Ethiopian Journal of Animal Production

Volume: 18 Number 1 2018 ISSN: 1607-3835

Official Journal of the Ethiopian Society of Animal Production (ESAP)

Ethiopian Journal of Animal Production

An Official Journal of the Ethiopian Society of Animal Production (ESAP)

Aims and Scope: The Ethiopian Journal of Animal Production is a peer reviewed journal publishing original basic and applied research articles, short communications, technical notes, and review articles dealing with livestock and livestock related issues. Although the journal focuses on livestock production in Ethiopia, papers from similar agro-ecological regions of the world are welcomed.

EJAP is published by the Ethiopian Society of Animal Production (ESAP)

©Ethiopian Society of Animal Production (ESAP) EJAP ISSN: 1607–3835 Volume 18, Number 1, 2018

Cover page by Wossene Abay

Table of Contents

We acknowledge the review services of the following reviewers (Volumes 17 and 18)

No.	Name	Institution
1	Dr Daniel Mushi	Sokoine University, Tanzania
2	Dr Tesesma Zewudu	Haramaya University
3	Dr Yayneshet Tesfay	Mekele University
4	Dr Abule Ebro	Ethiopian Institute of Agricultural Research
5	Prof. Solomon Demeke	Jimma University
6	Dr Diriba Diba	Wollega University
7	Dr Bimrew Asmare	Bahirdar University
8	Dr Aklilu Mekasha	Ethiopian Institute of Agricultural Research
9	Dr Mitiku Eshetu	Haramaya University
10	Dr Yeshambel Mekuria	Bahirdar University
11	Dr Yonas Hailu	Haramaya University
12	Dr Sandip Banerjee	Hawassa University
13	Prof. Taye Tolemariam	Jimma University
14	Prof. Tegene Negesse	Hawassa University
15	Prof. Berhan Tamir	Addis Ababa University
16	Dr. Tesfaye Getachew	ICARDA
17	Dr. Tesfaye Alemu	Oromia Agricultural Research Institute
18	Dr. Solomon Abegaz	Ethiopian Institute of Agricultural Research
19	Dr. Lema Fita	Ethiopian Institute of Agricultural Research
20	Dr. Firew Kassa	Ethiopian Institute of Agricultural Research
21	Dr. Mengistu Alemayehu	National Agricultural Research Council
22	Dr. Mitiku Eshetu	Haramaya Univeristy
23	Dr. Sintayehu Yigrem	Hawassa Univeristy

Indigenous Mineral Supplements of Livestock and Farmers' Perception on the Supplements in Wolaita Lowlands, Southern Ethiopia

Temesgen Desalegn[1, 2*], Adugna Tolera[2], Ajebu Nurfeta[2] and Terry Engle[3]

[1]Department of Animal and Range Sciences, Madawalabu University, Ethiopia
[2]School of Animal and Range Sciences, Hawassa University, Ethiopia
[3]Departments of Animal Sciences, Colorado State University, USA
*Corresponding author: temxdex@gmail.com;

Abstract

The objectives of this study were to assess the indigenous sources of mineral supplements for livestock and indigenous knowledge of farmers in Wolaita lowland on the mineral supplements. A total of 180 randomly selected households from three lowland districts (Humbo, Kindo Koysha and Offa) were interviewed. Most of the respondents (78.9%) reported Addua (mineral soil) is the major indigenous mineral supplement for livestock in Wolaita lowlands whereas 19.4% of respondents reported both Addua and Yogua (mineral water) as important mineral supplements. Addua is typically offered to livestock during wet season as per 65% of the respondents. About 30.6% of the respondents indicated that Addua is offered to livestock fortnightly. Drinking Yogua is practiced in the Humbo district and mainly offered to animals at monthly interval (58.3%). According to the respondents, physiological and physical appearance such as rough hair coat (55.6%), bad smell of ruminants upon eructation (53.3%), poor appetite (48.3%) and licking of soil/old clay pots (40.6%) were reported as the major symptoms of mineral deficiency of animals perceived by farmers. On the contrary, enhanced appetite (72.8%), smooth hair coat with shining appearance (68.9%), regularity in heat/estrus (48.3%) and improved milk yield (46.1%) were observed on animals that received adequate supplementation of Addua and/or Yogua. However, laboratory analysis followed by animal experiments is required to confirm whether the indigenous mineral supplements fulfill the requirement of different species and classes of livestock.

Keywords: *Addua, Ethiopia, indigenous knowledge, lowlands, Wolaita,*

Introduction

Under grazing systems, ruminants depend on forages and drinking water to satisfy all of their nutritional requirements. Unfortunately, grazing on natural pastures often does not provide all of the needed minerals, that animals require throughout the year. Many incidences of mineral inadequacies in forages and soils have been reported which are principal causes of reproductive failure and low overall production (McDowell 1992). Kabaija and Little (1988) noted that forages from the rangeland areas in Ethiopia are deficient in several essential minerals, especially sodium (Na), phosphorus (P) and copper (Cu). Similarly, most forages and crop residues used as livestock feed in the Rift Valley areas of Ethiopia were reported to be deficient or marginal in Na, P and Cu (Adugna and Said, 1994; Girma et al., 2000; Lemma et al., 2002). Findings of a study by Ajebu et al. (2008) indicated that some varieties of enset (*Ensete ventricosum*) in the Sidama Zone of southern Ethiopia had low Na and Cu content (except for leaf lamina).

To overcome the problem of mineral deficiencies, livestock owners in the lowlands of Ethiopia provide various types of indigenous minerals such as rock salt, mineral soils, and/or mineral water (Adugna, 2008; Temesgen and Mohammed, 2012). In Wolaita lowlands of Ethiopia, traditionally indigenous mineral supplements are widely used for different species of livestock by the local farmers (Personal communication). However, actual values of and the perception and practices of local farmers with the respect to indigenous mineral supplementation to ruminants in these areas had not been well documented except for the study by Muluken et al (2016) which was only in Humbo district. This study was conducted to investigate sources of indigenous mineral supplements, farmers' perception and practices of indigenous mineral supplementation to ruminants in Wolaita lowlands.

Materials and Methods

Descriptions of the study areas

The study was conducted in three lowland districts in the Wolaita Zone of Southern Ethiopia. The three study districts were Humbo, Offa and Kindo Koysha. Humbo district is located approximately at 6^0 34'N and 37^0 43'E latitude and longitude, respectively and at an altitude of 1100-2335 meters above sea level (masl). The mean annual rainfall of the district ranges from 840 to 1400 mm and the annual temperature ranges from 15°C to 29°C (HWARDO, 2014). Offa district is located at 7^O68'N and 38^O38'E latitude and longitude, respectively, with an altitude ranging from 1450 to 2800 masl. The mean annual minimum and maximum temperature of this area range between 22 to 25°C, respectively. The annual rainfall ranges from 800 to 1200 mm (Abrham et al., 2012). Kindo Koysha district is located at 7°58" N latitude and 37° 56"E longitude at an altitude of 600-1700 masl. The average annual rainfall is 904 mm, the minimum and maximum daily temperature are 21 and 29.2°C, respectively (Eyasu and Ahmed, 2013). The distribution of rain fall in the study districts is bimodal, with a short rainy season from January to April and longer rainy season from June to mid-September (WZARDO, 2012).

Selection of the study districts

To select the study districts, discussions were held with Wolaita Zone Administration, livestock experts and community elders about the livestock production in the lowlands, existence and usage of indigenous sources of minerals for livestock and accessibility of the areas for the study. Furthermore, in person visits were made to the suggested districts to assess each study site. Three potential districts, Humbo, Offa and Kindo Koysha, were selected on the basis of livestock production potential, usage of indigenous sources of minerals for livestock and accessibility of the study areas. From each district three representative study sites were selected for diagnostic survey in consultation with the respective districts administrators, livestock experts, development agents and community elders.

Nature of questionnaire and data collection

In order to gather primary data on indigenous mineral sources of livestock and indigenous mineral supplementation practices, both formal and informal surveys with a single-visit multiple subject (ILCA, 1990) questionnaires were carried out following random sampling of the livestock owners who are familiar in offering indigenous mineral supplements to their livestock. A total of 180 households from three districts (60 from each districts) were participated in the interview. The interview was conducted at the farmers' residences with the assistance of district's agriculture experts with close supervision of the researchers. The selected districts agriculture experts were oriented about the objectives of the study and trained on data collection prior to commencement of the interview. In each sampling site farmers were briefed about the objective of the study before starting the interview.

Structured and semi-structured questionnaires were prepared for the survey. Beside the structured questionnaire, group discussions (with key informants from the farmers in each study sites and districts livestock experts) and personal observations were conducted during the field work. The questionnaires were designed to gather information on indigenous mineral sources and indigenous knowledge of farmers on mineral nutrition, symptoms of mineral deficiencies and perceived importance of indigenous mineral supplementation on livestock performance. To ensure the compatibility of the response from the target group with the objectives of the study, the structured questionnaires were pre-tested prior to the actual survey by interviewing ten households and the questionnaires were corrected based on the outcomes of the pre-test. During the interview, each respondent was asked to provide the estimated amount of *Addua* offered to each cattle during the feeding time and those estimated amounts of *Addua* by farmers were collected and weighed to determine the mean amount of *Addua* offered to cattle per feeding time.

Statistical analysis

Descriptive statistics such as mean, frequency, percentage, standard deviation and Chi-square test were employed to analyze the data using Statistical Package for Social Sciences version 16 (SPSS, 1996).

Results

Indigenous mineral sources and supplementation practices of farmers

Indigenous mineral sources offered to livestock in the Wolaita lowlands are presented in Table 1.

Table 1. Supplemental mineral sources (% of households) of livestock in the study districts

Mineral sources of livestock	Humbo (n=60)	Offa (n=60)	Kindo Koysha (n=60)	Overall (N=180)	$\chi 2$	*p*
Table salt	3.3	1.7	0	1.7		
Addua	38.3	98.3	100	78.9	86.897	0.000
Both Addua and *Yogua*	58.3	0	0	19.4		

n=number of respondents of each study district; N= total number of respondents of the three study districts; $\chi 2$=Chi-square.

According to the respondent, *Addua* is naturally a salty soil located in the Humbo district specifically called *Chokare* and the mineral water *Yogua* is rain water standing on *Addua*. *Addua* was the major indigenous mineral supplements of livestock followed by both *Addua* and *Yogua*. Indigenous mineral supplementation practice of farmers varied ($p<0.01$) among the study districts. Kindo Koysha farmers solely use *Addua* as a mineral supplement for livestock. Humbo farmers use *Addua, Yogua* and table salt as mineral supplements for their livestock. Offa farmers primarily used *Addua* as mineral supplement for livestock followed by table salt. However, the use of table salt as mineral supplement was very limited in all study districts.

Season and frequencies of feeding indigenous mineral supplements to livestock

Season had a significant ($P<0.01$) effect on the use of indigenous mineral supplementation (Table 2). In the study districts, majority of the farmers reported *Addua* was primarily offered to livestock during the wet season. While majority of the respondents in Offa district and some of the respondents in Kindo

Koysha district reported that they use *Addua* irrespective of any season of the year. *Yogua* was used only in the Humbo district and offered to livestock only during the wet season.

Table 2. Season of feeding indigenous mineral supplements (percent of respondents)

Season of feeding	Humbo (n=60)	Offa (n=60)	Kindo Koysha (n=60)	Overall (N=180)	$\chi 2$	*p*
Mineral soil *(Addua)*						
• Wet season	96.7	31.7	66.7	65		
• Dry season	0	0	10	3.3		
• Season independent	3.3	68.3	23.3	31.7	82.316	0.000
Mineral water (*Yogua*)						
• Wet season	95	-*	-	31.7	-	-

n=number of respondents of each study district; N= total number of respondents of the three study districts; χ2=Chi-square; *=not available.

Table 3. Frequency of offering (% respondents) *Addua* and Yogua to livestock

Variables	Humbo (n=60)	Offa (n=60)	Kindo koysha (n=60)	Overall (N=180)	$\chi 2$	p
Frequency of feeding *Addua*						
• Daily	0	38.3	1.7	13.3		
• Twice a week	0	28.3	15	14.4		
• Weekly	8.3	25	25	18.9		
• 15 days interval	46.7	6.7	38.3	30.6		
• Monthly	5	1.7	20	9.4	147.0	0.000
• Once in three months	40	0	0	13.3		
Frequency of drinking *Yogua*	n=57	n=60	n=60	N=57		
• Weekly	6.7	-	-	6.7		
• 15 days interval	18.3	-*	-	18.3		
• Monthly	58.3	-	-	58.3	-	-
• Once in three months	16.7	-	-	16.7		

n=number of respondents of each study district; N= total number of respondents of the three study districts; χ2=Chi-square; *=not available.

The majority of respondents offer *Addua* to their livestock every 15 days followed by weekly, twice a week, daily, once every three months and monthly intervals (Table 3). There was a difference among study districts in frequency ($p<0.05$) of offering *Addua* to livestock. In Humbo district, the majority of the respondents supplemented *Addua* every 15 days followed by once in three months and weekly intervals. In Offa district, daily offering of *Addua* is a common practice followed by twice a week and weekly intervals. In the case of Kindo Koysha, offering *Addua* to livestock at 15 days intervals was the most common practice followed by weekly and monthly offering intervals. Drinking *Yogua* is practiced only in Humbo district, which usually occurs at monthly intervals followed by intervals of 15 days and once in three months.

The amount of *Addua* offered to cattle (Mean±SD) was 45.9±7.9, 22.0±11.1 and 47.8±20.4 g/head at each feeding in Humbo, Offa and Kindo Koysha districts, respectively, with a mean of 34.7 ±19.1 g/head/offering for all the three districts. Overall, the amount of *Addua* offered in the Offa district was lower ($p<0.05$) than the other two districts.

Farmers' perception of mineral deficiencies in livestock

The respondents identified several symptoms indicative of mineral deficiency in animals (Table 4).

Table 4. Mineral deficiency symptoms of animals perceived by respondents due to deficiency of *Addua* and/or *Yogua* supplementation

Category of Symptoms	Observed symptoms	Percentage of respondents			Overall (N=180)	$\chi 2$	*p*
		Humbo (n=60)	Offa (n=60)	Kindo Koysha (n=60)			
Production and reproduction	Decrease in milk yield	0	0	26.7	8.9		
	Weight loss	21.7	30	48.3	33.3		
	Delayed heat/estrus	13.3	50	30	31.1		
Physiological and physical appearance	Bad smell upon eructation	86.7	46.7	26.7	53.3		
	Rough hair coat	30	71.7	65	55.6		
	Poor appetite	5	41.7	98.3	48.3	401.8	0.000
Behavioral changes	Licking soil/old clay pots	58.3	46.7	16.7	40.6		
	Smelling to the direction of *Addua* location	15	-	-	5		
	Sudden running of animals to *Addua* sources	43.3	-	-	14.4		
	Frequent bellowing	8.3	0	0	2.8		
State of well-being	Susceptible to disease	0	13.3	5	6.1		
	Lush pasture bloating	28.3	0	5	11.1		
	Susceptible to mange mites	8.3	61.7	41.7	37.2		
	Occurrence of worms in dung	10	5	5	6.7		

n=number of respondents of each study district; N= total number of respondents of the three study districts; $\chi 2$=Chi-square.

These were grouped into four categories including symptoms pertaining to animal production and reproduction, physiological and physical appearance, behavior changes and state of animal well-being. Physiological and physical appearance such as rough hair coat, bad smell upon eructation, poor appetite and licking soil/old clay pots were reported as the major symptoms of mineral deficiency of animals perceived by farmers. There was a high ($p<0.01$) perception difference among study district in identifying perceived mineral deficiency symptoms. In Humbo district, bad smell upon eructation was perceived by respondent as the major indicator of mineral deficiency followed by licking soil/old clay pots and craving of animals for *Addua*. In Offa district farmers perceived a rough hair coat as the major indicator of mineral deficiency followed by mange mites and delayed heat/estrus. Poor appetite was reported as the major indicator of mineral deficiency by Kindo Koysha respondents followed by rough hair coat and weight loss.

