.

6.2 Recommendations for future studies

This study revealed a large reserve of Indigenous fodder species which contained more than 112 g/kg of CP, thus implying that they can used as nitrogen sources that could be used to supplement poor-quality roughage in dairy feeding for tropical regions. Thus, extension bodies, policymakers, and farmers should be aware of the importance of these fodder species, and they should be included in the feeding as supplemental diet for dairy production particularly in dry season. Hence, further study on the level of inclusion and animal response is highly recommended.

This study result revealed that adding PEG increased the in vitro digestibility and rumen fermentation characteristics of the tanniferous fodder species vary in relation to season. Thus, feeding trial for the practical utilization is needed both at on station and on farm demonstration level.

Based on the formulated diets result of this study, further investigation that aim to integrate feeds that have better nutritive values into the existing feeding system are required to evaluate feed intake, level of inclusion, and animal's responses for more efficient utilization of available feedstuffs for sustainable dairy production. Moreover, the NRC (2001) equation used for estimation of milk yield from the formulated diets should be further validated.

Feeding the improved forages and treated teff straw silage as a basal in total mixed ration diet for lactating dairy cows increase nutrient digestibility, milk yield and nitrogen utilization efficiency as well as reducing methane emission. Hence, these results should be demonstrated for the development of suitable feeding systems to improve productivity of dairy production.

In addition, it has been reported that there are a diverse crop residues feed resources in tropical regions. Thus, this study showed the great potential to explore those resources (e.g teff straw) using bio-chemical treatment with effective microbes, urea and molasses for improved feeding of dairy cows, which could increase milk yield as well as improve nitrogen utilization efficiency. Consequently, the animal performance evaluation of these recently introduced feeding technology should be further studied incorporating other crop residue varieties.

6.3 Limitation of the study

All formulated optimal diets in Chapter 4 were not tested by feeding trial for validation since there is time limitation as well as high cost demand to conduct the feeding experiment trial of dairy cows. However, the validation and evaluation of some of the formulated optimal diets in chapter 5 indicates the positive correlation with the actual tested diets on milk yield. Besides, further investigation and generation of more data on the effects of all formulated diets using in vivo experiment on the animal performance is highly appreciated and recommended for precision validation.

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SUMMARY

Dairy production is one of the most important agricultural sectors for global food security and nutrition. Though, the average per capita global milk consumption amounts at 113 kg of milk/year, this is significantly much less in developing countries such as Ethiopia, with a per capita milk less than 20 kg per annum (2018). The major constraints affecting milk production in Ethiopia are attributed to the dominant free grazing feeding practice of poor-condition natural pasture feed sources combined with lack of improved feeding practice which is influenced by feed shortage both in quality and quantity. Free grazing cause low productivity, increase overgrazing and aggravate land degradation. Moreover, the indigenous dairy cow breeds (98%) being characterized by low milk yield (average 1.5 kg/cow/day). To enhance dairy production improving feeding and designing appropriate feeding strategies are crucial. On the other hand, stall-feeding increase potential productivity.

A stall-feeding system has been promoted by the Ethiopian government for improving livestock productivity to improve feeding management as well as reduce land degradation by avoiding free grazing. The key research gaps identified in this study are two folds: lack of information on the type, nutritive value, anti-nutritional factors of locally available or improved feedstuffs and feeding regimen, an optimal diet that specifies the amount and schedule of nutritional intake. Thus, the overall goal of this thesis is to improve the productivity of indigenous dairy cows through formulating stall-feeding regimen taking a case study of the Fogera breed in northwest Ethiopia. The specific objectives were: 1) to identify the available feedstuffs and evaluate the nutritive values, 2) to improve feed quality through mitigating the anti-nutritional factors, 3) to formulate optimal diet for lactating indigenous dairy cows and 4) to evaluate and validate the effect of some selected diets containing improved grasses hays and treated teff straw silage on milk yield, nitrogen utilization, and methane emission. These objectives cover chapters 2–5 of this thesis summarized as follows:

Chapter 1 explains the introductory section of the study. It presents an overview of the background for the study, focusing on dairy production, feed resource, past stall feeding practice, and methane emission from dairy cows based on the existing literature. Subsequently, it presents the background, problem statement, objectives, study areas description and outline of the thesis.

Chapter 2 identifies the available feed resources in the study sites and analyze the nutritive values. A total of 32 feedstuffs are found in the studied sites, of which natural pasture and crop residue are the most dominant (43% and 25%, respectively). On the other hand, both natural pasture and crop residue are poor quality feeds due to their low nutritive value and digestibility hence they cannot fulfill the maintenance nutrient requirement of lactating dairy cows. Whereas, improved forages, agro-industrial by-products and green fodders have high crude protein (CP) value (>100 g/kg DM) with better digestibility that can be used as supplementation for poor-quality feeds. This study also revealed a number of potentially valuable indigenous fodder species which can be used as feed supplementation for CP particularly in the dry season. The mineral profile result also indicates that most of the feedstuffs are rich in calcium, magnesium, potassium, phosphorus and iron content but deficient poor in sodium, cobalt and copper. Then, it is necessary to supplement those deficient minerals in the diet of dairy cow by providing a mineral mixture.

Chapter 3 deals with the evaluation of polyethylene glycol (PEG) to reduce the anti-nutritional effects of polyphenols on in vitro digestibility and fermentation characteristics of fodder plant species. The study was designed as a 10 x 2 x 2 factorial arrangement with 10 fodder species, 2 seasons (wet and dry), and 2 states of PEG (with and without PEG). The result showed that addition of PEG improves the in vitro organic matter digestibility (IVOMD), metabolizable energy (ME) and volatile fatty acids (VFA) production on average by 48%, 42%, and 20%, respectively. Moreover, the ant-nutritional factors such as phenols and tannins were (negatively correlated $p<0.001$) with IVOMD, ME, and VFA. In summary, PEG markedly reduced the anti-nutritional effects of polyphenols on in vitro fermentation and improve the nutritive value of fodder species.

Chapter 4 evaluates the existing lactating dairy feeding practices and formulated optimized diet formulation. The result indicates that the existing feeding practice doesn’t satisfy the nutrient demand for lactating dairy cows resulted in low milk yield. This implies that dairy cows should be supplemented for optimum milk production. The optimized diet formulation indicates that with a combination of high-quality feed ingredients it can be possible to fulfill the maintenance as well as the lactation nutrient requirements that enhances the productivity of indigenous dairy cows.