Farmers' perceptions on Addua supplementation on animal performances

The farmers listed the factors which they perceive are signs of animals with good mineral intake as indicated in Table 5.

Table 5. Perceived importance of *Addua* supplementation in ruminants as per the respondents

Category of changes	Perceived effects	Percentage of respondents Humbo (n=60)	Offa (n=60)	Kindo-Koysha (n=60)	Overall (N=180)	$\chi 2$	*p*
Production and reproduction	Increases milk yield	36.7	43.3	58.3	46.1		
	Improves weight gain	41.7	0	88.3	43.3		
	Regular heat/estrus	51.7	46.7	46.7	48.3		
	Improves meat taste	5	0	0	1.7		
	Increases bulls sexual desire	0	0	5	1.7		
	Improves butter quality and yield	3.3	0	3.3	2.2		
	Thick milk with good smell	0	5	0	1.7	404.4	0.000
Physiological and physical appearance	Increases appetite	76.7	75	66.7	72.8		
	Smoothes hair coat with shining appearance	70	83.3	53.3	68.9		
	Avoids bad smell upon eructation	45	15	5	21.7		
Behavioral changes	Maintains normal behavior	0	0	28.3	9.4		
	Stops licking soil/ old pots	0	3.3	0	1.1		
State of well-being	Kills internal worms	56.7	0.0	3.3	20		
	Avoids lush feeds bloating	23.3	0	0	7.8		
	Clears digestive tract through diarrhea	8.3	0	0	2.8		
	Removes mange mites	6.7	30	13.3	16.7		

n=number of respondents of each study district; N= total number of respondents of the three study districts; χ2=Chi-square.

They reported easily observable and measurable signs through assessments. In the order of importance improved appetite, smooth hair coat with shining appearance, regular estrus cycle and improved milk yield were perceived as the major signs of animals receiving in adequate supplementation of *Addua*.

Regarding the effects of mineral supplementation on livestock, respondents from the Humbo district perceived improvement in the appetite of livestock as the first sign of optimum mineral status of the animals followed by a smooth hair coat with a shining appearance and the disappearance of internal parasites in dung. Farmers from Offa district perceived smooth hair coat with a shining appearance as the first indicator of animals with better mineral status followed by increased appetite and improved onset of estrus. In Kindo Koysha district, improved weight gain was perceived as the first sign of animals having better mineral supplementation followed by increased appetite and increased milk yields.

Discussion

Indigenous mineral sources and supplementation practices of farmers

Addua was the major indigenous source of mineral supplement in Wolaita lowlands. In similar studies different authors reported wide utilization of mineral soil for livestock. Muluken et al. (2015) reported mineral soil/ *Bole* and mineral water as sources of indigenous mineral supplements of sheep in Humbo district, Wolaita. A study conducted in Somali region of eastern Ethiopia (Sisay et al., 2007; Temesgen and Mohammed, 2012) showed that natural mineral soil known locally as Carro/ Biy'ada are found in vast area of the region and widely utilized by pastoralists. Temesgen and Mohammed (2012) reported mineral water as source of mineral supplement to camels and cattle in Jijiga district, eastern Ethiopia.

Addua is fed to livestock mainly during wet season. Farmers perceive that *Addua* is very salty and that feeding it to animals during the dry season when they do not get adequate feed and water supply could harm the animal; i.e, they think that it could cause emaciation and may ultimately kill the animals. On the contrary, the respondents perceive that feeding mineral soil during wet/rainy season when there is adequate or surplus green feed can improve feed intake, productivity and well-being of the animals. Some farmers feed *Addua* independent of the season. These farmers mostly feed *Addua* for fattening and/or milking animals that get special care and treatment than the other herds in order to consume more feed and water and produce more (weight gain or milk yield). This finding is in line with the findings of previous studies of Temesgen and Mohamed (2012) and Sisay et al. (2007). However, Muluken et al. (2015) reported that most of farmers in Humbo district feed sheep mineral soil in the dry season which contradicts with the current finding. The possible reasons for the discrepancy of the results are not clear. Among the study districts, *Yogua* (rain water standing on *Addua*) is found only in Humbo district in the wet season and farmers of the district especially in the vicinity of *Yogua* allow their animals to drink from it at monthly intervals. Majority of the farmers preferred monthly interval of drinking Yogua due to its high salt content which could harm their animals if they drink under monthly interval. Farmers' practice of offering Yogua at monthly interval could be reasonable as Youga was high in electrical conductivity (EC), total dissolved solids (TDS), Na and potassium (K) concentration (unpublished data). According to NRC (2001) high EC and TDS in *Yogua* indicates that *Yogua* could be used with reasonable safety for adult ruminants and should be avoided for pregnant and baby calves. However, the report of offering sheep *Yogua* mainly in the dry season (Muluken et al., 2015) is also not in line to the current findings and the discrepancy of the results are not clear as from the respondents points of view and of our field observation during the dry season where we observed *Youga* got dried and not available in the dry season due to higher evaporation.

Frequency of feeding Addua and Yogua to livestock and the amount of Addua offered to cattle

Through focus group discussions farmers stated different reasons responsible for frequency of offering *Addua* to their livestock such as: 1) the effect of *Addua* on animal well-being; 2) cost of *Addua;* 3) animal numbers, and 4) the interest of the animals for *Addua.* The farmers perceived that feeding of *Addua* on at daily or weekly interval could harm/ emaciate the animals due to its salty nature. Salty nature of *Addua* could be related with high content of Na in the *Addua* (unpublished data) and also could be related with high chloride content in it. In line with farmers view, McDowell (2003) reported high intake of sodium chloride characterized by increases in water consumption, anorexia, weight loss, edema, nervousness, paralysis and a variety of signs that are dependent on the animal species involved. The farmers also indicated that feeding of *Addua* at daily or weekly intervals would be very expensive for a large number of animals due to high cost of mineral soil which is in agreement the findings of Muluken et al. (2015). Thus, as the number of animals increases, the frequency and amount of *Addua* offered decreases. Some farmers indicated that some animals are not interested in consuming *Addua* when *Addua* is offered daily. Muluken et al. (2015) reported weekly intervals of supplementation as the most common feeding frequency of mineral soil/*Bole* to sheep in the Humbo district, which contradicts with the current finding. Temesgen and Mohammed (2012) reported monthly intervals of feeding mineral soil/Biya'ada and mineral water for camels in the Jijiga district, Ethiopia.

Farmers' perception of mineral deficiency symptoms in the livestock

Farmers in the study districts are traditionally aware of the importance of mineral and lack of which could result in mineral deficiency. From their experiences, farmers related easily observable symptoms of *Addua / Yogua* deficiency. Licking soil/old clay pots observed by farmers as mineral deficiency symptom could be associated with P deficiency in the feeds and indigenous mineral supplements in the study districts (unpublished data) which corresponds with P deficiency symptoms reported by McDowell and Valle (2000) and McDowell (2003). From the study in Somali, mineral deficiency symptoms of camel were reduced feed intake, reduced milk yield, restlessness and chewing construction/woody materials as the top symptoms of mineral deficiency listed by pastoralists (Temesgen and Mohammed, 2012). In a similar study, Rendille pastoralists in Kenya also listed inadequate rumen fill, reduced milk yield and licking of urine as the top three mineral deficiency symptoms in camels (Kaufmann, 1998; Kuria et al., 2004). Farmers/pastoralists perception of mineral deficiency symptoms more or less corresponds to scientific reports. All mineral deficiencies and most excesses, in their more severe forms, are manifested by clinical and physiological disturbances in the animal, but these are rarely specific for a single element (Underwood and Suttle, 1999). Many of the most obvious manifestations, such as subnormal growth, reduces appetite, anemia, bone abnormalities, structural defects in the skin and skeleton, impaired lactation and poor reproductive performance occur to varying degrees with deficiencies of a wide range of mineral elements (McDowell, 1992; Underwood and Suttle, 1999). In the current study, farmers reported a bad smell upon eructation as a symptom of mineral deficiency when animals are not in adequate supplementation of *Addua* and/or *Yogua* in the study districts. They also reported that supplementing animals with adequate *Addua* and/or *Yogua* avoids bad smell during eructation. In a similar study, Temesgen and Mohammed (2012) also reported perceptions of pastoralists regarding bad smell during eructation of camel can be related with mineral deficiency caused by inadequate supplementation of mineral soil lick Biya'ada. Thus, *Addua* supplementation could have effect on rumen function and manipulating the proportion of volatile fatty acids which are responsible for a bad smell upon eructation.

Farmers' perceptions on Addua supplementation effects on animal performances

Traditionally farmers assume *Addua and/or Yogua* are important to improve the well-being and productivity of animals. They also traditionally learnt from their ancestors about the importance of *Addua and/or Yogua* supplementation to their livestock. As farmers' livelihoods are directly or indirectly associated with the livestock they are interested in each observable and/or measurable changes in their livestock. In the order of importance they reported increased appetite, smooth hair coat with a shining appearance, regular onset of estrus and increased milk yield as the major perceived signs of animals in adequate supplementation of *Addua and/or Yogua*. Indigenous knowledge of farmers corresponds well to the scientific evidences of nutritional significance of mineral to the livestock. Our unpublished data showed that calcium (Ca), magnesium (Mg), Na, iron (Fe), manganese (Mn), molybdenum (Mo) and cobalt (Co) concentrations of *Addua* were in adequate amounts according to ruminants requirements (McDowell, 2003; Puls, 1994) and *Yogua* had a good potential to contribute Na, potassium (K), P, Cu, Mo, Co and Fe for the daily requirements of the livestock in the Wolaita lowlands. Both these macro and trace elements found in the indigenous mineral supplements are vital for normal growth, milk and meat yield, reproduction, health and proper functioning of the animal's body, regulation of body fluids, transport of gases and muscle contractions, immune development, onset of estrus, better appetite and skin/wool pigmentation and pliability (McDowell 1992; Malhotra, 1998; Underwood and Suttle, 1999; Murray et al., 2000; McDowell and Valle, 2000).

Conclusion

The mineral soil locally known as *Addua* and the mineral water known as *Yogua* are the major sources of mineral supplements for livestock in Wolaita low lands. Farmers in the study districts are knowledgeable about the mineral deficiency symptoms and nutritional importance of minerals to livestock health and production, which corresponds well with scientific evidences of mineral deficiency signs and nutritional significance of minerals to livestock. Farmers also reported that *Addua* is important in reducing bad smell upon eructation which could be related with manipulation of volatile fatty acid proportion in the rumen. However, laboratory and animal experiments are required to confirm whether the indigenous mineral supplements fulfill the mineral requirements of the animals and could manipulate volatile fatty acid proportion in the rumen and reduce a bad smell of eructation in the study districts.

Acknowledgements

The authors wish to acknowledge Hawassa University for its support during data collection and for the provision of facilities; they also acknowledge the Ethiopian Ministry of Education for funding this study; and all those who have contributed to the study.

References

Abrham Shumbulo, Yishak Gecho and Melese Tora. 2012. Diversity, challenges and potentials of Enset (Ensete ventricosum) production: Case of Offa Woreda, Wolaita Zone, Southern Ethiopia. Food Science and Quality Management 7: 24-31.

Adugna Tolera and Said, A.N. 1994. Assessment of feed resources in Welayta Sodo: Quantity estimation and laboratory evaluation. Ethiopian Journal of Agricultural Sciences 14: 69-87.

Adugna Tolera. 2008. Feed resources and feeding management: A manual for feedlot operators and development workers. SPS-LMM Program. Addis Ababa, Ethiopia, pp.17.

Ajebu Nurfeta, Adugna Tolera, Eik, L.O. and Sundstol, F. 2008. Yield and mineral content of ten enset (Ensete ventricosum) varieties. Tropical Animal Health and Production 40:299-309.

Eyasu Adale and Ahmed Yasine. 2013. Prevalence of bovine trypanosomiasis in Wolaita Zone Kindo Koish District of Ethiopia. African Journal of Agricultural Research 8(49): 6383-6387.

Girma Abebe, Merkel, R.C., Tilahun Sahlu. 2000. Enhancing food security and income generating potential of families in southern Ethiopia through improved goat production and extension. Proceedings of a workshop held at Debub University, Awassa, Ethiopia. Debub University, Awassa, Ethiopia and Langston University, Langston, Oklahoma, USA.

HWARDO. 2014 Humbo Woreda Agricultural and Rural Development Office. Wolaita Zone, Ethiopia.

ILCA. 1990. Livestock Research Manual. ILCA, Addis Ababa, Ethiopia 2: 31-54.

Kabaija, E. and Little, D .1988. Nutrient quality of forages in Ethiopia with particular reference to mineral elements. In: Pastures Network for Eastern and Southern Africa (PANESA). Proceedings of the 3rd workshop held at the international conference center, Arusha, Tanzania. ILCA, Addis Ababa, pp.423-427.

Kaufmann, B. 1998. Analysis of pastoral camel husbandry in northern Kenya. Hohenheim University, Tropical Agricultural Series 5. Center for Agriculture in the Tropics and Sub-tropics, University of Hohenheim, pp.194.

Kuria, S.G., Wanyoike, M.M., Gachuiri, C.K. and Wahome, R.G. 2004. Indigenous camel mineral supplementation knowledge and practices on manyatta based camel herds by the Rendille pastoralists of Marsabit district, Kenya. Livestock Research for Rural Development. Vol. 16, Art. # 51. Retrieved September, 2017, from http://www.lrrd.org/lrrd16/7/kuri16051.htm.

Lemma Gizachew, Adane Hirpha, Fikadu Jalata and Smit G.N. 2002. Mineral element status of soils, native pastures and cattle blood serum in the mid-altitude of western Ethiopia. African Journal of Range and Forage Sciences 19: 147-155.

Malhotra, V.K. 1998. Biochemistry for Students. 10thed. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.

McDowell, L.R. and Valle, G.C., 2000. Major minerals in forages. pp 373-375.In: D.I. Givens, E.Owen, R.F.E. Axford and H.M. Omed (eds.). Forage Evaluation in ruminants Nutrition. Biddles Ltd, Guildford and King’s Lynn, UK

McDowell, L.R. 1992. Minerals in Animal and Human Nutrition. Academic Press Inc. Harcourt Brace Jovanovich Publishers, San Diego, CA.

McDowell, L.R. 2003. Minerals in animal and human nutrition. 2nd ed. The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK.

Muluken Zeleke, Yisehak Kechero and Mohammed Yusuf. 2015. Effects of dietary inclusion of soil as mineral supplements with concentrates on nutrient utilization and economic efficiency of sheep fed hay as basal diet. MSc Thesis. Haramaya University, Ethiopia.

Muluken Zeleke, Yisehak Kechero and Mohammed Yusuf. 2016. Practice of local mineral supplementation to livestock's and perception of farmer's in Humbo Woreda, Wolaita Zone, Ethiopia. Global Veterinaria 17 (2): 114-121.

Murray, R.K. , Granner, D.K., Mayes, P.A., Rodwell, V.W. 2000. Harper's Biochemistry. 25th ed. McGraw-Hill, Health Profession Division, USA.

Puls, R. 1994. Mineral levels in animal health: Diagnostic data. 2nd ed. Clear book, BC, V2T 4X2, Canada.

Sisay Tilahun, Vijchulata, P., Chairatanayuth, P. and Swasdiphanich, S. 2007. Effects of natural mineral soils on body weight and liver minerals of black head Somali sheep in Ethiopia. Kasetsart Journal of Natural Sciences 41:288-299.

SPSS (Statistical Packages for the Social Sciences). 1996. Version16.0. The Apasche software foundation.