Chapter 5 investigates the evaluation and validation of selected diets containing improved forage grasses hay and treated teff straw silage on milk yield, nitrogen utilization and methane emission using eight lactating indigenous Fogera cows. The following four roughage basal dietary treatments supplemented with formulated concentrate in total mixed ration (TMR) were evaluated: control (natural pasture hay (NPH)); treated teff straw silage (TTS); Napier grass hay (NGH), and brachiaria hybrid grass hay (BhH). The results showed that compared with the NPH based TMR diet, the daily milk yield increased ($P < 0.05$) by 32%, 53% and 89% with TTS, NGH and BhH diets, respectively. Cows fed BhH had the highest dry matter intake (8.8 kg/d), followed by NGH (8.1 kg/d) and TTS (7.7 kg/d); all of these intakes were greater ($P < 0.05$) than that of NPH (6.2 kg/d). Nitrogen digestibility increased ($P < 0.05$) from the NPH diet to TTS (by 28%), NGH (22%) and BhH (40%). The milk urea nitrogen (MUN) concentration found higher ($P < 0.05$) in cows fed TTS (975.6 μmol/L) than other diets. Remarkably, cows fed NPH had higher ($P < 0.05$) non-esterified fatty acid (NEFA) (0.5 mmol/L) and β-hydroxybutyrate (BHBA) (0.2 mmol/L) than those fed other TMR diets. Feeding TTS, NGH, and BhH hay as a basal diet changed the nitrogen excretion pathway from urine to feces, which can benefit the protection against environmental pollution. Dairy cows fed BhH, NGH and TTS emitted less daily CH4 expressed as per unit of milk yield ($P < 0.05$) than NPH diet. The validation of the formulated diet milk yield has a positive relation (r2 = 0.61) with the actual milk yield. Consequently, this study indicated the possibility of increasing milk yield and reducing methane emissions in countries like Ethiopia, where precision-feeding is a limitation. Hence, the results provide feeding regimen through feeding nutritionally upgraded roughages basal diet that can improve milk productivity of Fogera breeds on average up to 58%.

Chapter 6 provides the general conclusion and recommendations of the whole thesis based on the key findings from Chapters 2–5. If the stall-feeding system is integrated with feeding optimal diet it would be possible to increase milk productivity of Fogera cow breeds on average by 58%, while significantly reducing N fecal excretion (23%) and methane emissions per kilogram of milk yield (28%).

摘要

酪農生産は、世界の食糧安全保障と栄養にとって最も重要な農業部門の一つである。世界の一人当たりの平均的な牛乳消費量は 113kg/年だが、エチオピアのような発展途上国では、同消費量は年間 20kg 未満である（2018 年）。エチオピアにおける乳生産上の主な制約は、在来種（98％）が優勢で乳生産性が低い（平均乳量は 1.5kg/頭/日）という特徴にある。この生産性の低さは、質量の両面での飼料不足に左右されるような、飼養改善の不足に加え、状態の悪い天然草地での自由放牧が主流であることが原因である。酪農生産を向上させるためには、給餌方法の改善と給餌戦略の設計が非常に重要である。

エチオピア政府は、家畜の飼養管理を改善し、自由放牧を回避して土地の劣化を軽減するため、舎飼いでの飼養システムを推進している。本研究で明らかになった、研究上の主な課題は 2 つある。それは、現地で入手可能な飼料や改良された飼料の種類、栄養価、抗栄養因子、および栄養摂取量とスケジュールを明確にした最適な飼料設計に関する情報が不足していることである。したがって、本論文の全体的な目標は、エチオピア北西部のフォガラ種を用いて、舎飼いでの飼養法を策定し、在来乳牛の生産性を向上させることにある。具体的な目的は以下のとおり。１）入手可能な飼料の特定と栄養価の評価、２）抗栄養因子による作用の緩和と飼料品質の向上、３）泌乳期の在来乳牛飼料の最適化、４）改良草種乾草や処理すみテフ藁サイレージを含む飼料配合による、乳量・窒素利用率・メタン排出量に及ぼす影響の評価・検証。これらの目的は、本論文の第 2 ～ 5 章を構成する。

第 1 章は、本研究の紹介である。既存文献に基づき、乳生産・飼料・飼養方法・メタン発生に関するこれまでの研究の背景を述べ、問題の所在と本論文の目的を説明する。

第 2 章では、対象地域における入手可能な飼料原料を同定し、その栄養価を分析する。その結果、調査地域では計 32 種の飼料源が確認され、天然草種と農産物残渣が最も多かった（それぞれ 53%、25%）。これら飼料源は栄養価と消化率が低いため、泌乳牛の維持栄養要求量を満たすことができず、飼料としての品質が低い。一方、改良牧草や食品工業残渣、青刈飼料は、粗タンパク質（CP）値が高く（100 g/kg DM 以上）、消化率が良いため、低品質飼料を補う飼料源としての活用が考えられた。また、特に乾期にCP補給源となりうる、潜在的価値のある在来飼料源が多数存在することも明らかになった。微量要素分析の結果からは、ほとんどの飼料で Ca, Mg, K, P, Fe を豊富であるが、Na, Co, Cu の不足が明らかとなり、飼料設計においてこれら微量要素を配合飼料で補う必要性が認められた。

第 3 章では、飼料草の試験管内（in-vitro）消化率と発酵性状に対するポリフェノールの抗栄養作用を軽減するためのポリエチレングリコール（PEG）の添加効果を扱う。草種・季節（乾季と雨季）・PEG濃度を要因とする 10×２×２要因計画とした。PEG添加は、試験管内有機物消化率（IVOMD）・代謝エネルギー（ME）・乾物消化率（DMD）・揮発性有機酸含量（VFA）をそれぞれ、47.9%、42.2%、20.2% 増加させた。これら抗栄養因子（フェノール化合物、タンニンほか）含有量は、IVOMD, ME, VFA と有意な負の相関が見られた（$p<0.001$）。PEG添加は、ポリフェノールによる抗栄養作用を顕著に減らし、飼料草の栄養価を改善した。

第 4 章では、対象地域における泌乳牛の飼養（給餌）実態を評価し、飼料配合条件の最適化について検討

した。その結果、現行の配合条件は泌乳牛の栄養要求量を満たしておらず、メタン排出量も多いことが判明し、その最適化には補助飼料の給与が必要性と考えられた。飼料の最適化において、高品質の飼料原料を組み合わせることで維持および泌乳に必要な栄養要求量を満たし、乳量の増加に貢献しうることが示された。

第 5 章では、フォガラ種の泌乳牛 8 頭を用いて、改良草種乾草とテフ藁サイレージを含む、選定した 4 飼料配合条件での乳量・窒素利用・メタン発生量を評価した。濃厚飼料を補助飼料とする粗飼料主体の 4 処理区を設けた。天然草乾草給与区（対照区、NPH）、テフ藁サイレージ給与区（TTS）、ネピアグラス乾草給与区（NGH）、ブラキアリア給与区（BhH）である。その結果、NPHと比べて TTS, NGH, BhHではそれぞれ、乳量が 24.2%, 34.6%, 47.0% 増加した。BhHでは乾物摂取量が最も高く（8.8kg/日）、NGH（8.1kg/日）、TTS（7.7kg/日）が続いた。これら 3 処理区の乾物摂取量は NPH（6.2kg/日）より大きかった（$p<0.05$）。窒素消化率は、NPHから、TTS（21.7%）、NGH（17.9%）、BhH（28.3%）へと増加した（$p<0.05$）。乳中尿素窒素濃度は、TTS（975.6μmol/L）で高かった（$p=0.041$）。血中の非エステル脂肪酸（0.5mmol/L）および β-ハイドロキシ酪酸含量（0.2mmol/L）は、NPH で高かった。基礎飼料としての TTS, NGH, BhH の給与は、窒素排出の経路を尿から糞に変えることで、環境汚染防止の点からも望ましいと思われた。BhH, NGH, TTS では、NPHと比べ、乾物摂取量あたりのメタン排出量（$p<0.05$）、エネルギー摂取量（$p<0.05$）、乳量（$p<0.05$）が低かった。乳量の推定量と実測値との間には有意な正の相関（$r^2 =0.61$）があった。これらの結果は、栄養面で強化した粗飼料を基礎とする新たな飼料配合が、フォガラ種の乳生産効率を最大で57.6%、改善することを示唆した。

第 6 章では、第 2 ～ 5 章で得られた所見をもとに、本論文における包括的な結論と提言を示す。最適化した飼料配合の下では、フォガラ種乳牛の生産性を57.8%向上させ、糞からの窒素排泄量（22.7%）と乳量 1kg あたりのメタン排出量（27.2%）を大幅に削減できることが明らかになった。

LIST OF PUBLICATIONS

Mekuriaw, S., Tsunekawa, A., Ichinohe, T., Tegegne, F., Haregeweyn, N., Nobuyuki, K., Tassew, A., Mekuriaw, Y., Walie, M., Tsubo, M. and Okuro, T., 2019. Mitigating the anti-nutritional effect of polyphenols on in vitro digestibility and fermentation characteristics of browse species in north western Ethiopia. Tropical Animal Health and Production, 52(3), 1287-1298.

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APPENDICES

Appendix table 1 List of indigenous trees and shrubs, abundance patterns, growth forms, and palatability

No.	Scientific Name	Family	Local name	Abundance D	A	G	GF	Palatability	Reference
1	*Acacia abyssinica*	Fabaceae	Girar	+	+	+	T	HP	(Sisay and Mekonnen, 2013)
2	*Acacia diccurens*	Fabaceae	Chigign	–	–	+	T	UP	Unregistered
3	*Acacia lahi*	Mimosoideae	Cheba	–	–	+	T	MP	(Girma et al., 2015)
4	*Acacia nilotica*	Fabaceae	Tikur girar	–	–	+	T	LP	(Derero and Kitaw, 2018)
5	*Acacia sali*	Fabaceae	Girar	–	+	–	T	HP	(Mekoya *et al.*, 2008)
6	*Acacia saligna*	Fabaceae	Saligina	–	+	–	T	MP	(Mekoya *et al.*, 2008)
7	*Acanthus sennii*	Acanthaceae	Kosheshilie	+	+	+	C	LP	Unregistered
8	*Achyranthes aspera*	Amaranthaceae	Tej	–	+	–	S	MP	(Azene, 2007)
9	*Adhatoda schimperiana*	Acanthaceae	Simiza	–	–	+	S	UP	(Birhanu et al., 2015)
10	*Agrocharis* sp.	Apiaceae	Chegogot	+	–	–	S	LP	(Mohammed and Abraha, 2013)
11	*Albizia malacophylla*	Fabaceae	Sendel	+	–	–	T	HP	(Mulugeta and Admassu, 2014)
12	*Albizia schimperiana*	Fabaceae	Sesa	+	+	–	T	HP	(Mulugeta and Admassu, 2014)
13	*Apodytes dimidiata*	Metteniusaceae	Dong	–	+	–	T	HP	(Kidane and Tesfaye, 2006)
14	*Asparagus africanus*	Asparagaceae	Kesit	–	+	–	S	HP	(Birhanu et al., 2018)
15	*Asystasia mysorensis*	Acanthaceae	Unknown2	+	+	–	S	LP	Unregistered
16	*Buddleja polystachya*	Loganiaceae	Anfer	–	–	+	T	MP	(Kassa et al., 2015)
17	*Buddlejapoly stachya*	Loganiaceae	Ashiqaurie	+	–	–	S	MP	(Haile and Tolemariam, 2008)
18	*Calpurnia aurea*	Fabaceae	Zigita	–	+	–	T	HP	(Tefera et al., 2014)
19	*Caparis tomentosa*	Caparidaceae	Gumero	–	+	–	S	HP	(Birhanu *et al.*, 2018)
20	*Carduus nutans*	Asteraceae	Yeahiya eshoh	+	–	+	S	LP	Unregistered
21	*Carissa edulis*	Apocynaceae	Agam	+	+	+	S	HP	(Getahun, 1976)
22	*Catha edulis*	Celastraceae	Chat	–	+	–	S	UP	Unregistered
23	*Chamaecrista mimosoides*	Fabaceae	Unknown1	+	–	–	T	UP	Unregistered
24	*Cheilanthes farinosa*	Sinopeteridaceae	Unknown7	+	–	–	S	MP	Unregistered
25	*Clausena anisata*	Rutaceae	Limich	–	–	+	S	HP	(Birhanu *et al.*, 2018)