Temesgen Desalegn and Mohammed Yusuf. 2012. Indigenous sources of minerals and mineral supplementation practices of pastoralists to camels in Jijiga district, Eastern Ethiopia. African Journal of Animal and Biomedical Sciences 7(1): 65-70.

Underwood, E.J. and Suttle, N.F. 1999. The Mineral Nutrition of Livestock 3rd Ed. CABI Publishing, CAB International, Wallingford, Oxon, UK.

WZARDO (Wolaita Zone Agriculture and Rural Development Office). 2012. Socio-economic Study Document. Wolaita, Ethiopia. pp 47.

Hygienic Handling Practices and Quality of Ethiopian Traditional Butter (*Kibe*) along the Value Chain in Selected Areas of the Central Highlands

Abebe Bereda[1], Zelalem Yilma[2], Mitiku Eshetu[3], Mohammed Yousuf[3]

[1]Department of Animal Sciences, Debre Berhan University, Debre Berhan, Ethiopia
[2]Land O'Lakes IDF - PAID Ethiopia Program, Ethiopia
[3]School of Animal and Range Sciences, Haramaya University, Ethiopia

Abstract

This study was conducted to understand production and market conditions and determine the microbial quality and safety of traditional butter in eight selected dairy potential sites in the Ethiopian central highlands. A total of 320 smallholder farmers, 8 primary dairy cooperatives, 80 open market butter traders and 40 dairy product shops were interviewed using a semi structured questionnaire. A total of 160 butter samples (from 80 smallholder farmers, 40 shops and 40 butter traders) were collected from a subset of previously interviewed respondents for microbial analysis. All respondent from smallholder producers, shops, and dairy cooperatives reported that they wash their hands prior to butter handling with about 74 - 100% reporting to wash their hands with cold water and detergent. However, none of the respondents from open market butter traders washed their hands and butter storage containers. About 75 - 88% of respondents from dairy producers, shops and cooperatives used plastic containers for storing butter, while the remaining used stainless steel. The majority of the sample smallholder dairy farmers, open market traders, shops, and cooperatives had no access to refrigerator instead store containers holding butter in cold water (5 - 30%) or at ambient temperature (67.7 - 92%). The average aerobic mesophilic bacterial, coliform, yeast and mould and lipolytic bacterial counts observed in the present study were 6.23, 2.5, 4.6 and 3.98 log cfuml^{-1}, respectively. Out of the 160 butter samples, on average, 3.88% were positive for Listeria monocytogenes, while no sample was positive for Salmonella test. Generally, the microbial quality of butter obtained in this study failed to comply with the minimum standard values set for quality butter. Therefore, care should be taken in the raw material used to producing butter, the equipment used for processing and storage, personnel hygiene of personnel handling the product in all the way from production to marketing.

Key words: *Butter, hygienic practices, microbial quality*

Introduction

A wide variety of dairy products are manufactured by processing milk, among which, butter is one of the primary source of fat and dietary energy. Milk fat being its major component, butter contains small percentages of proteins, lactose and water, which make it a suitable substrate for many bacteria, and yeast and mould that have been implicated in spoilage and lipolysis of butter even at low temperature (Rady and Badr, 2003). Traditionally, butter has been viewed as a microbiologically safe or low risk product. However, internationally, a number of public health problems have been reported in connection with pathogens such as *Listeria monocytogenes* in Finland, USA and England (Chun *et al.,* 1990; ACMSF, 2003).

There is limited information on the microbial quality and safety of Ethiopian traditional butter (*Kibe*). Earlier reports revealed that the microbial quality of butter is substandard (Almaz *et al.,* 2001; Wondu2007, Zelalem *et al.,* 2007; Mekdes, 2008; Zelalem, 2010; Debela, 2015) and samples contained bacteria of public health concern. For instance, studies conducted by Zelalem *et al.* (2007) and Liyuwork*et al.* (2013) who identified *Enterobacter sakazakii, Klebsiella pneumoniae, Klebsiella oxytoca* and *Salmonella* from butter samples collected from the central highlands of Ethiopia. The microbiology of butter usually reflects the microflora present in the raw material, milk, the sanitary conditions of processing equipment, manufacturing environment and conditions under which the product is stored. Hence, controlling microbial contamination of butter and their subsequent growth can be achieved through properly keeping the hygienic quality of milk at farm level as well as control during processing, storage and packaging (Pandey and Voskuil, 2011).

Butter is one of the food items used in most traditional dishes on a regular basis thus is one of the most marketable dairy commodities in Ethiopia. Likewise, the most important selling attributes of butter is its unique and pleasant flavor, which is the main reason for its higher selling price than other fat sources (Rady and Badr, 2003). However, the flavor of butter considered to be of good quality is very delicate as even small amounts of bacterial growth can change its pleasant flavor and aroma. Information on the microbial quality and safety of traditional butter (*Kibe*) produced and marketed in the study areas is essential to understand the overall quality of the butter being consumed. Therefore, the objective of this study was to assess hygienic handling practices and evaluate the microbial quality and safety of Ethiopian traditional butter along its value chain.

Materials and Methods

Description of the study areas

The study was conducted in eight selected sites in the Ethiopian central highlands namely Debre Berhan, Sheno, Sendafa, Chancho, Fiche, Degem, Debre Zeit and Asella. These areas are located within a radius of about 175 km from Addis Ababa, capital city of the country with elevations ranging from 1600 to 3000 meters above sea level. According to each of the respective district level Bureaus of Livestock and

Fisheries, the mean annual temperature ranges from 2.4 to 28°C while the rainfall ranges from 860 to 1200 mm.

Study methodology and approach

Two methods/approaches were used to collect the required information;

Survey using a questionnaire

Three hundred twenty dairy herd owners, 40 from dairy product shops and 80 from open market butter traders as well as 8 primary dairy cooperatives were interviewed using a semi structured questionnaire. The assessment mainly focused on hygienic practices during butter handling, source of water used for cleaning (containers and hands), storage conditions and materials of butter along the butter value chain.

Microbial analysis

A total of 160 butter samples; 80 from smallholder farmers (10 per each of the 8 study sites), 40 from dairy product shops (5 per each of the 8 study sites) and 40 from open market butter traders (5 per each of the 8 study sites) were collected from previously surveyed respondents. Approximately 250g butter sample was aseptically sampled from containers of each individual selected sample respondent into a sterile bottle. The butter samples were labeled, securely capped and kept in ice-cooler box immediately following collection and transported to Dairy Microbiology Laboratory of the Holetta Agricultural Research Center for analysis. Samples were refrigerated and analyzed within 24hrs of sampling. Butter samples were heated in a water bath at 37°C for ease of handling (HPA, 2003).

Serial dilutions of the samples (10^{-1} - 10^{-7}) were made and about 0.1ml (surface plating) and 1 ml (pour plating) dilutions were mixed with culture medium in pre-labeled sterile plates. For each sample analysis was made in duplicate plates. For all tests, the media were prepared according to the guidelines given by manufacturers. Except VRBA which was sterilized by boiling, peptone water and other media prepared for each test were autoclaved for 15 min at 121°C (Richardson, 1985). All samples were analyzed for Aerobic Mesophilic Bacterial Count (AMBC), Coliform Count (CC), Yeast and Mould Count (YMC) and Lipolytic Bacterial Count (LPBC). Samples were also tested for the prevalence of selected bacterial pathogens namely *Listeria monocytogenes* and *Salmonella* spp. The microbial loads, except for *Listeria monocytogenes* and *Salmonella* spp were counted and expressed as Colony Forming Units (CFU) per gram of butter samples.

Aerobic Mesophilic Bacterial Count (AMBC): With a sterile pipet, 1ml of test portion of sample dilutions were added into pre-labeled plates and 15 - 20 mlPlate Count Agar (PCA) (cooled to 45°C) was immediately added and then mixed thoroughly. Plated samples were then allowed to solidify and counts were made after incubating plates at 30ºC for 48hrs in an inverted position.

Coliform Count (CC): Appropriate dilutions of butter samples (1 ml) were placed in sterile plates and 15 - 20 ML Violet Red Bile Agar (VRBA) was added and mixed. The dried plates were incubated in an inverted position for 24hrs at 32^{o}C (Richardson 1985). Typical dark red colonies normally measuring at least 0.5 mm in diameter were considered as coliform colonies.

Yeast and Mould Count (YMC): One ml test portion of sample dilutions was added into pre-labeled sterile plates, and 15 - 20 mltempered Potato Dextrose Agar (PDA) was added with streptomycin and chloramphenicol. Sample dilutions and agar medium were then thoroughly mixed by alternate back and forth rotation of plates on the flat leveled surface and allowed to set. The dried plates were then incubated at 25^{0}C for 3 to 5 days (Yousef and Carlstrom 2003).

Lipolytic Bacteria Count (LPBC): After autoclaving, Tributyrin agar was cooled in a water bath to45^{0}C.About15 - 20 mlof the medium was added into a sterile plate and allowed to solidify. From an appropriate dilution 0.1 ml of inoculum was then spread over the surface of the solidified medium using a sterile bent glass rod. Finally, the plates were incubated at 32^{o}C for 48hrs in an inverted position (Richardson 1985).

Listeria monocytogenes**:** well mixed testing butter samples (25ml) were homogenized in 225 mlof Listeria Enrichment Broth A and B and incubated for 24hrs at 37^{o}C. After 24hrs, the enrichment culture broth was transferred using a loop and streaked over the surface of PolymyxinAcriflavin-Lithium Chloride Ceftazidime AesculinMannitol (PALCAM) agar plate and incubated for 48hrs at 37^{o}C. Colonies that are gray-green with black precipitate were considered as *Listeria monocytogenes*. Suspected *Listeria monocytogenes* colonies were then further characterized using gram staining and catalase test.

***Salmonella* spp**: twenty-five mlof butter samples was pre-enriched in 225 mlof Buffered Peptone Water (BPW) and incubated for 24hrs at 37^{o}C. A portion (0.1 ML) of the pre-enriched culture was transferred to 10 mlRappaport-Vassiliadis (RVs) broth and incubated at 42^{o}C for 24hrs. A loop full of a culture from the enrichment broth was streaked on the surface of Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37^{o}C for 24hrs (El-Shamy *et al.,*2008). Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish color and black center, were sub-cultured on nutrient agar and confirmed biochemically using Triple Sugar Iron (TSI) and Simon citrate agar.

Data analysis

Percentage was used to compute different parameters related to hygienic practices involved in butter production using SPSS software version 16. Bacteria counts of each variable were first converted into logarithmic values ($\log_{10}$ cfu g^{-1}) and these transformed values were analyzed using the General Linear Model procedure of analysis of variance using SPSS version 16. Mean comparison was done using Duncan test for variables whose F value appeared to be statistically significant. Differences were considered as significant when P-value is <0.05.The model used for this study is $Y_{ij}=\mu+\beta_i+e_{ij}$, where, Y_{ij} = microbial count, μ = overall mean, β_i = butter sampling sources and e_{ij} = random error.

Results

Hygienic practices during traditional butter handling

The entire sample producers, dairy product shops and primary dairy cooperatives reported to wash their hands prior to butter handling (Table 1). Other related hygienic practices such as dressing gown and hair cover were usually common with primary dairy cooperatives and to some extent with dairy product shops and open market butter traders. However, none of the sample open market butter traders reported to wash hands prior to handling butter due to inaccessibility of water at market place. Sources of water used for cleaning (hands and utensils) varied along the butter value chain. For instance, only about 54% of the producers used tap water for the purpose; however, all of the sampled dairy cooperatives and dairy product shops reported that they had access to tap water for cleaning.

Up to 74% of the sample farm owners washed their hands with cold water and soap to remove the milk fat residuals whereas the proportion that used cold water and soap was higher in dairy product shops (95%) and primary dairy cooperatives (100%) (Table 1). The majority of the respondents use plastic materials to store butter along the butter value chain. However, 12 - 25% of the sample smallholder farmers, dairy shops and primary dairy cooperatives use stainless steel (Table 1). Concerning cleaning practices (hands and utensils), about 79 - 100% of the respondents used warm water and soap to clean butter storage containers. The rest (20.7%) of the sample dairy farmers and dairy product shops (11%) reported to use cold water and soap (Table 1).

Table 1: Butter hygienic practices followed along the butter value chain (%) (N=448)

Parameters	Producers (n=320)	Traders (n=80)	Dairy Shops (n=40)	Dairy cooperatives (n=8)
Sanitary practices				
Hand washing	100	0.0	100	100
Wearing gown	0.0	25.0	35.0	100
Cover hair	0.0	10.0	30.0	100
Water sources				
Tap	54.47	0.0	100	100
River	20.15	0.0	0.0	0.0
Spring	12.87	0.0	0.0	0.0
Bore well	12.50	0.0	0.0	0.0
Methods of hand washing				
Cold water	25.1	-	4.8	0.0
Cold water with soap	74.4	-	95.2	100
Storage equipment				
Plastic	87.8	100	83.8	75.0
Stainless steel	12.2	0	16.2	25.0
Methods of equipment cleaning				
Cold water and soap	20.7	-	11.2	0.0
Warm water and soap	79.3	-	88.8	100

Table 2: Butter storage conditions (%) (N = 448)

	Producers (n=320)	Traders (n=80)	Shops (n=40)	Cooperatives (n=8)
Storage conditions				
Refrigerators	2.3	0.0	21.0	25.0
Cold water	30.0	7.6	5.2	0.0
Ambient temperature	67.7	92.4	74.8	75.0
Methods of extension shelf life				
Mixing with spice	100	5.0	11.0	0.0
Convert to ghee	100	0.0	0.0	0.0
Mixing salt	0.0	0.0	9.0	0.0
Butter storage time before sale				
One week	45	30.3	28.3	98.2
Two weeks	54	58.4	51.1	1.8
Three weeks	0.0	11.3	9.0	0.0
One month - two months	0.0	0.0	9.0	0.0
Longer than two months	0.0	0.0	2.6	0.0

The study revealed that about 67.7 - 92% of the interviewed respondents usually kept butter at room temperature until marketing, while about 21 and 25% of the sample dairy product shops and dairy cooperatives kept butter refrigerated, respectively (Table 2). The majority (54%) of the sample farmers supply butter to market within two weeks after processing, and about 58% and 51% of the butter traders and dairy product shops, respectively kept the product up to two weeks. In the case of dairy cooperatives butter is usually sold within a week.

A few sample producers reported to store butter intended for their own family consumption as long as over a year. The respondent from smallholder farmers reported that they traditionally preserve butter using a combination of different plant spices such as *Tikur azmud (Nigella sativa),Besobla (Ocimum sanctum), Zengible (Zingiber officinale), Kororima (Aframomum angusti-folium), Garlic (Allium sativum), Rue/Tenadam (Ruta chalepensis), Abish (Trigonella foenum), Nech azmud (Trachyperimum copticulm), Kosert (Ocimum hardiense)* and *Tossign (Thymus vulgaris).* As noted from the farm households, converting butter into traditional ghee was one of the best options to extend the shelf life of butter using aforementioned traditional spices.

Microbial quality and safety of butter

Lipolyitc and aerobic mesophilic bacteria counts of butter samples significantly varied ($P<0.05$) among sampling sources. Butter sampled from producers had the lowest LPBC and AMBC, while highest counts were recorded for samples collected from open market butter traders (Table 3). Butter samples collected from producers had 2.39 and 4.49 log cfu g^{-1} of CC and YMC, respectively, which were not significantly different ($P>0.05$) from butter sampled from open markets and dairy product shops (Table 3). The highest prevalence of *Listeria monocytogenes* was observed in butter samples taken from open markets (6.2%) followed by shops (3.2%) and producers (2.26%). However, none of the butter samples were positive for *Salmonella* (Table 3).