26	*Clausena rutaceae*	Rutaceae	Yelam btir	–	–	+	T	HP	(Birhanu *et al.*, 2015)
27	*Clematis simensis*	Ranunculaceae	Azohareg	–	+	–	C	HP	(Tilahun et al., 2015)
28	*Clerodendrum myricoides*	Umbelliferae	Missiritch	–	+	–	S	LP	Unregistered
29	*Clutia lanceolata*	Myrtaceae	Fiyele fej	+	+	+	S	HP	(Shenkute *et al.*, 2012)
30	*Coffea arabica*	Rubiaceae	Buna	–	+	–	S	UP	Unregistered
31	*Combretum molle*	Combretaceae	Abalo	+	+	+	T	UP	Unregistered
32	*Cordea africana*	Boraginaceae	Wanza	+	+	+	T	HP	(Girma *et al.*, 2015)
33	*Croton crostachyus*	Euphorbiaceae	Baguri	+	–	–	T	MP	(Mekuanent et al., 2015)
34	*Croton macrostachyus*	Euphorbiaceae	Bisana	+	+	+	T	UP	Unregistered
35	*Diospyros abyssinica*	Euphorbiaceae.	Marmeta	+	–	–	T	HP	(Berhane et al., 2006)
36	*Discopodium penninervium*	Solanaceae	Aluma	–	+	–	T	MP	(Yirga et al., 2019)
37	*Dodonaea angustifolia*	Sapindaceae	Kitikita	–	+	–	T	HP	(Birhanu *et al.*, 2018)
38	*Dombeya torrida*	Solanaceae	Wulkifa	–	–	+	T	HP	(Birhanu *et al.*, 2018)
39	*Echinochloa frumentacea*	Poaceae	Sama	–	–	+	S	UP	Unregistered
40	*Ekebergia capensis*	Meliaceae	Lol	–	+	–	T	HP	(Girma *et al.*, 2015)
41	*Embelia schimperi*	Myrsinaceae	Enkoko	–	–	+	S	HP	(Berhane *et al.*, 2006)
42	*Ensete ventricosum*	Musaceae	Enset	+	+	+	T	MP	(Berhane *et al.*, 2006)
43	*Entadopsis abyssinica*	Fabaceae	Kentafa	–	+	–	S	LP	Unregistered
44	*Ervthrina abvssinica*	Fabaceae	Korchi	+	–	+	T	HP	(Yisehak and Janssens, 2013)
45	*Eucalyptus globulus*	Myrtaceae	Eculptus	+	+	+	T	UP	Unregistered
46	*Euphorbia abyssinica*	Euphorbiaceae	Kulkuwal	–	–	+	S	MP	(Abebe, 2016)
47	*Ficus ovata*	Moraceae	Kef	–	+	–	T	HP	(Giday *et al.*, 2018)
48	*Ficus sur*	Moraceae	Shola	+	+	+	T	HP	(Tefera *et al.*, 2014)
49	*Ficus sycomorus*	Moraceae	Banba	–	+	–	T	HP	(Asmare and Mekuriaw, 2019)
50	*Ficus thonningii*	Moraceae	Chibaha	+	+	+	T	HP	(Birhanu *et al.*, 2018)
51	*Ficus vasta*	Moraceae	Warka	+	–	+	T	HP	(Birhanu *et al.*, 2018)
52	*Flacourtia indica*	Flacourtiaceae	Jangurt	–	+	–	T	LP	Unregistered
53	*Grevillea robusta*	Protacea	Gravilla	–	+	–	T	HP	(Ayana et al., 2017)
54	*Grewia ferruginea*	Moraceae	Lenquata	+	+	+	S	HP	(Girma *et al.*, 2015)

55	*Hageniaia abyssinica*	Rosaceae	Koso	–	–	+	T	HP	(Seegeler, 1983)
56	*Hypericum revolutum*	Clusiaceae	Amja	–	–	+	T	MP	Unregistered
57	*Jasminum grandiflorum*	Oleaceae	Tenbelel	–	+	–	S	HP	(Yohannis et al., 2018)
58	*Kalanchoe petitiana*	Crassulaceae	Andawul	–	+	+	S	LP	(Birhanu *et al.*, 2018)
59	*Lantana camara*	Verbenas	Wefkolo	–	+	–	S	UP	Unregistered
60	*Launaea massauensis*	Asteraceae	Unknown3	–	+	–	S	MP	Unregistered
61	*Leonotis ocymifolia*	Lamiaceae	Unknown6	+	–	–	S	MP	Unregistered
62	*Maesa lanceolata*	Myrsinaceae	Kilaba	–	+	+	T	HP	(Mekuriaw et al., 2012)
63	*Malus domestica*	Rosaceae	Apple	–	–	+	T	UP	Unregistered
64	*Mangifera indica*	Anacardiaceae	Mango	–	+	–	T	UP	Unregistered
65	*Maytenus obscura*	Celastraceae	Qoba	+	–	+	T	HP	(Birhanu *et al.*, 2018)
66	*Maytenus undata*	Celastraceae	Atat	–	+	–	S	HP	(Mekoya et al., 2008)
67	*Millettia ferruginea*	Fabaceae	Birbira	–	+	–	T	MP	(Alemu et al., 2013)
68	*Mimusops kummel*	Sapotaceae	Eshie	–	+	–	T	HP	(Mekoya *et al.*, 2008)
69	*Momordica foetida*	Moraceae	Hareg	–	+	+	C	HP	(Teklehaymanot, 2009)
70	*Musa acuminata*	Musa	Banana	+	–	–	T	HP	(Negesse *et al.*, 2009)
71	*Mytenus undata*	Celastraceae	Chocho	–	+	–	T	MP	Unregistered
72	*Ocimum lamiifolium*	Lamiaceae	Damakesie	–	+	–	S	LP	(Mengistu and Asfaw, 2016)
73	*Osyris quadripartita*	Santalaceae	Keret	–	+	–	S	HP	(Yohannis et al., 2018)
74	*Oxytenanthera abyssinica*	Poaceae	Shimel	+	–	–	T	HP	(Mekuriaw *et al.*, 2012)
75	*Pavetta abyssinica*	Rubiaceae	Unknown4	–	–	+	S	MP	Unregistered
76	*Pavonia burchellii*	Meliaceae	Nacha	–	+	+	S	MP	(Giday *et al.*, 2018)
77	*Persea americana*	Lauraceae	Abokado	+	–	–	T	UP	Unregistered
78	*Phytolaca dodecandra*	Phytolacaceae	Endod	–	+	+	S	HP	Giday *et al.*, 2018)
79	*Piliostigma thonningii*	Fabaceae	Lafidi	–	+	–	T	MP	Sosona, 2018
80	*Premna schimperi*	Verbenaceae	Checho	–	+	–	S	MP	(Enyew et al., 2014)
81	*Prunus africana*	Kalkm rosaceae	Koma	–	–	+	T	HP	(Birhanu *et al.*, 2018)
82	*Rhamnus prinoides*	Gesho	Rhamnaceae	+	+	+	S	UP	Unregistered
83	*Rhus ruspolii Engl.*	Anacardiaceae	Unknown5	–	–	+	S	UP	Unregistered

84	*Rosa abyssinica*	Rosaceae	Kega	–	–	+	S	LP	Unregistered
85	*Rubus apetalus*	Rosaceae	Enjori	–	–	+	S	HP	(Birhanu *et al.*, 2018)
86	*Rumex abyssinicus*	Polygonaceae	Mokimoko	–	+	–	S	MP	(Getie et al., 2003)
87	*Rumex nervosus*	Polygonaceae	Embacho	–	–	+	S	MP	(Birhanu *et al.*, 2018)
88	*Rytigynia neglecta*	Rubiaceae	Dingay seber	+	+	+	T	HP	(Birhanu *et al.*, 2018)
89	*Salix mucronata*	Salicaceae	Shunshura	–	+	–	T	HP	(Giday et al., 2007)
90	*Samanea saman*	Fabaceae	Washint	+	–	+	T	HP	(Birhanu *et al.*, 2018)
91	*Sansevieria erythraeae*	Asparagaceae	Chiret	+	–	–	S	MP	Unregistered
92	*Sapium ellipticum*	Euphorbiaceae	Arboch	–	+	–	T	MP	Unregistered
93	*Schefflera abyssinica*	Araliaceae	Getum	–	–	+	T	HP	(Birhanu *et al.*, 2018)
94	*Senna singueana*	Fabaceae	Gufa	–	+	–	T	LP	Unregistered
95	*Sapium ellipticum*	Fabaceae	Guancho	–	+	–	T	HP	(Mekoya *et al.*, 2008)
96	*Sida schimperiana*	Malvaceae	Chifirg	+	+	–	S	MP	(Limenih et al., 2015)
97	*Solanecio gigas*	Asteraceae	Dengorita	+	+	+	S	UP	Unregistered
98	*Solanum capylacanth*	Melianthaceae	Azamir	–	+	+	S	UP	Unregistered
99	*Stereospermum kunthianum*	Bignoniaceae	Zana	+	+	–	T	HP	Unregistered
100	*Syzigium guineense*	Bignoniaceae	Dokima	+	–	+	T	HP	(Seegeler, 1983)
101	*Terminalia laxiflora*	Rosaceae	Bagura	–	–	+	T	MP	Unregistered
102	*Terminalia macroptera*	Combretaceae	Kokora	+	–	+	T	HP	(Birhanu *et al.*, 2018)
103	*species unknown*	Unidentified	Serkabeba	–	+	–	S	UP	Unregistered
104	*species unknown*	Unidentified	Bitezaf	–	+	–	S	UP	Unregistered
105	*species unknown*	Asteraceae	Canabari	–	–	+	T	MP	Unregistered
106	*species unknown*	Bignoniaceae	Abari	+	–	–	T	MP	Haile and Tolemariam, 2008)
107	*species unknown*	Unidentified	Aborka	–	–	+	T	LP	Unregistered
108	*species unknown*	Unidentified	Ader	+	–	–	S	LP	Unregistered
109	*species unknown*	Unidentified	Anburka	+	–	–	S	Unknown	Unregistered
110	*species unknown*	Unidentified	Arboch	–	+	–	T	MP	Unregistered
111	*species unknown*	Unidentified	Ashikontir	+	–	–	S	Unknown	Unregistered
112	*species unknown*	Unidentified	Awura	–	+	–	S	LP	Unregistered

113	*species unknown*	Unidentified	Bakusti	+	–	+	T	MP	Unregistered
114	*species unknown*	Fabaceae	Chimlik	+	–	–	T	HP	Unregistered
115	*species unknown*	Unidentified	Kamo	–	+	–	T	LP	Unregistered
116	*species unknown*	Unidentified	Kutikoto	–	–	+	S	LP	Unregistered
117	*species unknown*	Unidentified	Midekurta	–	+	–	S	LP	Unregistered
118	*species unknown*	Unidentified	Tembesaba	+	–	–	S	LP	Unregistered
119	*species unknown*	Unidentified	Tikintik	–	+	–	T	MP	Unregistered
120	*species unknown*	Unidentified	Tsedakie	+	–	–	T	UP	Unregistered
121	*species unknown*	Unidentified	Yenebir tifir	+	–	+	S	MP	Unregistered
122	*species unknown*	Unidentified	Yileho	–	–	–	S	LP	Unregistered
123	*Urera hypselodendron*	Urticaceae	Lankisho	–	–	+	C	HP	(Mohammed and Abraha, 2013)
124	*Vernonia amygdalina*	Asparagaceae	Girawa	–	+	+	T	HP	(Tibbo et al., 2006)
125	*Viscum tuberculatum*	Viscaceae	Boke	–	+	–	T	MP	Unregistered
126	*Ximenia caffra*	Olacaceae	Enkuwai	+	–	+	S	HP	(Tibbo *et al.*, 2006)
127	*Xylopia aethiopica*	Annonaceae	Gambilo	+	–	+	T	MP	(Girma *et al.*, 2015)
128	*Yushania alpina*	Poaceae	Bamboo	–	–	+	T	HP	(Mekuriaw *et al.*, 2012)
129	*Ziziphus spina-christi*	Rhamnaceae	Kikira	+	–	+	T	HP	(Yirga *et al.*, 2019)