Table 3: Microbial quality and safety butter collected from different sources - mean (SE)

Variables	Sample sources (log cfu g^{-1})			Overall mean (N=160)
	Producers (n=80)	Open market butter traders (n=40)	Dairy product shops (n=40)	
AMBC(log cfu/ml)	5.84(0.09)[a]	6.54 (0.07)[b]	6.22(0.09)[c]	6.23 (0.05)
CC(log cfu/ml)	2.39(0.14)	2.61(0.21)	2.61(0.21)	2.50 (0.11)
YMC(log cfu/ml)	4.49(0.11)	4.67(0.08)	4.57(0.12)	4.60(0.61)
LPBC(log cfu/ml)	3.64(0.06)[a]	4.07(0.04)[b]	4.12(0.06)[bc]	3.98(0.04)
L.monocytogenes(%)	2.26	6.20	3.20	3.88
Salmonella (%)	0.00	0.00	0.00	0.00

AMBC - Aerobic Mesophilic Bacterial Count, CC - Coliform Count, YMC - Yeast and Mould Count, LPBC - Lipolytic Bacterial Count. Mean with different letters within the same row show significant difference (P<0.05)

Discussion

Hygienic practices during traditional butter handling

Washing hands with clean water and detergent followed by drying with a clean towel before handling butter helps to protect the product from microbial and dirt contamination (Richard, 2002). All of the sample respondents reported that they wash their hands before handling the butter with the exception of the interviewed butter traders. Covering hair and dressing clean gown during handling of milk and milk products are also important practices that handlers need to practice. However, none of surveyed households followed this practice. In contrary, all sample dairy cooperatives, and about 25 and 35% butter traders and dairy product shops, respectively reported to wear gown and hair cover.

Concerning sources of water used for cleaning (hands and containers), noticeable differences were observed along the butter value chain. Only about 54% of smallholder farmers had access to tap water, while all of the interviewed dairy cooperatives and dairy product shops used tap water for the same purpose. Previous study in the central highlands of Ethiopia also reported the use of tap water by 53% of the farmers (Zelalem and Faye, 2006). The quality of water sources other than tap water used for cleaning may not fulfill the required standard thus can contribute to the poor quality of milk and milk products (Zelalem, 2010). Therefore, it is important that producers should at least filter and heat treat it before use since the quality of water determines the number of bacterial counts (Biruk *et al.,* 2012).

The major sources of microbial contaminations of butter are related to unclean surfaces of the churn, storage utensils and wash water (O'Connor, 1995). With regard to storage containers, the majority (75 - 100%) of the respondents used plastic containers; this is in agreement with earlier reports in the central highlands of Ethiopia (Zelalem, 2010; Hiwot, 2013). Contrary to the current study, in East Shewa zone of Oromia, clay pot is commonly used for butter storage (Lemma, 2004). A few (12%) farmers, dairy product shops (16%) and primary dairy cooperatives (25%) used stainless steel for butter storage.

Plastic materials used for butter storage and transportation are not generally easy to clean, and are scratched by repeated cleaning, which provides a hiding place for microbial multiplication. Such conditions can represent a potential source of microbial contamination of butter, despite washing with hot water (Pandey and Voskuil, 2011). Therefore, replacements of plastic containers with stainless steel or aluminum containers can improve the flavor and hygienic conditions of the product considerably (Budhkar *et al.,* 2014).

The use of detergent and good quality water for cleaning the equipment could remove butter residues together with potentially existing spoilage as well as pathogenic microorganisms. As understood from the current study, most (79 - 100%) of the respondents used warm water and soap to clean butter containers, however, all the surveyed butter traders did not practice any butter storage container cleaning.

Butter holding materials such as cups and leaves can also be important sources of contamination (O'Connor, 1995). Butter producers use cups, plastic containers and other local containers to transport butter by wrapping/topping with plastic sheets or leaves of *Enset (Ensete ventricosum)*, castor bean and eucalyptus (*nech bahirzaf)*. These leaves can usually harbor several kinds of worms such as snails infested with dirt and microbes, thus may affect the quality of product as well as consumers' health. Asrat (2009) reported that *Enset* leaf used for cheese packaging is usually wilted using fire, thus can have the advantage of destroying potentially existing worm and/or insect larva.

Storage temperature of the product should get the highest priority to ensure safe foods with low risk of contamination. In this study, smallholder farmers used different mechanisms to maintain the keeping quality of butter before being supplied to the market. Out of the interviewed dairy cattle producers, about 30% stored butter in containers with cold water, while the remaining 67.7% of the respondents kept the product at room temperature. The high percentage of value chain actors storing butter at ambient temperature observed in the current study is similar to that practiced by smallholder farmers (68.6%) reported in the central highlands of Ethiopia (Biruk *et al.,* 2012). However, 21 - 25% of the sample dairy product shops and primary dairy cooperatives kept butter in refrigerator until sale.

The majority (92.4%) of open market butter traders reported to keep butter at ambient temperature. Moreover, the marketing of butter had been taking place where a lot of dirt arises due to motor traffic, garbage dumps and open sewage. Such practice can result in butter contaminated with various microorganisms. Across the study areas, butter produced by smallholder farmers was either sold or preserved by mixing with different traditional spices to extend its shelf life and eventually improve its flavor. Farmers fetch butter to market at varying frequencies. For instance, about 45% of the sample households sold butter once a week, while the remaining (54%) sold butter fortnightly. The majority of open market butter traders and shops reportedly complete selling the butter within two weeks following collection.

As reported by sample smallholder farmers, the minimum and maximum shelf life of butter treated with traditional spices and stored under ambient temperature in this study was one month and 18 months, respectively. Other findings showed that, the keeping quality of butter can be increased by adding salt (O'Connor, 1995). However, salting butter was not observed to be a common practice among sample respondents in the current study, which is in line with that reported by Lemma (2004). Moreover, higher cooking temperatures when combined with different traditional spices are also reported to enhance the keeping quality of butter. As is the case in many other parts of the country, a variety of plant species such as *Nigella sativa, Ocimum sanctum, Zingiber officinale, Aframomum angusti-folium, Allium sativum, Ruta chalepensis, Trigonella foenum, Trachyperimum copticulm, Ocimum hardiense* and *Thymus vulgaris* are the most commonly reported butter preservatives in the study areas.

Microbial properties of butter

Aerobic Mesophilic Bacteria Count (AMBC): The number of bacteria present in any given food product indicates the conditions under which it was produced and handled, and it also determines the food's keeping quality. The average values of AMBC (6.23 log cfu g^{-1}) found in the current study was lower than that of earlier findings (6.67 - 8.71 log cfu g^{-1}) reported in different parts of the country (Wondu, 2007; Mekdes, 2008; Zelalem, 2010; Debela, 2015).

The discrepancy in counts of aerobic mesophilic bacteria in butter sampled from different sources in this study might be attributed to differences in cleanliness of storage equipment and conditions, wash water used, handling materials and market place hygienic conditions. However, the overall mean AMBC observed in the present study failed to comply with the standard value (4.6 log cfu g^{-1}) given for good quality butter (COMESA, 2003).

The high counts of aerobic mesophilic bacteria observed in the present study could probably be related to the high initial microbial load in the raw material milk, absence of pasteurization, poor sanitary conditions of equipment used to manufacture the product, storage and transportation conditions, inadequate market facilities and the poor sanitary conditions practiced during butter handling. It is therefore advisable to adopt strict hygienic measures during milk handling to prevent contamination and improve the quality of its derivative products, in addition to proper heat treatment of milk and subsequent product handling (Meshref, 2010).

Coliform Count (CC): The average CC of the butter samples considered in this study was estimated at 2.5 log cfu g^{-1}. Higher CC (4.0 - 5.62 log cfu g^{-1}) was, on the contrary, reported for butter samples in the central highlands of Ethiopia (Zelalem, 2010; Debela, 2015). Aleme *et al.* (2013) also reported higher CC of (3.07 log cfu g^{-1}) butter samples made from the blend of goat and camel milk. However, lower coliform counts (0.90 to 1.66 log cfu g^{-1}) were reported for traditional butter made from camel milk in four regions of Algerian Sahara (Kacem and Karam, 2006).

The average CC observed in the present study is much higher than the acceptable limit of 1log cfu g^{-1} (Mostert and Jooste, 2002).This high count could be considered as indicative of potential product contamination with enteric pathogens thus represent potential health risk to consumers. The occurrence of coliform in a high number in this study could show substandard sanitation measures taken during product handling (milking, processing and packaging); fecal contamination or improper storage; and/or contamination while transportation following manufacturing. Moreover, wash water used from sources other than tap could also be a source of contamination by coliform bacteria.

Yeast and Mould Count (YMC): Yeast and mould are considered to be the major group of microorganisms causing butter quality deterioration during storage. Such quality deterioration could lead not only to a reduction of the nutritional value of the product but also render the product to produce undesirable flavor (Idoui *et al*., 2013). The average YMC observed in this study was estimated to be 4.6 log cfu g^{-1}. This figure is much higher than the maximum (50 cfu g^{-1}) value set for acceptable quality butter in the COMESA countries (COMESA, 2003). Values of YMC (ranging from 5.58 to 8.32 log cfu g^{-1}) higher than that observed in the current study were, however, reported for butter samples collected from various regions of Ethiopia (Mekdes, 2008; Zelalem, 2010; Debela, 2015). On the contrary, lower average values (2.08 - 3.0 log cfu g^{-1}) of YMC were also reported in Algerian traditional butter manufactured from goat and camel milk (Kacem and Karam, 2006; Idoui *et al.,* 2013). The overall mean counts of yeast and mould observed in this study are in congruent with the value (4.80 log cfu g^{-1}) reported by Samet-Bali *et al.* (2009) in Turkish butter samples.

In a similar growth environment, yeast and mould can usually grow faster than bacteria and accordingly can cause rapid food spoilage with high acidity, low moisture and water activity (Walstra *et al.,* 2006). In addition to causing food spoilage, some yeast and mould species are capable of producing dangerous mycotoxins (Ramazan *et al.,* 2010), aflatoxin being an example worth mentioning of the reports in all the milk and dairy feeds samples in Addis Ababa milk shed (Dawit *et al.,* 2016). The presence of high counts of yeast and mould in butter could likely be related among others to lack of hygienic practices during butter making process and/or packaging. Yeast and mould are widely distributed in the environment and can enter into food products such as butter through inadequately sanitized equipment and/or as airborne contaminants. Warm weather and improper storage conditions can also be considered to be among the principal causes for butter contamination (Moreira *et al.,* 2001).

Lipolytic BacteriaCount (LPBC): Being psychotropic and natural inhabitants of soil, water and animals, lipolytic bacteria count are ubiquitous and able to grow at refrigeration temperature. Lipolytic bacteria are the most frequently occurring spoilage microorganisms in butter therefore are used to predict the keeping quality of butter (Lejko *et al.,* 2009). Lipolytic bacteria have lipolytic activity often responsible for rancidity or loss of flavors (Idoui *et al.,* 2013). Minimizing lipolytic microorganisms therefore means increasing fat retention and high butter yield with desirable flavor.

The average LPBC of butter samples collected from producers is significantly lower than that obtained from dairy product shops and open markets. Under acceptable condition, counts of lipolytic bacteria should not exceed 1.69 log cfu g^{-1} if the butter is to be considered as good quality (Richard, 2002). However, in the current study the observed counts are much higher in lipolytic bacteria (3.98 log cfu g^{-1}) than the acceptable limit. Studies carried out by Kacem and Karam (2006) and Rady and Badr (2003) reported lower average LPBC of 2.33 and 3.07 log cfu g^{-1}, respectively. However, no lipolytic bacteria were detected in butter manufactured from cow's milk in East Algeria (Adams and Moss 2008). The cleanliness of the milkers, the udder of the cow, the washing water, the milking environment, and processing and storage equipment represent major sources of contamination of the raw material milk as well as the final processed product.

Listeria monocytogenes: A food unfit for human consumption may not necessarily be spoiled and contain pathogens. *Listeria monocytogenes* is considered as one of the most important pathogens responsible for food-borne infection (Ryser and Marth, 2007). The prevalence of *Listeria monocytogenes* is higher (6.2%) in butter samples obtained from open market butter traders compared with those sampled from dairy product shops (3.2%) and smallholder farmers (2.26%). Similar to the present study, several positive *Listeria monocytogenes* results were reported in different countries such as Finland, Turkey and Iran (Lyytikainen *et al.,* 2000; Aygun and Pehlivanlar, 2006; Rahimi *et al.,* 2012). Detection of *Listeria monocytogenes* in product samples can reflect fecal and environmental contamination during milking, storage and transport as well as infected cows in dairy farms and poor silage quality (Husu, 2010). The presence of *Listeria monocytogenes* in butter may also indicate a significant failure of hygiene standards during the preparation and/or storage of such products.

***Salmonella* spp:** Studies made on pathogens isolation and food borne illness associated with the consumption of butter contaminated with *Salmonella* and *Listeria monocytogenes* had not been fully documented in Ethiopia. *Salmonella* species are of public health concern given the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life-threatening typhoid fever (Fook *et al.,* 2004). In this study, *Salmonella* was not detected in 160 tested butter samples collected from different sources, which contrasts with the 1% prevalence of *Salmonella* in butter sampled from Addis Ababa (Liyuwork *et al.,* 2013). Foods of animal origin, particularly milk and milk products, meat and egg, are considered to be primary sources of human *Salmonellosis* (Acha and Szyfers, 2001). Most of these food products become contaminated during milking, slaughter, processing in contaminated environment and because of faulty during transportation, handling and storage facilities.

Conclusion

Based on results of the current study, butter produced and marketed in the study areas can generally be considered to be of poor quality, which is evidenced by the observed microbial quality that did not fulfill the required acceptable limits set for quality butter. The microbial contamination of butter could be attributed to factors such as the raw material (unpasteurized whole milk) coupled with traditional methods of processing, handling, storage and marketing. Therefore, it is essential to consider all butter quality affecting factors and accordingly take corrective measures to avoid microbial contamination of butter. Such quality measures should be taken all the way starting from the production of raw milk till the butter is consumed. This is important in terms of ensuring food security, public health and better financial return for all market actors involved in the butter value chain in the current study areas.

Acknowledgments

The authors are grateful to all enumerators involved in survey and Dairy Microbiology Laboratory staffs of the Holetta Agricultural Research Center for their kindness and support during the laboratory work. The financial assistance of Debre Berhan University, Haramaya University, and Ethiopian Institute of Agricultural Research (EIAR) are also highly appreciated.

References

Acha, P.N. and Szyfres, B. 2001. Zoonoses and communicable diseases common to man and animals (3rd edn). Washington DC: Pan American Health Organization 1, 233-246.

ACMSF. 2003. Information Paper. Recent trends in listeriosis in the UK. ACM⁄667. London, UK: Advisory Committee on the Microbiological Safety of Foods (ACMSF).

Adams, M.R. and Moss, M.O. 2008. Food microbiology (3rd edn). (RSC publishing, Cambridge, UK).

Aleme Asresie, Eyasu Seifu and Mohammed Yousuf. 2013. Churning efficiency and microbial quality of butter made from camel milk alone and blending it with goat milk. Net Journal of Agricultural Science1(3): 75-80.

Almaz Gonfa, A., Foster, H.A. and Holzapfel, W.H. 2001. Field survey and literature review of Ethiopian traditional fermented milk products. International Journal of Food Microbiology 68: 173-186.

Asrat Guja. 2009. Evaluation of traditional practices and effects of lacto peroxidase system on keeping quality of cottage cheese. MSc Thesis, University of Hawassa, Ethiopia.

Aygun, O. and Pehlivanlar, S. 2006. Listeria spp. in the raw milk and dairy products in Antakya, Turkey. Food Control 17: 676 - 679.

Biruk Hailu, Samson Ghilu, Emmanuelle, G., Zelalem Yilma and Eyasu Seifu. 2012. Traditional knowledge for hygienic milk production in central highlands and Arsi zone dairy producers. In proceedings of the 19th annual Ethiopian Society of Animal Production conference. pp 429-441. Ethiopian society of animal production (ESAP), Addis Ababa.