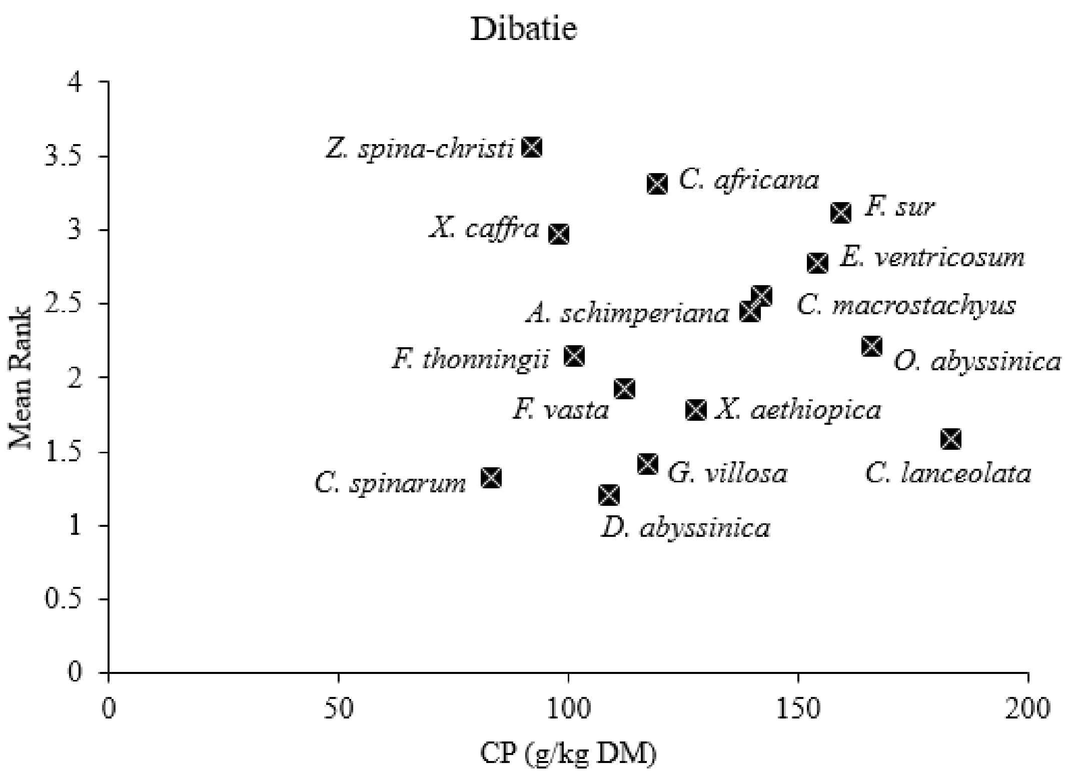
A, Aba Gerima; C, climbing; D, Dibatie; G, Guder; GF, growth form; HP, highly palatable; LS, less palatable; MP, moderately palatable; S, shrub; T, tree; UP, unpalatable.

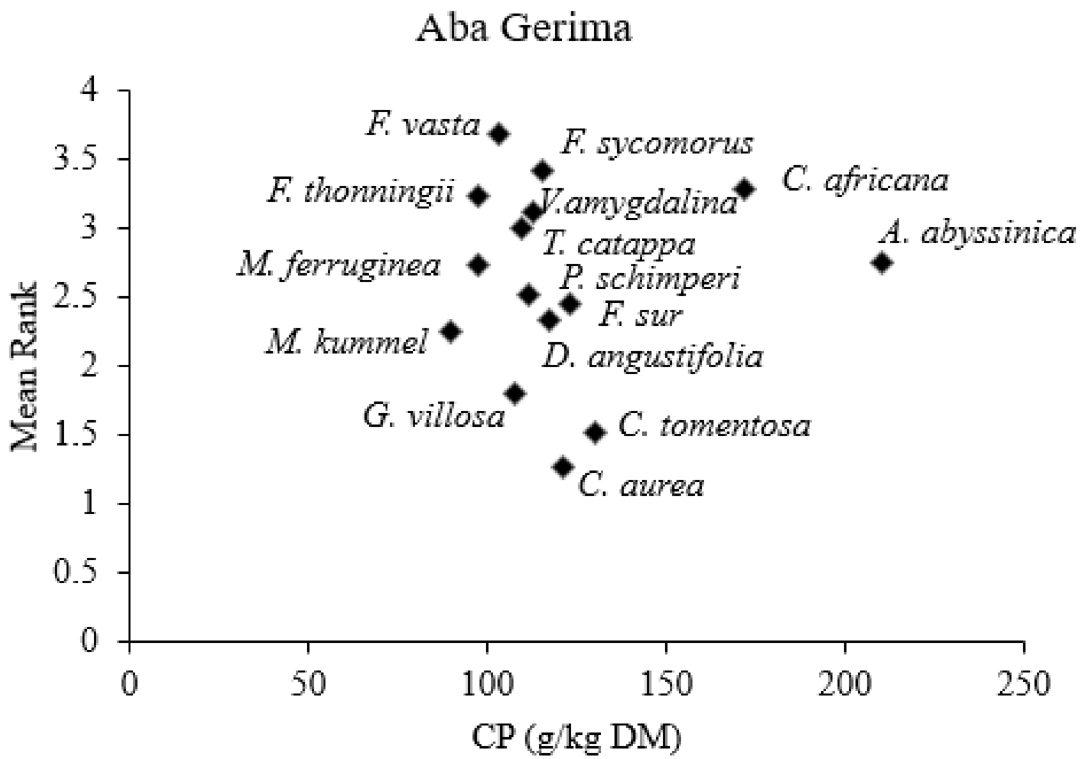
Appendix table 2. Nutritive value of selected IFTS species for ruminant animal feed