Budhkar, Y. A., Bankar, S.B. and Singhal, R.S. 2014. Milk and milk products: microbiology of cream and butter. In: Batt, C.A., Tortorello, M.L. (Edn.), Encyclopedia of food microbiology 2, 728–73. Elsevier Ltd, Academic Press.

Chun, L., Mascola, L., Thomas, J.C., Bibb, W.F., Schwartz, B., Salminen, C. and Heseltine, P. 1990. A case-control study of a cluster of perinatal listeriosis identified by an active surveillance system in Los Angeles County, California, September 1987 - February 1988. In Food-borne Listeriosis Topics in Industrial Microbiology Edn AJ Miller, JL Smith, GA Somkuti PP. 75 - 79.

COMESA. 2003. Butter specification. Draft COMESA/East African Standard No. CD/K03-2 - 3. Common market for Eastern and Southern Africa, Lusaka, Zambia.

Dawit Gizaw, Barbara, S., Azage Tegegne, Jean, H. and Delia, G. 2016. Aflatoxin contamination of milk and dairy feeds in the greater Addis Ababa milk shed, Ethiopia. Food Control59: 773-779.

DebelaBayu. 2015. Traditional butter preservation techniques and comparison of their efficiency through determination of microbial quality and organoleptic properties of butter in West Shewa zone, Oromia regional state, Ethiopia. MSc Thesis, University of Ambo, Ethiopia.

El-Shamy, H.A., Bakr, W.I., Gomaa, N.F. and Barheem, O.H. 2008. Evaluation of two enrichment broths, three plating media and ELISA technique for the isolation of Salmonella from dairy products. Journal of the Egyptian Public Health Association 83: 133-137.

Fook, Y., Aminah, A. and Mohd, K.A. 2004. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiology21: 535-541.

Hiwot Desta. 2013. Hygienic practices during milking and bacteriological quality of the milk in raw bovine bulk milk in the selected milk collection centers: smallholder dairy processing Ethiopia. PhD Dissertation, Università Degli Studi di Milano, Germany.

HPA. 2003. Preparation of samples and decimal dilutions. D1. Issued by standard unit, evaluation and standards laboratory. Health Protection Agency (HPA). Specialist and Reference Microbiology Division 2(3).

Husu, J.R. 2010. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. Journal of Veterinary Medicine37: 276-282.

Idoui, T., Rechak, H. and Zabayou, N. 2013. Microbial quality, physicochemical characteristics and fatty acid composition of a traditional butter made from goat milk. Food Science and Technology14(1): 108-114

Kacem, M. and Karam, N.E. 2006. Physicochemical and microbiological study of "shmen", a traditional butter made from camel milk in the Sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. Grasas Y Aceites 57(2): 198-204.

Lejko, D.N., Grega, T., Sady, M. and Domagała, J. 2009. The quality and storage stability of butter made from sour cream with addition of dried sage and rosemary. Journal of Biotechnology Animal Husbandry 25(6): 753-76.

Lemma Fita. 2004. Assessment of butter quality and butter making efficiency of new churns compared to smallholders' butter making techniques in East Shewa zone of Oromia. MSc Thesis, University of Haramaya, Ethiopia.

Liyuwork Tesfaw, Biruhalem Taye, Sefinew Alemu, Haile Alemayehu, Zufan Sisay and Haileleul Negussie. 2013. Prevalence and antimicrobial resistance profile of *Salmonella* isolates from dairy products in Addis Ababa, Ethiopia. African Journal of Microbiology Research **7**(43): 5046-5050.

Lyytikainen, O., Autio, T., Maijala, R., Ruutu, P., Honkanen-Buzalski, T., Miettinen, M., Hatakka, M., Mikkola, J., Anttila, V.J., Johansson, T., Rantala, L., Aalto, T., Korkeala, H. and Siitonen, A. 2000. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. Journal of Infectious Disease 181(5): 1838-1841.

MekdesAsfaw. 2008. Assessment of processing techniques and quality attributes of butter produced in Delbo watershed of Wolayta zone, Southern Ethiopia. MSc Thesis, University of Hawassa, Ethiopia.

Meshref, S.M. 2010. Microbiological quality and safety of cooking butter in Beni-Suef governorate, Egypt. African Health Science10(2): 193-198.

Moreira, S.R., Schwan, R.F., Carvalho, E.P. and Wheals, A.E. 2001. Isolation and identification of yeasts and filamentous fungi from yoghurts in Brazil. Brazil Journal of Microbiology 32(2): 117-122.

Mostert, J.F. and Jooste P.J. 2002. Quality control in the dairy industry. In: Robinson, R.K. (ed.) Dairy microbiology handbook, 3rd eds., John Wiley, New York 655–736.

O'Connor, C.B. 1995. Rural dairy technology. ILRI training manual 1. International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, 133p.

Pandey, G.S. and Voskuil, G.C. 2011. Manual of milk safety, quality and hygiene. Golden Valley Agricultural Research Trust, Lusaka Zambia.

Rady, A.H. and Badr, H.M. 2003. Keeping the quality of cows' butter by γ–irradiation. Grasas y Aceites54: 410-418.

Rahimi, E., Momtaz, H., Sharifzadeh, A., Behzadnia, A., Ashtari, M.S., Zandi Esfahani, S., Riahi M. and Momen, M. 2012. Prevalence and antimicrobial resistance of listeria species isolated from traditional dairy products in Chahar Mahal and Bakhtiyari, Iran. Bulgarian Journal of Veterinary Medicine15(2): 115-122.

Ramazan, G., Yesim, A. and Emine, N.H. 2010. Microbiological quality of karın butter, a traditionally manufactured butter from Turkey. Rasas Y Aceites 61(2): 121-125.

Richard, K.R. 2002. Dairy microbiology hand book 3rd edition. A John Wiley and Sons, Inc., Publication.

Richardson, G.H. 1985. Standard methods for the analysis of dairy products, 15th edition. American Public Health Association, Washington, DC., USA.

Ryser, E.T. and Marth, E. 2007. Listeria, listeriosis and food safety 3rd edition. Taylor and Francis, Boca Raton, FL.

Samet-Bali, O., Ayadi, M.A. and Attia, H. 2009. Traditional Tunisian butter, physicochemical and microbial characteristics and storage stability of the oil fraction. Food Science and Technology, 42, 899-905.

Walstra, P., Jan, W. and Tom, G. 2006. Dairy science and technology second edition. CRC Press Taylor and Francis Group, England.

Wondu Mamo. 2007. Composition, microbial quality and acceptability of butter produced from cow's milk in Awassa, Southern Ethiopia. MSc Thesis, University of Hawassa, Ethiopia.

Yousef, A.E. and Carlstrom, C. 2003. Food Microbiology, a Laboratory Manual. A John Wiley and Son, Inc., Hoboken, New Jersey, Canada.

Zelalem Yilma and Faye, B. 2006. Handling and microbial load of cow's milk and Ergo (fermented milk) collected from different shops and producers in central highlands of Ethiopia. Ethiopia Journal of Animal Production 6(2): 67-82.

Zelalem Yilma, Loiseau, G. and Faye, B. 2007. Manufacturing efficiencies and microbial properties of butter and Ayib - Ethiopian cottage cheese. Livestock Research for Rural Development19(7).

Zelalem Yilma. 2010. Quality factors that affect Ethiopian formal milk business: Experiences from selected dairy potential areas, Netherlands Development Organization, Addis Ababa, Ethiopia.

Haplotype Variation, Linkage Disequilibrium and Neutrality Test Analyses of Detected Single Nucleotide Polymorphisms in Kisspeptin Gene of Selected Indigenous Goat Populations in Ethiopia

Getinet Mekuriaw Tarekegn[1,2,*], Tadelle Dessie[3], Okeyo Mwai[4], Appolinaire Djikeng[5], Joram M. Mwacharo[6], Sarah Osama[7], Kassahun Tesfaye[8]

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]Department of Animal Production and Technology, Bahir Dar University, Ethiopia
[3]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia
[4]International Livestock Research Institute (ILRI), Nairobi, Kenya
[5]Centre for Tropical Livestock Genetics and Health, The University of Edinburgh, UK
[6]Small Ruminant Genetics and Genomics Group, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia
[7]The University of Queensland, Australia
[8]Department of Microbial, Cellular and molecular Biology Addis Ababa University, Ethiopia
Corresponding author: * = yafetgetinet@gmail.com

Abstract

The study was aimed to evaluate the haplotype diversity and patterns of linkage disequilibrium (LD) of Kisspeptin **(***KISS1) gene in Woyto-Guji and Gondar goat populations in Ethiopia. Target regions of the KISS1 gene which include Exon1 (1210 bp) and Exon2 (325 bp) were amplified. A total of 29 haplotypes in exon1 and three haplotypes in exon2 were detected. The first haplotype of exon2 registered highest haplotype frequency (f=0.947) of all. In exon1, seven haplotypes showed haplotype frequency <1%. Lowest (0.083 for Woyto-Guji and 0.081 for Gondar goats) and modest (0.656 for Woyto-Guji and 0.635 for Gondar goats) average estimates of* R^2 *and |D'| were obtained in LD decay analysis of the haplotypes observed in exon1, respectively. Based on the loci linkage combination analysis, loci 3649, 3808, 3963 and 3989 showed highly significant LD accumulation. The neutrality tests showed significant and negative values of Fs (Fs = -8.098 for Woyto-Guji and Fs = -12.08 for Gondar goat populations) in the goat populations studied; whereas, the Tajima's D test was positive and non-significant. Overall, remarkable haplotype diversity was observed on the KISS1 gene in the goat populations studied, and measures of LD decays revealed effect of selection implying importance of the target region as a candidate gene for the fecundity trait.*

Key words: *Haplotype heterozygosity, Indigenous goats, Linkage disequilibrium, Neutrality test*

Introduction

Apart from the study of genomic DNA (nuclear as well as mitochondrial DNAs), the study of association of alleles, which is non random, plays fundamental role in evolutionary and history of demographic expansion of population genetics (Fields, 2014). Linkage disequilibrium (LD) plays a pivotal role in genomic selection, mapping quantitative trait loci (QTL), estimates for effective population size, marker assisted selection and association study (Nachman, 2002; Khatkar et al., 2008; Zhu et al., 2013). At genome wide scale, LD can serve to uncover the population history, population characteristics, the breeding system, patterns of gene exchange and geographic subdivision (Zhu et al., 2013); whereas, at the level of genomic region/s it reflects the history of natural selection, gene conversion and mutation (Slatkin, 2008). Linkage disequilibrium is facilitated by genetic and non-genetic factors like, genetic drift, genetic linkage, mutation, selection, population structure, demographic expansion and non random mating (Majo, 2008; Zhu et al 2013). However, these forces which affect LD in the genomic region depends on rate of recombination (Slatkin, 2008) and the extent of their effect vary they each other. For instance, natural selection affects only one or a small number of loci; by contrast, population subdivision, changes in population size and the exchange of individuals among populations affect LD throughout the genome.

Methods that directly evaluate LD by using haplotype data are more powerful than methods that examine multiple loci without evaluation of haplotype sharing (Service et al., 1999). It is believed that true haplotypes are more informative than genotypes (Gong et al., 2007; Pei et al., 2009) and are more powerful than single markers for genetic association analysis due to the highest statistical power haplotype based association test has than tests using single SNPs (Shifman et al., 2002; Pei et al., 2009). Balding (2006) also mentioned LD will remain crucial to the design of association studies until whole-genome re-sequencing becomes routinely available. However, there is the issue of uncertainty of individual haplotype in haplotype based analysis, which can be resolved by haplotype phasing algorithm like haplotype trend regression analysis, which is an efficient genetic association analysis method (Pei et al., 2009). In addition, the information loss that arises from phasing is small when linkage disequilibrium (LD) is strong (Balding 2006). The pattern of LD varies across chromosomes and genomic regions (Zhu et al., 2013).

The extent and distribution of linkage disequilibrium (LD) in livestock is becoming a center of discussion. It is because of the fact that it plays a fundamental role in gene mapping, both as a tool for fine mapping of complex disease genes and in proposed genome wide association studies (Service et al., 1999; Nachman, 2002; Slatkin, 2008). In line with this, number of markers required for a purpose like marker-trait association study and mapping is determined by the extent of LD (Abecasis et al., 2001; Khatkar et al., 2008). Moreover, if alleles at two loci are in LD and they both affect reproductive fitness, the response to selection on one locus might be accelerated or impeded by selection affecting the other (Slatkin, 2008). The ultimate value of SNPs for linkage and association mapping studies depends, in part, on the distribution of SNP's allele frequencies and inter-marker linkage disequilibrium (LD) across populations (Goddard et al., 2000).

Analyses of haplotype and linkage disequilibrium have been carried out in various farm animals like cattle, sheep, pig and chicken. However, there is limited effort conducted on domestic goats. On the other hand, smaller effective population size and selection practices led LD to be far reached in farm animals than in human (McRae et al., 2002). However, analysis of haplotype and linkage disequilibrium at a segment of the genome has been rarely carried out in livestock species. Instead, genome wide analysis of LD has been extensively done by various scholars. This is because of the fact that the number of haplotypes would be small in a segment of DNA (Shifman et al., 2002; Beaty et al., 2005; Balding, 2006). Moreover, due to the smallest size of sequenced region which leads to have shortest physical distances among segregating sites, short physical distances among polymorphic regions are not suggested for association study (Pritchard and Przeworski, 2001). Therefore, scholars prefer to focus analysis of LD at genome wide level.

However, it does not necessary mean that a segment of DNA; i.e. a target gene, cannot be useful for association study. There are genes, called polygenes or pleiotrphy, which help for the expression of a phenotype trait/s. Moreover, literatures confirmed that haplotypes can explain more information about an unobserved causal variant by identifying it uniquely or by identifying related haplotypes which are overrepresented among cases (Beaty et al., 2005). Therefore, polymorphism analysis on specific genes and their associations to targeted traits of interest have been carried out by various scholars. GPR54 and KISS1 genes for litter size in goats and sheep, major histocompatibility complex (MHC) gene for immunity in goats are some of the genes which the polymorphism and gene-trait association studies were carried out (Cao et al., 2010 and 2011; Grossen et al., 2014). In a recent study, on the same target regions of the KISS1 gene and the SNPs observed, we carried out the polymorphism analysis and association of the KISS1 gene with twining ability in Gondar and Woyto-Guji goat populations of Ethiopia (Getinet Mekuriaw et al., 2017). As a follow up, this study was initiated to evaluate the haplotype diversity and extent of linkage disequilibrium of detected loci in KISS1 gene in Woyto-Guji and Gondar goat populations in Ethiopia.

Materials and Methods

In this study Woyto-Guji and Gondar goat populations were included. These populations were selected for this study because parts of the goat community based breeding program supported by BecA-Sweden partnership program. The sample size included in the study were 115 sequences for exon1 and 130 sequences for exon2 of Woyto-Guji goat and 58 sequences for exon and 112 sequences for exon2 of Gondar goat. The sample size difference between the exon regions in both goat populations was due to poor quality of some sequences. Information related to the agro-ecology and the production systems the populations are being reared, the sample type and size, the DNA extraction and extraction protocol, primers employed, PCR conditions, target regions for sequencing and data management are described in our former study (Getinet Mekuriaw et al., 2017).

Estimates of haplotype frequencies, measures and patterns of pairwise LD and neutrality test were computed for both goat populations using the built-in infinite site models of Tajima's D and Fisher's exact tests. To investigate the variability associated with fine-scale measures of LD, measures of pairwise LD decays (*R*, R^2, *D*, *D'* and |*D'*|) between adjacent markers were calculated in both populations. Measures of pairwise LD decays, recombination rate, number of haplotypes, haplotype diversity estimates and neutrality test were analyzed by DnaSP 5.0 software (Rozas et al., 2003); whereas, loci linkage, heterozygosity estimation of haplotypes were analyzed by Arlequin ver. 3.0 (Excoffier et al., 2005). Measures of haplotype diversity were evaluated based on estimated haplotype frequencies (Beaty et al., 2005). This measure of gene diversity is analogous to the heterozygosity at a single locus and attains its maximum when haplotypes observed in the sample occur at equal frequencies. The number of different haplotypes in each population reflects this haplotype diversity. To understand whether the observed number of unique haplotypes was different among populations, we first calculated the number of expected haplotypes for each population sample. To calculate F_{ST}, single-nucleotide polymorphisms (SNPs) contain much less information when taken one at a time (Browning and Wei et al., 2010); and hence F_{ST} values have not been tested for this study. Moreover, the preliminary analysis indicated negative result, which is unexpected that might be because of the least information each SNP contain when it is taken at a time and the overall smaller numbers of SNPs obtained in the target regions. In relation to this, it is suggested to calculate averages over windows of markers or even over the whole genome (Weir et al., 2005), which is far from the objective, approach and target regions of this study.

Results

Estimation of haplotype frequencies and heterozygosities

A total of 29 haplotypes in exon1 and three in exon2 were obtained in both populations, of which only 10 of them in exon1 and two in exon2 were shared haplotypes by both populations (Table 1).

Table 1. Haplotype frequency of KISS1 gene of the goat populations studied

	Exon1		Exon2	
Haplotype	Woyto-Guji (n=116)	Gondar (n=62)	Woyto-Guji (n=133)	Gondar (n=117)
Hap-1	0.0435	0.0172	0.9470	0.9830
Hap-2	0.2430	0.2410	0.0301	0.0171
Hap-3	0.1910	0.1550	0.0226	
Hap-4*	0.0087			
Hap-5	0.1480	0.2240		
Hap-6	0.0174	0.0172		
Hap-7	0.0261	0.0345		
Hap-8*	0.0087			
Hap-9	0.0609	0.0172		
Hap-10	0.0783	0.0690		
Hap-11*	0.0522			
Hap-12	0.0261	0.0172		
Hap-13*	0.0435			
Hap-14	0.0087	0.0345		
Hap-15*	0.0870			
Hap-16*	0.0087			
Hap-17*	0.0087			
Hap-18**		0.0172		
Hap-19* *		0.0172		
Hap-20**		0.0172		
Hap-21* *		0.0172		
Hap-22* *		0.0172		
Hap-23* *		0.0172		
Hap-24* *		0.0172		
Hap-25 **		0.0172		
Hap-26 **		0.0172		
Hap-27 **		0.0172		
Hap-28 *	0.0087			
Hap-29 *	0.0087			

Key:- *= Private haplotypes in Woyto-Guji population; **= private haplotypes in Gondar population

The remaining haplotypes were uncommon for both populations. These 29 haplotypes were generated from previously reported 20 segregating sites (Getinet Mekuriaw et al., 2017). The haplotype frequencies in exon1 range from 0.0087-0.243 and from 0.0171-0.983 in exon2. The 2^{nd}, 3^{rd} and 5^{th} haplotypes in exon1 and the 1^{st} haplotype in exon2 registered the highest haplotype frequencies in both goat populations.

Haplotype diversity

The overall gene (haplotype) diversity was 0.870 ± 0.014 for exon1 and 0.0703 ± 0.022 for exon2; whereas the mean nucleotide diversity estimated to be 0.00275 ± 0.00157 for exon1 and 0.00029 ± 0.00002 for exon2 of both populations. The lowest haplotype diversity obtained in exon2 might be because of the smallest number of haplotypes observed in this target region. On the other hand, the overall mean expected and observed haplotype heterozygosities were 0.034482755 and 0.034482731, respectively with a range of 0.0057803 - 0.2665826 in exon1 and 0.0225564 - 0.9473684 in exon2 (Table 2 and 3). The highest haplotype heterozygosity was observed in the first haplotype, and the second and third haplotypes follow in exon1 and in the first haplotype in exon2. Low values of both heterozygosity estimates (H_O and H_E) were observed in most of the haplotypes. The expected heterozygosity (H_E) estimations were not consistently higher than observed heterozygosity (H_O) estimates (Supplementary Figure S1) implying the absence of sampling bias (Dorji et al., 2012).

Table 2. Estimates of haplotype heterozygosity (H_O and H_E) in Exon2

Hap	Over all		Woyto-Guji		Gondar	
	H_O	H_E	H_O	H_E	H_O	H_E
1	0.9640000	0.7891760	0.9473684	0.7799023	0.9829060	0.8716068
2	0.0240000	0.1760640	0.0300752	0.1803008	0.0170940	0.1283932
3	0.0120000	0.0347600	0.0225564	0.0397970	-	-

Table 3. Estimates of haplotype heterozygosity (H_O and H_E) in Exon1

Hap	Over all		Woyto-Guji		Gondar	
	H_O	H_E	H_O	H_E	H_O	H_E
1	0.2427746	0.2043179	0.2434783	0.2665826	0.2413793	0.2015172
2	0.1791908	0.1294046	0.1913043	0.1590348	0.2241379	0.1310172
3	0.1734104	0.0978786	0.1478261	0.1118696	0.1551724	0.0997586
4	0.0751445	0.0779538	0.0782609	0.0859478		
5	0.0462428	0.0642428	0.0608696	0.0678000	0.0689655	0.0810000
6	0.0346821	0.0542601	0.0521739	0.0550870	0.0344828	0.0669310
7	0.0346821	0.0460000	0.0434783	0.0448870	0.0344828	0.0572931
8	0.0289017	0.0396069	0.0434783	0.0374522		
9	0.0289017	0.0347341	0.0260870	0.0310087	0.0172414	0.0492241
10	0.0231214	0.0303988	0.0260870	0.0260435	0.0172414	0.0427931
11	0.0173410	0.0267052	0.0173913	0.0215739		
12	0.0173410	0.0236821	0.0086957	0.0182522	0.0172414	0.0376379
13	0.0057803	0.0208266	0.0086957	0.0153043		
14	0.0057803	0.0184624	0.0086957	0.0127391	0.0172414	0.0330862
15	0.0057803	0.0164971	0.0086957	0.0106087		
16	0.0057803	0.0147572	0.0086957	0.0094696		
17	0.0057803	0.0130000	0.0086957	0.0088957		
18	0.0057803	0.0116012			0.0172414	0.0288103
19	0.0057803	0.0103584			0.0172414	0.0251207
20	0.0057803	0.0090462			0.0172414	0.0219310
21	0.0057803	0.0078555			0.0172414	0.0192759
22	0.0057803	0.0069480			0.0172414	0.0181207
23	0.0057803	0.0064162			0.0172414	0.0174828
24	0.0057803	0.0060347			0.0172414	0.0172759
25	0.0057803	0.0058613			0.0172414	0.0172414
26	0.0057803	0.0058035			0.0172414	0.0172414
27	0.0057803	0.0057861			0.0172414	0.0172414
28	0.0057803	0.0057803	0.0086957	0.0087391		
29	0.0057803	0.0057803	0.0086957	0.0087043		

Analysis of linkage disequilibrium and neutrality test

Linkage disequilibrium (LD): it is a sensitive indicator of the population genetic forces that structure a genome (Slatkin, 2008). In this study, most estimates of *D'* and *R* were obtained below and close to zero (Supplementary Table S1). This could be contributed by ceiling effect of *D'* and is largely responsible for the low rank correlation between populations for the *D´* measure (Evan and Cardon, 2005). Similarly, the average estimates of coefficient of LD (R^2) which is the major measure of LD, was very low.

The average R^2 values were 0.083 and 0.081 for Woyto-Guji and Gondar goat populations, respectively (Table 4). Whereas, the mean value of |*D'*| were 0.656 for Woyto-Guji and 0.635 for Gondar goat. However, most relationships of the SNPs and LD measures are concentrated at the maximum value for |*D'*| (|*D'*| = 1) and minimum value for R^2 (R^2 = 0). The average distance among segregating/polymorphic sites is comparable for both goat populations. It was estimated 226.19 bp for Woyto-Guji and 234.86 bp for Gondar goat populations. On the other hand, the |*D'*| regression showed positive relationship whereas the R^2 had negative but weak relationship with respect to the physical distances of polymorphic sites in all categories of the goat populations (Figure 1). It is believed that the regression analysis helps to indicate the relationship between linkage disequilibrium with physical distance (Sokal and Rohlf, 1981).

Table 4. Descriptive statistics of measures of linkage disequilibrium

	Woyto-Guji				Gondar			
	Mean±sd	Range	Min	Max	Mean±sd	Range	Min	Max
D	0.00440±0.06	0.31	-0.14	0.17	0.01500±0.05	0.26	-0.080	0.18
D'	-0.18052±0.74	2.00	-1.00	1.00	-0.11405±0.72	1.85	-1.000	0.85
|*D'*|	0.65633±0.36	0.86	0.14	1.00	0.63462±0.32	0.97	0.033	1.00
R	0.02410±0.29	1.26	-0.57	0.69	0.07643±0.28	1.08	-0.338	0.74
R^2	0.08334±0.15	0.47	0.0006	0.47	0.08111±0.15	0.55	0.00005	0.55
Dis.	226.19±161.86	570.00	3.00	573.00	234.86±170.64	570.00	3.00	573.00

Key: Dis.= distance among segregating sites, Measures of LD

Table 5. Correlation analysis of LD measures: Woyto-Guji (below diagonal) and Gondar (above diagonal)

	D	*D'*	|*D'*|	*R*	r^2
D		0.665^{**}	0.013^{ns}	0.952^{**}	0.719^{**}
D'	0.588^{**}		-0.562^{**}	0.795^{**}	0.461^{*}
|*D'*|	-0.105^{ns}	-0.465^{*}		-0.092^{ns}	0.148^{ns}
R	0.972^{**}	0.728^{**}	-0.146^{ns}		0.759^{**}
r^2	0.204^{ns}	0.260^{ns}	0.224^{ns}	0.272^{ns}	

Key: *=significant at 5% significant level; **= significant at 1% significant level; ns=non significant

On the other hand, the correlation analysis indicates that there is modest to highest correlation among most of the LD measures in the goat populations studied (Table 5). For instance, *R* and *D* had shown strong correlation whereas modest correlation was observed between *R* and *D'*. However, R^2 showed non-significant correlations with all the LD measures. Negative correlations with variable power of correlations were observed between |*D'*| and *D'*, and *R* and |*D'*| in both goat populations.

Among the twelve loci (polymorphic sites which occurred in more than 1% of the sequences; Getinet Mekuriaw et al., 2017) combinations in both goat populations 16.67% of them had significant marker-marker linkage disequilibrium in exon1 of both goat populations (Table 6). Comparatively, highest LD accumulations were detected in associations at 3649, 3808, 3963 and 3989 loci. The detection of significant linkages observed in few of the loci goes in line with the percentage estimations of linked loci per locus (Table 7). Similarly, association of the nine polymorphic sites in each goat population indicated that there was similar (16.67%) accumulation of linkage disequilibrium (Table 6). The highest significant LD accumulation was observed at 3989 locus association in Gondar goat population and at 3649 and 3963 loci in Woyto-Guji goat population. There was no any LD accumulation detected at loci 3416, 3533 and 3770 with the respective loci combinations in Woyto-Guji goat population and at loci 3354, 3416, 3696 and 3963 in Gondar goat population. On the other hand, the overall estimated recombination rate was 0.0567 in this study. Based on Hudson (1987) test, in exon1, the estimated recombination rate was detected in five adjacent sites: (3416, 3649), (3783, 3808), (3808, 3811), (3811, 3963), (3963, 3989).

Table 6. Linkage analysis observed among loci in exon1

L/L	Both goat populations simultaneously												Woyto-Guji (below diagonal) and Gondar (above diagonal)									
	3354	3416	3533	3649	3696	3770	3783	3808	3811	3927	3963	3989	3416	3533	3649	3770	3783	3808	3811	3927	3963	3989
3354	*												-		-			-	-	-	-	-
3416	-	*											*		-			-	-	-	-	-
3533	-	-	*										-	*	-			-	-	-	-	-
3649	+	-	-	*									-	-	*			+	-	-	-	+
3696	-	-	-	-	*													-	-	-	-	-
3770	-	-	-	+	-	*							-	-	-	*		-	-	-	-	-
3783	-	-	-	+	-	-	*						-	-	+	-	*	-	-	-	-	-
3808	-	-	-	+	-	-	-	*					-	-	+	-	-	*	-	-	-	+
3811	-	-	-	-	-	-	-	-	*				-	-	-	-	-	-	*	-	+	+
3927	-	-	-	-	-	-	-	-	-	*										*	-	-
3963	-	-	-	-	-	-	-	+	+	-	*		-	-	-	-	-	-	+		*	+
3989	+	-	-	+	-	+	-	+	-	-	+	*	-	-	+	-	-	+	-	-	+	*

Key: L=locus

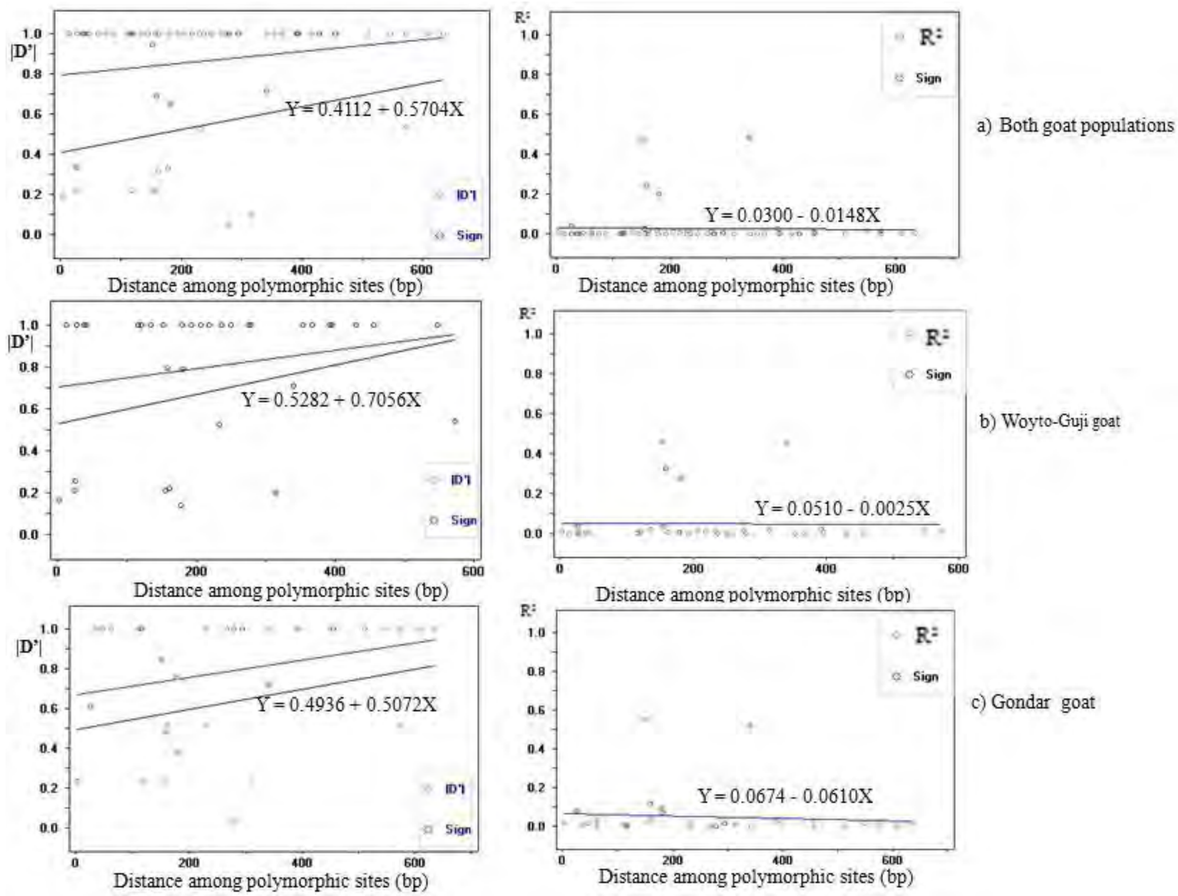


Figure 1. Measures of LD decays (|*D'*| and R^2) with respect to distances among segregating/polymorphic sites

Neutrality test evaluation*:* the randomly evolving mutations are called "neutral", while mutations under selection are "non-neutral" (Tajima, 1989b). The neutrality tests used for the study were Tajima's *D* and Fu's *F*s, which both assume infinite-site model. In our study, Fu's *F*s test showed significant negative values for both goat populations (*F*s = -8.098 for Woyto-Guji; *F*s = -12.080 for Gondar goat population), providing evidence for the effects of selection on KISS1 gene, especially in Gondar goat population. It was also consistent with the haplotype frequency differences obtained (Table 1); the increased inter population haplotype frequency differences are indications of selection pressures (Voight et al., 2006; Weir et al., 2005). The *ZnS* test, which is a measure of allele frequency equivalency across polymorphic sites in the absence of recombination (Kelly, 1997), is also very minimal (0.0265; Table 8). This suggests that there was almost absence of recombination rather presence of selective sweeps (Kelly, 1997).