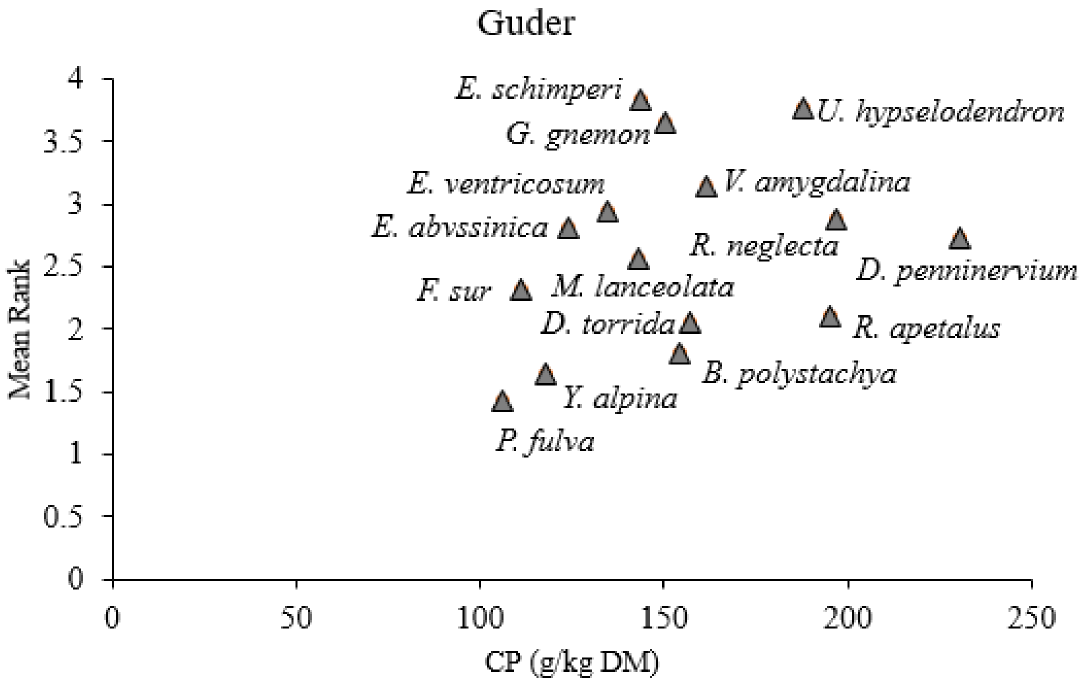
Scientific name	CP	NDF	ADF	ADL	Ash	GE	CT	IVOMD	EDDM	ME	TVFA
IFTS in Dibatie											
A. schimperiana	139.6^{d}	384.5	277.9	211.0	165.0^{ab}	16.0	4.1^{e}	22.3^{de}	36.9	3.3^{e}	137.8
C. africana	142.1^{ed}	295.9	298.7	231.9	71.6^{g}	13.1	23.2^{cd}	33.1^{c}	23.1	3.5^{e}	107.4
C. adonsonia	183.1^{a}	253.5	164.5	90.2	72.9^{fg}	17.1	3.5^{e}	43.1^{cd}	26.0	6.5^{bc}	112.9
C. macrostachyus	83.3^{g}	366.3	236.6	133.9	85.2^{ef}	19.6	210.9^{a}	34.8^{cd}	56.0	4.6^{d}	129.9
C. spinarum	119.4^{h}	450.2	367.7	201.3	94.4^{e}	16.6	28.9^{cd}	36.2^{bc}	29.2	5.6^{cd}	133.2
D. abyssinica	108.7^{f}	298.1	216.3	134.9	133.9^{cd}	17.4	42.2^{c}	31.1^{cd}	28.8	5.1^{cd}	125.4
E. ventricosum	154.1^{c}	300.2	288.9	126.8	65.0^{eg}	18.4	75.4^{b}	47.1^{bc}	31.7	7.1^{b}	113.2
F. sur	159.4^{b}	344.3	226.8	123.6	134.8^{c}	13.5	5.6^{e}	67.4^{a}	35.3	10.2^{a}	144.5
F. thonningii	101.2^{f}	446.4	311.0	145.1	129.0^{d}	16.2	18.0^{d}	22.4^{e}	35.3	3.2^{e}	72.1
F. vasta	112^{ef}	409.1	311.9	118.3	90.1^{e}	13.2	3.5^{e}	46.1^{bc}	38.8	5.9^{c}	136.0
G. villosa	117.2^{ef}	444.8	316.1	140.2	94.8^{e}	10.4	46.7^{c}	49.8^{bc}	34.3	6.5^{bc}	126.3
O. abyssinica	165.9^{b}	513.2	350.2	267.7	172.4^{a}	15.6	29.8^{cd}	25.4^{d}	31.2	3.8^{de}	98.0
X. aethiopica	127.6^{e}	266.6	216.3	66.5	84.0^{f}	17	88.7^{b}	53.6^{b}	25.4	3.8^{de}	105.9
X. caffra	97.9^{f}	488.3	290.9	151.5	157.7^{be}	13.5	13.4^{d}	23.5^{de}	35.1	3.5^{de}	133.8
Z. spina-christi	92.2^{fg}	402.2	304.1	151.7	82.1^{f}	17.7	39.3^{cd}	23.2^{de}	30.5	3.5^{de}	124.1
SEM	8.9	29.2	20.8	21.7	16.9	0.7	16.0	4.1	2.3	0.6	5.7
P value	0.048	0.377	0.307	0.297	0.001	0.407	0.048	0.032	0.617	0.007	0.163
IFTS in Aba Gerima											
A. nilotica	210.5^{a}	350.9	254.3	108.6	73.5^{de}	12.1	37.1	38.9	41.2	5.9^{c}	126.3
C. aurea	171.9^{b}	443.3	280.3	153.4	64.9^{de}	15.5	28.9	36.1	28.8	5.1^{cd}	116.9
C. tomentosa	121.1^{cd}	209.0	145.3	42.5	105.5^{cd}	16.1	86.3	21.6	35.8	2.5^{e}	131.1
C. africana	130.3^{c}	302.9	167.6	107.1	48.2^{f}	18.4	28.6	53.9	33.8	8.2^{b}	130.3
D. angustifolia	117.6^{cd}	272.8	230.8	119.0	48.5^{f}	12	40.1	38.3	32.1	5.8^{c}	86.83
F. sur	122.8^{cd}	406.5	334.5	196.3	111.2^{cd}	7.8	43.5	54.89	32.8	8.1^{b}	129.4
F. sycomorus	114.6^{cd}	370.2	275.3	120.5	169.5^{a}	18.9	51.8	38.1	56.9	5.8^{c}	133.1
F. thonningii	97.6^{f}	445.0	331.7	184.3	111.5^{cd}	14.5	18.0	21.9	35.3	3.1^{de}	72.1
F. vasta	103.1^{ef}	342.2	253.9	90.5	119.1^{c}	15.4	34.9	43.4	32.4	6.8^{bc}	136.8
G. villosa	107.7^{ef}	367.8	268.7	79.9	85.7^{e}	17.1	41.4	42.8	31.2	5.2^{c}	123.7
M. ferruginea	97.6^{f}	445.0	331.7	184.3	121.5^{c}	14.5	33.4	37.8	32.8	5.1^{cd}	121.8
M. kummel	90^{f}	474.0	328.4	180.1	49.2^{f}	19.0	36.2	37.8	41.8	5.1^{cd}	121.4