Table 7. Percentage of linked loci per locus (α (*P*)=0.05)

	y\Locus	**3416**	**3533**	**3649**	**3770**	**3783**	**3808**	**3811**	**3963**	**3989**	**No. of loci**
Woyto-Guji	1	0.0	0.0	37.5	0.0	12.5	25.0	12.5	25.0	37.5	9
	2	0.0	*	50.0	*	16.7	33.3	16.7	33.3	50.0	7
	3	0.0	*	40.0	*	*	40.0	20.0	40.0	60.0	6
	4	0.0	*	40.0	*	*	40.0	20.0	40.0	60.0	6
	5	*	*	50.0	*	*	50.0	25.0	50.0	75.0	5
	y\Locus	**3354**	**3416**	**3649**	**3696**	**3808**	**3811**	**3927**	**3963**	**3989**	**No. of loci**
Gondar	1	0.0	0.0	25.0	0.0	25.0	25.0	0.0	25.0	50.0	9
	2	*	0.0	33.3	*	33.3	33.3	0.0	33.3	66.7	7
	3	*	0.0	40.0	*	40.0	40.0	*	40.0	80.0	6
	4	*	0.0	40.0	*	40.0	00.0	*	40.0	80.0	6
	5	*	*	50.0	*	50.0	50.0	*	50.0	100.0	5

Table 8. Neutrality test in exon1 across populations

Neutrality model	**Over all**	**Woyto-Guji**	**Gondar**
ZnS (Kelly, 1997)	0.0265	0.0504	0.0509
Za (Rozas et al 2001)	0.0064	0.0636	0.0203
ZZ (Rozas et al 2001)	-0.0200	0.0132	-0.0306
*F*s values (Fu, 1997).	-17.7960^{**}	-8.0980^{*}	-12.0800^{**}
Tajima's *D* (Tajima, 1989b)	0.19900^{ns}	0.85931^{ns}	0.46476^{ns}
Fay and Wu's *H* (Fay and Wu, 2000)	-2.11640		

The neutrality estimates vary among polymorphism and divergence, and overview of polymorphism in all tests of both populations. The neutrality test for polymorphism and divergence were 0.82933 (Tajima's *D* test), -0.22458 (Fu and Li's *D** test), 0.16986 (Fu and Li's *F** test) (Fu and Li, 1993) and -7.114 (Fu's *F*s test). Whereas, the overview of polymorphism was 0.19900 (Tajima's *D* test), -1.29583 (Fu and Li's *D** test, -0.89153 (Fu and Li's *F**) and -17.796 (Fu's *F*s test).

Discussion

Haplotype analysis

In these days, fine-mapping studies and identification of candidate genes are conducted by haplotype analysis of the SNPs detected in the target regions (Beaty et al., 2005). However, it is mentioned that there has been surprisingly little work done on haplotype based multivariate association analyses (Pei et al., 2009). Haplotype based analysis of the kisspeptin gene was carried out in this study. The result indicated that more number of rare haplotypes was detected in Woyto-Guji goat population. This might be due to relatively more sample size used in Woyto-Guji goat population compared to Gondar goat. Large sample size in a population is more likely includes more rare haplotypes (Beaty et al., 2005). In the shared haplotypes, relatively more haplotype frequencies were obtained in Woyto-Guji population than Gondar except the 1st haplotype of exon2 (Table 2). However, almost all non-shared haplotypes have less than 2% haplotype frequency estimates, and all private haplotypes except hap-13 registered frequencies closer to 1% (Table 1). However, no haplotype was observed having a frequency of <1% in Gondar goat population. This is contrary to small sample size used for the latter goat population. In line with this, three nonsynonymous mutations showed a frequency <1% in populus nigra cinnamyl alcohol dehydrogenase (CAD4) gene and stated that it would not have been identified by studies using smaller sample size (Marroni et al., 2011).

Linkage disequilibrium

The study of variations in both linkage disequilibrium (LD) and haplotype frequencies within and across populations is highly relevant in the choice of "tagging" SNPs for candidate gene or whole-genome association studies (Beaty et al., 2005). This is due to the fact that some markers will not be polymorphic in all samples and some haplotypes will be poorly represented or completely absent. In the LD measures, very low estimated *D'* and *R* were obtained the current study. This might be because of the shortest size of target region that leads to short physical distances among segregating sites (Supplementary Table S1). The average distances among segregating sites are 226.19 bp for Woyto-Guji and 234.86 bp for Gondar goat populations (Table 4). When markers are separated by <1 kb of DNA, *D'* values could be on average <1 (Abecasis et al., 2001) implying that an excess of LD does not appear in short physical distance (e.g.

<10kb) (Pritchard and Przeworski, 2001). However, it's agreed that, in analysis of whole genome or large size target region, measures of LD decay decreases as physical distance among loci increases. This is due to the fact that the recombination events will make the distribution of alleles at linked loci occur independently of each other (Lin, 2005). Another argument is, the low estimates of *D'* can be explained H_O were lower than H_E (i.e; $\Theta_\pi < \Theta_K$) (Table 3; Supplementary Figure S1) in most of heterozygosity estimates. This could be due to presence of more rare alleles at low frequencies and there might have been recent selective sweep and population expansion (Tajima, 1989a). In another study of Ethiopian goat population we found that there was high level of population migration per generation (*N*m = 24) (Getinet Mekuriaw Tarekegn, 2016) and recent and rapid *bi-modal* demographic expansion events (Getinet Mekuriaw Tarekegn et al., 2018).

Similarly, the average R^2 value was very small ($R^2 = 0.08334 \pm 0.15$) (Table 4) suggesting the little power of coefficient of correlation to detect association among the loci (Pritchard and Przeworski, 2001) and no difference (P>0.05) between both populations. However, in terms of magnitude, there was slightly higher estimate |*D'*| for Woyto-Guji goat population. This might be explained by the highest flock size farmers owned in Woyto-Guji area than Gondar (Netsanet Zergaw, 2014; Alubel Alemu, 2015) which could provide better selection practice in the latter goat population studied. Population growth leads to an excess of low-frequency variants (Tajima, 1989a); whereas, population structure tends to increase levels of LD (Pritchard and Przeworski, 2001). Similarly, in other reports, the source of variation of LD measures among populations could be selective sweeps, history of natural selection, gene conversion, mutation, genetic drift and other forces that cause gene-frequency to evolute (Abecasis et al., 2001; Slatkin, 2008). Geographical distance (>1500 km) among Gondar and Woyto-Guji goat populations might be one possible reason which contributed for the LD variation observed among themselves. Though the biological reasons have not been known yet, majority of residual variation for the distribution of LD is explained by physical distance among study populations (Abecasis et al., 2001; Pritchard and Przeworski, 2001).

Trends of linkage disequilibrium decays for all SNPs detected are illustrated at figure 1. Both |*D'*| and R^2 suffer ceiling and floor effects, respectively. Most of the pairwise comparisons of polymorphic sites are concentrated at maximum value for |*D'*| (|*D'*| = 1) and at minimum value for R^2 ($R^2 = 0$) (Marroni et al., 2011). This could be because of one of the four possible haplotypes is not observed in the sample for the former (Mueller, 2004; Marroni et al., 2011) and presence of excess rare alleles for the latter (Hedrick and Kumar, 2001). The abundance of pairwise comparisons of the ceiling and floor effects are irrespective of they each other (the combination graph of |*D'*| and R^2 is not indicated).

On the other hand, very low recombination rate (*c* = 0.0567) was observed in both goat populations indicating the non significant contribution of genetic drift on LD accumulation rather it could be happened by selection and migration or population expansion. The lowest neutral estimate of *ZnS* (*ZnS* = 0.0265), discussed below, strengthened this argument. When a favorable mutant at the locus under selection sweeps detected in the population, it drags along the neutral locus and therefore the pattern of polymorphism at the neutral locus can be strongly affected by the linkage to the selected locus (Fu, 1997). However, this recombination evaluation in this specific segment of the DNA does not represent the status of the recombination in the whole genome. It is because of that the rate of recombination varies across the genomic regions (Payseur and Nachman, 2000; Yu et al., 2001).

Most of the SNPs detected have no/lack strong linkage disequilibrium indicating they are not likely appropriate for genetic association studies. In regions of high LD, a reduced set of haplotype tag SNPs may be selected to detect efficient associations between variations in that gene or region and a trait of interest (Beaty et al., 2005; Gong et al., 2007). Another possible reason could be, variability in LD is also a function of sample size (Beaty et al., 2005). Sample size of Gondar goat population is by half smaller than that of sample size of Woyto-Guji goat population. In the shared loci, both goat populations have almost similar patterns of pairwise LD accumulation except at two loci combination in each population; this can ease to identify the minimum number of SNPs that tag the most common haplotypes, termed "tagging SNPs". The similar trends of haplotype frequencies of shared haplotypes of both goat populations (Table 2) strengthened this argument. However, according to Evans and Cardon (2005), whenever haplotype frequencies vary considerably across populations, it becomes more difficult to predict which SNPs will identify enough of the existing haplotypes in all subpopulations to ensure adequate coverage, and the chance of spurious findings due to confounding increases in tests of association. Of course, factors such as sample size become important when estimating haplotype frequencies too; but, the key determinant of differences remains underlying level of haplotype diversity and LD across populations (Beaty et al., 2005). The relative higher estimates of LD in the study conducted (Table 4), is because the more practice of selection than the effect of genetic drift. The later argument can be strengthened by the relative low estimate of recombination of linked loci obtained and is supported by Slatkin (2008). Genetic drift which can create small amounts of LD, interacts with selection (Hill and Robertson, 1966; Slatkin, 2008) and this reduces the response to selection. The low recombination rate has also an implication that the common ancestor in the sequences was created recently which was initially linked to the selectively favored mutation (Kelly, 1997).

In addition, it is explained that changes in population size, particularly an extreme reduction in size (a population bottleneck), can increase LD (Slatkin, 2008). With respect to this, the population/flock size of Gondar per household is lowest compared to Woyto-Guji. On the other hand Netsanet Zergaw (2014) reported that in Woyto-Guji area, the maximum goat holding in her study group was 200 per house hold; however, we had also observed up to 400 heads of goats per house hold during the field work.

From the total SNPs detected, the association analysis among loci indicated that only four loci showed highest and significant LD accumulation (Table 6 and 7). Strong LD is expected in tightly linked loci (Pritchard and Przeworski, 2001). Variability at linked markers will be higher on chromosomes bearing that allele than other chromosomes whenever an advantageous allele is fixed (Slatkin, 2008). In another association study of the KISS1 gene of the same goat populations and the same loci studied, we observed significant (P<0.001) contribution of mutations at g.950T>C, g.3416G>C, g.3811G>T and g.3963T>C on multiple birth ability of Gondar and Woyto-Guji goat populations (Getinet Mekuriaw et al., 2017). It is reported that strong positive selection quickly increases the frequency of an advantageous allele (Slatkin, 2008). This results linked loci to remain in strong LD with that allele, which is called genetic hitch-hiking (Maynard and Haigh, 1974). The second primarily route of selection, epistatic selection, might have its own contribution for relatively higher estimates of measures of LD in Woyto-Guji goat population than Gondar. The latter selection type leads to have the association of particular alleles at different loci that provide motivation of historical studies of LD in the study population. The insignificant recombination rate estimate strengthens this argument. It is explained that epistatic selection would have to be very strong to maintain allelic associations at the scale of megabases, in the face of substantial recombination (http://www.as.wvu.edu/~kgarbutt/QuantGen/Gen535-2-2004/Linkage-disequilibrium.htm).

In general, the significant population variation in that candidate gene was also observed, particularly in the shared haplotypes, in haplotype diversity and in differences in LD implying that some of the SNPs and haplotypes are ‘‘useful’’ for association studies (Beaty et al., 2005). Woyto-Guji goat populations had fairly similar haplotype diversity but slightly higher levels of measures of LD than did Gondar goat population. The rate of recombination is also relatively lower in Woyto-Guji goat population (c=0.0505) than in Gondar goat population (c=0.0765) and strengthened the idea that the recombination rate decreases as LD accumulation increases.

Neutrality test

In the current study, the estimates in polymorphism and divergence were higher than the polymorphism overview estimates in all neutrality tests. Negative and highly significant *F*s values were obtained in both goat populations studied. According to Fu (Fu, 1997), *F*s test is especially sensitive to population demographic expansion, which generally leads to have large negative *F*s values. However, Gondar goat showed higher significant negative value of *F*s than Woyto-Guji. This could be because of high demographic expansion towards Gondar area. As result, a recent genome wide study revealed that Gondar goat has more than four genetic backgrounds, which could let this goat population to have higher negative values whereas Woyto-Guji has only two genetic backgrounds (Getinet Mekuriaw Tarekegn, 2016). However, all the estimates, except the Tajima's *D* test, were negative values in the latter group (Table 9). Large negative value which indicates a one-sided test, for in instance in F_S**,** is an indicator against the neutrality of mutations implying an excess of number of rare alleles and a reduction of the number of common alleles (Fu, 1997). Fu *ibid* proved that in showing the effect of population growth on neutrality test, the *F*s test is the most powerful one; in fact, it is often more than twice as powerful as any other test examined. On the other hand, Watterson's test *W* is the least powerful test. In between are Tajima's test *T*, *F*u and Li's tests *D** and *F** and the new test *F'* (-1, 1).

Moreover, negative values of Tajima's *D* in particular, which is non-significant positive value in our study, shows presence of negative selection, population growth and genetic hitchhiking (Tajima, 1989b). In the current study, we obtained negative value of Fay and Wu' *H* (*H*= -2.11640) suggesting genetic hitchhiking (Fay and Wu, 2000). In another study, we observed positive (*D* = 0.10) and negative (*D* = -0.22) values of Tajima's *D* estimates for Gondar and Woyto-Guji goat populations (Getinet Mekuriaw Tarekegn et al., 2017). The coexistence of negative values for both Tajima's *D* and *H* could be related to demographic history of the population (Marroni et al., 2011) that could be explained by a bottleneck event (Heuertz et al., 2006).