P. schimperi	111.7^{de}	231.4	199.3	65.7	99.8^{e}	18.0	34.2	41.8	36.9	4.1^{d}	117.8
T. catappa	115.6^{d}	360.2	265.3	110.5	149.5^{b}	18.7	33.5	37.8	31.8	5.5^{c}	128.4
V. amygdalina	109.6d^{ef}	361.1	286.6	88.4	75.0^{ef}	18.1	50.9	70.1	28.4	10.6^{a}	148.9
SEM	9.4	23.8	17.7	14.3	11.7	1.0	4.6	3.8	2.1	0.6	5.8
P value	0.037	0.702	0.847	0.129	0.045	0.099	0.662	0.702	0.496	0.041	0.382
IFTS in Guder											
B. polystachya	154.5^{cd}	361.7	221.0	89.6	62.9^{fg}	18.7	66.8^{c}	57.7^{c}	29.1	2.9^{f}	135.4
D. penninervium	230.5^{a}	265.4	241.1	111.6	54.1^{g}	19.0	2.5^{f}	65.9^{bc}	27.6	10.0^{b}	160.2
D. torrida	196.7^{b}	476.8	318.1	214.3	186^{a}	12.6	139.3^{a}	25.6^{de}	38.3	3.8^{e}	130.9
E. capensis	123.8^{cd}	401.8	345.6	191.0	106.3^{e}	15.8	42.3^{de}	44.2^{cd}	56.9	2.8^{f}	150.1
E. ventricosum	143.8^{d}	346.4	328.4	187.4	94.2^{ef}	18.1	84.8^{bc}	41.9^{de}	32.8	6.3^{c}	153.2
E. brucei	154.1^{cd}	300.2	409.0	285.8	120^{d}	14.3	27.5^{ef}	29.5^{a}	41.8	4.4^{de}	110.4
F. sur	110.9^{e}	575.3	277.2	108.7	82.8^{ef}	12.9	24.5^{ef}	48.8^{e}	28.2	6.8^{c}	97.5
G. gnemon	134de	418.2	317.3	168.9	81.2^{ef}	17.3	28.8^{ef}	33.5^{c}	37.4	5.2^{de}	125.8
M. lanceolata	143.2^{d}	564.0	262.8	105.0	153.4^{bc}	16.5	73.1^{bc}	83.9^{cd}	31.7	12.8^{a}	144.4
P. fulva	106.2^{eb}	401.7	202.9	86.7	128.8^{cd}	14.8	33.4^{de}	22.8^{e}	26.3	2.9^{f}	125.6
R. apetalus	195^{c}	231.6	190.4	67.7	162.7^{b}	18.1	35.3^{de}	42.0^{cd}	45.0	6.4^{c}	138.9
R. neglecta	157^{d}	347.0	217.5	128.2	50.2^{g}	18.2	82.1^{bc}	41.1^{d}	31.4	4.7^{de}	117.2
U. hypselodendron	188.1^{bc}	283.2	272.8	239.8	139.2^{c}	15.5	107.7^{b}	71^{b}	31.4	10.8^{b}	161.6
V. amygdalina	161.8^{c}	271.6	298.1	198.7	68.8^{fg}	17.1	47.9^{d}	65.3^{bc}	34.7	9.6^{b}	138.9
Y alpina	118de	627.0	318.0	135.0	75.1^{f}	18.6	31.4^{e}	45^{cd}	25.4	3.8^{e}	98.0
SEM	10.7	36.8	18.2	19.1	12.7	0.6	11.0	5.4	2.5	1.0	6.1
P value	0.046	0.540	0.577	0.213	0.026	0.253	0.037	0.035	0.557	0.031	0.273

ADF, acid detergent fiber (g/kg); ADL, acid detergent lignin (g/kg); CP, crude protein (g/kg DM); CT, condensed tannins (g/kg); EDDM, effective degradability of dry matter (%); GE, gross energy (MJ/kg); ME, metabolizable energy (MJ/kg); NDF, neutral detergent fiber (g/kg); OMD, organic matter digestibility (%); TVFA, total volatile fatty acid (mmol/L); SEM, standard error of mean. Means followed by the same letter in the column do not differ ($P > 0.05$) statistically by the turkey test of IFTS species with in each site.


Dibatie
Mean Rank
CP (g/kg DM)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
50
100
150
200
Z. spina-christi
C. africana
F. sur
X. caffra
E. ventricosum
A. schimperiana
C. macrostachyus
F. thonningii
O. abyssinica
F. vasta
X. aethiopica
C. spinarum
G. villosa
C. lanceolata
D. abyssinica


Aba Gerima
Mean Rank
CP (g/kg DM)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
F. vasta
F. sycomorus
F. thonningii
V.amygdalina
C. africana
T. catappa
A. abyssinica
M. ferruginea
P. schimperi
F. sur
M. kummel
D. angustifolia
G. villosa
C. tomentosa
C. aurea



Appendix figure 1. Complementarities between farmers' preference and crude protein (CP) content of selected fodder species in Dibatie, Aba Gerima and Guder.

a) Rumen fruid collection and i*n vitro* digestibility using syringe method

b) Tannin extraction using spectrophotometer

c) Polyethylene glycerol (PEG 6000)

d) Experimental Fogera lactating dairy cows

e) Feeding experiment setup supervision

f) Napier grass

g) *Brachiaria* grass

h) Stall feeding using improved forage

Appendix figure 2. Pictures showing laboratory procedures and experimental setup