On the other hand, the lowest *ZnS*, where *ZnS* has a range of 0 to 1 estimate, obtained in the current study implies acceptance of the neutral model and encourage to use it as a test (Kelly, 1997). According to Kelly (1997), the values *ZnS* measures declines as asymmetry among loci increases; when natural selection acts on a polymorphism that is closely linked to neutral sites, allele frequency asymmetries may be reduced. For this reason, higher expected values of *ZnS* may represent a molecular signature of natural selection. The considerable codon bias index (CBI = 0.301) obtained could also strengthen the effect of natural selection.

In General, the overall neutrality evaluation of the KISS1 gene shows influence of selection on the goat population studied. However, it is mentioned that neutrality tests are quite sensitive to variations in sample size (Marroni et al., 2011). The reason is small sample sizes lead to a relatively large variance of π and D (Lohse and Kelleher, 2009). However, how small sample size is small and how variable sample size differences among study populations need to be defined. For instance, in the current study the average estimate of π (π = 0.00275) and its variance were very low and neutrality test was detected in contrast to highest variation of sample size between the two goat populations included in the study.

From the total 29 haplotypes, only 12 of them are common for both goat populations studied. Majority of them are not shared haplotypes resulted from the rare alleles. Gondar and Woyto-Guji goat populations are geographically isolated as described in the methods section. It is noted that individuals from different geographic areas could cause allelic frequencies to be skewed toward rare alleles resulting in the detection of negative Tajima's *D* values due to population structure (Städler et al., 2009). However, the positive Tajima's *D* value detected in the KISS1 gene could be due to high estimation of level of population migration per generation (Nm =18.17) and this is strengthened by lowest pairwise F_{ST} distance (F_{ST} = 0.0267) between the two goat populations (Getinet Mekuriaw Tarekegn, 2016).

Conclusion

Kisspeptin can be is an essential gene for fecundity trait. It plays a role of secretion of kisspeptin protein in the hypothalamus region of the brain and facilitates hormonal regulation in the female reproduction system. In the current study, haplotype frequencies, together with patterns of pairwise LD, were used to assess genetic variation in Woyto-Guji and Gondar goat populations. These goat populations showed fairly similar haplotype frequencies and heterozygosity. However, relatively higher LD decays were observed in Woyto-Guji goat population than Gondar. In addition, the neutrality tests confirmed effect of natural selection on the former goat population. In general, some of the polymorphic loci detected in the target regions showed comparatively highly significant linkages among themselves suggesting the importance of the gene for multiple births, as confirmed in former study (Getinet Mekuriaw Tarekegn et al., 2017). Therefore, the kisspeptin gene can be suggested to be part of the designs of improvement program in goat breeding. However, it is wise to note that sequencing the whole length of KISS1 gene and testing both the haplotype and measures of LD decays with more sample size may help to suggest strong recommendation.

Acknowledgements

The project was part of the BecA-ILRI Hub livestock productivity program (entitled: Harnessing genetic diversity for improving livestock productivity: goats) funded by the Sida (Sweden Government offices decision UF2011/55504/UD/UP). This project was also supported by the BecA-ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program. The ABCF Program is funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA-CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill and Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID), and the Swedish International Development Cooperation Agency (Sida).

References

Abecasis, G.R., Noguchi, E., Heinzmann, A., Traherne, J.A., Bhattacharya, S., Leaves, N.I., Anderson, G.G., Zhang, Y., Lench, N.J., Carey, A., Cardon, L.R., Moffatt, M.F. and Cookson, W.O. 2001. Extent and distribution of linkage disequilibrium in three genomic regions. American Journal of Human Genetics 68:191–197.

Alubel Alemu. 2015. On-farm phenotypic characterizations and performance evaluation of Abergelle and Central highland goat breeds as an input for designing community-based breeding program. MSc theis. Haramaya University Haramaya, Ethiopia. 2015.

Balding, D.J. 2006. A tutorial on statistical methods for population association studies. Nature review, genetics 7: 781–791.

Beaty, T.H., Fallin, M.D., Hetmanski, J.B., McIntosh, I., Chong, S.S., Ingersoll, R., Sheng, X., Chakraborty, R. and Scott, A.F. 2005. Haplotype Diversity in 11 Candidate Genes Across Four Populations. Genetics 171: 259–267.

Browning, S.R. and Weir, B.S. 2010. Population Structure With Localized Haplotype Clusters. Genetics 185: 1337–1344.

Cao, G.L., Chu, M.X., Fang, L., Di, R., Feng, T. and Li, N. 2010. Analysis on DNA sequence of KiSS-1 gene and its association with litter size in goats. Molecular Biolology Report 37(8): 3921-3929.

Cao, G.L., Chu, M.X., Fang, L., Feng, T., Di, R. and Li, N. 2011 Analysis on DNA sequence of GPR54 gene and its association with litter size in goats. Molecular Biology Report 38:3839–3848.

Dorji, N., Duangjinda, M. and Phasuk, Y. 2012. Genetic characterization of Bhutanese native chickens based on an analysis of Red Junglefowl (Gallus gallus gallus and Gallus gallus spadecieus), domestic Southeast Asian and commercial chicken lines (Gallus gallus domesticus). Genetics and Molecular Biology 35(3): 603-609.

Evans, D.M. and Cardon, L.R. 2005. A comparison of linkage disequilibrium patterns and estimated population recombination rates across multiple populations. American Journal of Human Genetics 76: 681–687.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online. 2005, 1:47-50.

Fay, J.C. and Wu, C.I. 2000 Hitchhiking under positive Darwinian selection. Genetics 155:1405–1413.

Fields, P.D., McCauley, D.E., McAssey, E.V. and Taylor, D.R. 2014. Patterns of cyto-nuclear linkage disequilibrium in Silene latifolia: genomic heterogeneity and temporal stability. Heredity 112: 99–104.

Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997, 147:915–925.

Fu, Y.X. and Li, W.H. 1993. Statistical Tests of Neutrality of Mutations. Genetics 133:693-709

Getinet Mekuriaw Tarekegn. 2016. Molecular characterization of Ethiopian indigenous goat populations: Genetic diversity and structure, demographic dynamics and assessment of the kisspeptin gene polymorphism. PhD dissertation. Addis Ababa University. https://core.ac.uk/download/pdf/46137491.pdf

Getinet Mekuriaw, Mwacharo J.M., Tadelle Dessie, Mwai, O., Djikeng, A., Osama, S,, Grum Gebreyesus, Alayu Kidane, Solomon Abegaz and Kassahun Tesfaye 2017. Polymorphism analysis of Kiss peptin (KISS1) gene and its association with litter size in Ethiopian indigenous goat populations. African Journal of Biotechnology. 2017, 16:22. DOI: 10.5897/AJB2016.15750

Getinet Mekuriaw Tarekegn, Kassahun Tesfaye, Tadelle Dessie , Mwai, O., Djikeng, A., Osama, S., Netsanet Zergaw, Alubel Alemu, Achieng, G., Tutah, J., Mutai, C., Njuguna, J., Birungi, J. and Mwacharo, M.J. 2018. Mitochondrial DNA analysis of Ethiopian indigenous goat populations revealed maternal origins and recent demographic expansion with high within genetic diversity. Ecol Evol. 00:1–11. https://doi.org/10.1002/ece3.3710

Goddard, K.A.B., Hopkins, P.J., Hall, J.M. and Witte, J.S. 2000. Linkage Disequilibrium and Allele-Frequency Distributions for 114 Single-Nucleotide Polymorphisms in Five populations. American Journal of Humam Genetics66:216–234

Gong, Y., Beitelshees, A.L., Wessel, J., Langaee, T.Y., Schork, N.J. and Johnsona, J.A. 2007. Single nucleotide polymorphism discovery and haplotype analysis of Ca2+-dependent K+ channel beta-1 subunit. Pharmacogenet Genomics 17(4): 267–275.

Grossen, C., Keller, L., Biebach, I., The International Goat Genome Consortium and Croll, D. 2014. Introgression from Domestic Goat Generated Variation at the Major Histocompatibility Complex of Alpine Ibex. PLoS Genetenetics 10:6. e1004438. doi:10.1371/journal.pgen.1004438

Hedrick, P. and Kumar, S. 2001. Mutation and linkage disequilibrium in human mtDNA. European Journal of Human Genetics 9:969–972

Heuertz, M., De Paoli, E., Källman, T., Larsson, H., Jurman, I., Morgante, M., Lascoux, M. and Gyllenstrand, N. 2006. Multilocus patterns of nucleotide diversity, linkage disequilibrium and demographic history of Norway spruce. Genetics 174:2095–2105.

Hill, W.G. and Robertson, A. 1966. The effect of linkage on limits to artificial selection. Genet. Res8: 269–294.

Kelly, J.K. 1997. A Test of Neutrality Based on Interlocus Associations. Genetics 146:1197-1206.

Khatkar, M.S., Nicholas, F.W., Collins, A.R., Zenger, K.R., Cavanagh, J.A.L., Barris, W., Schnabel, R.D., Taylor, J.F. and Raadsma, H.W. 2008. Extent of genome-wide linkage disequilibrium in Australian Holstein-Friesian cattle based on a high-density SNP panel. BMC Genomics 9: 187.

Li, W.H. and Sadler, L,A, 1991, Low nucleotide diversity in man. Genetics 129:513–23.

Lohse, K. and Kelleher, J. 2009. Measuring the degree of star shape in genealogies—summary statistics and demographic inference. Genetics Research 91:281–292.

Majo, F.C. 2008. Selection and linkage disequilibrium tests under complex demographies and ascertainment bias. Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra (UPF). PhD dissertation. Barcelona, Juliol 2008.

Marroni, F., Pinosio, S., Zaina, G., Fogolari, F., Felice, N., Cattonaro, F. and Morgante, M. 2011. Nucleotide diversity and linkage disequilibrium in Populus nigra cinnamyl alcohol dehydrogenase (CAD4) gene. Tree Genetics and Genomes 7:1011–1023.

Maynard, S.J. and Haigh, J. 1974. The hitch-hiking effect of a favourable gene. Genetics Research 23: 23–35.

McRae, A.F., McEwan, J.C., Dodds, K.G., Wilson, T., Crawford, A.M. and Slate, J. 2002. Linkage disequilibrium in domestic sheep. Genetics 160(3): 1113-1122.

Mueller, J.C. 2004. Linkage disequilibrium for different scales and applications. Brief Bioinformatics, 5:355–364.

Nachman, M.W. 2002. Variation in recombination rate across the genome: evidence and implications. Current Opinion in Genetics & Development 12:657-663.

Netsanet Zergaw. 2014. On-farm phenotypic characterizations and performance evaluation of Central highland goat and Woyto-Guji goat types for designing community-based breeding strategies. MSc theis. Haramaya University Haramaya, Ethiopia.

Payseur, B.A. and Nachman, M.W. 2000. Microsatellite variation and recombination rate in the human genome. Genetics 156:1285–1298.

Pei, Y.F., Zhang, L., Liu, J. and Deng, H.W. 2009. Multivariate Association Test Using Haplotype Trend Regression. Ann Hum Genet 73(4): 456–464.

Pritchard, J.K. and Przeworski, M., 2001. Linkage disequilibrium in humans: models and data. American Journal of Human Genetics 69:1–14.

Rozas, J., Gullaud, M., Blandin, G. and Aguade, M. 2001. DNA variation at the rp49 gene region of Drosophila simulans: Evolutionary inferences from an unusual haplotype structure. Genetics 158: 1147-1155.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X. and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496-2497.

Service, S.K., Temple, L.D.W., Freimer, N.B. and Sandkuijl, L.A. 1999. Linkage-disequilibrium mapping of disease genes by reconstruction of ancestral haplotypes in founder populations. American Journal of Human Genetics 64:1728–1738.

Shifman, S., Bronstein, M., Sternfeld, M., Pisante-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B. and Darvasi, A. 2002. A highly significant association between a COMT haplotype and schizophrenia. American Journal of Human Genetics 71:1296–1302.

Slatkin, M. 2008. Linkage disequilibrium: understanding the evolutionary past and mapping the medical future. Nature Reviews Genetics 9: 477-485. doi:10.1038/nrg2361

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. Second Edition. W. H. Freeman and Company. New York. 1981.

Städler, T., Haubold, B., Merino, C., Stephan, W. and Pfaffelhuber, P. 2009. The impact of sampling schemes on the site frequency spectrum in non-equilibrium subdivided populations. Genetics 182:205-216.

Tajima, F. 1989a. The effect of change in population size on DNA polymorphism. Genetics. 123:597–601.

Tajima, F. 1989b. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:3, 585–95.

Voight, B.F., Kudaravalli, S., Wen, X. and Pritchard, J.K. 2006. A map of recent positive selection in the human genome. PLoS Biology 4:72.

Weir, B.S., Cardon, L.R., Anderson, A.D., Nielsen, D.M. and Hill, W.G. 2005. Measures of human population structure show heterogeneity among genomic regions. Genome Research 15:1468–1476.

Yu, A., Zhao, C., Fan, Y., Jang, W., Mungall, A.J., Deloukas, P., Olsen, A., Doggett, N.A., Ghebranious, N., Broman, K.W. and Weber, J.L. 2001. Comparisons of human genetic and sequence-based physical maps. Nature 409:951–953.

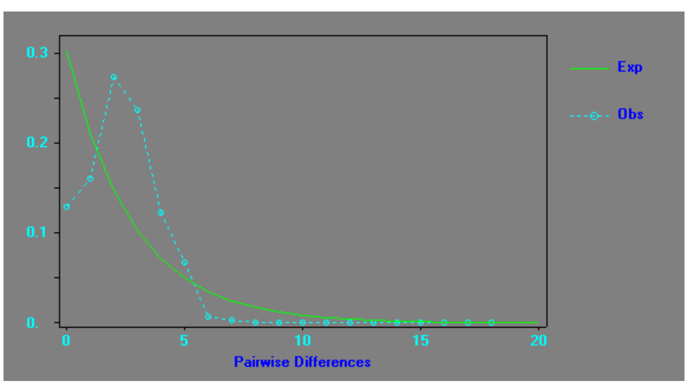
Zhu, M., Zhu, B., Wang, Y.H., Wu, Y., Xu, L., Guo, L.P., Yua., Z.R., Zhang, L.P., Gao, X., Gao, H.J., Xu, S.Z. and Li, J.Y. 2013. Linkage Disequilibrium Estimation of Chinese Beef Simmental Cattle Using High-density SNP Panels. Asian-Australasian Journal of Animal Sciences 26(6): 772-779.

Supplementary Table S1. Summary of linkage disequilibrium measures of exon1 for both goat populations

Locus1	Locus2	Dist	D	D'	R	Fisher	Chi-sq	Locus1	Locus2	Dist	D	D'	R	Fisher	Chi-sq
74	136	62	0.000	-1.000	-1.000	1.000	0.049	369	528	159	-0.118	-0.694	-0.492	0.000***B	41.907***B
74	253	179	0.000	-1.000	-0.006	1.000	0.006	369	531	162	0.024	0.316	0.137	0.085	3.230
74	369	295	0.003	1.000	0.080	0.474	1.116	369	647	278	0.000	0.049	0.006	1.000	0.005
74	416	342	0.000	-0.000	-0.006	1.000	0.006	369	683	314	-0.011	-0.096	-0.052	0.595	0.459
74	490	416	0.000	-1.000	-0.006	1.000	0.006	369	709	340	0.174	0.715	0.698	0.000***B	84.390***B
74	503	429	0.000	-1.000	-0.008	1.000	0.012	416	490	74	0.000	-1.000	-0.006	1.000	0.006
74	528	454	0.004	1.000	0.102	0.358	1.801	416	503	87	0.000	-1.000	-0.008	1.000	0.012
74	531	457	-0.001	-1.000	-0.031	1.000	0.170	416	528	112	-0.002	-1.000	-0.057	1.000	0.562
74	647	573	0.000	-1.000	-0.008	1.000	0.012	416	531	115	-0.001	-1.000	-0.031	1.000	0.170
74	683	609	-0.001	-1.000	-0.043	1.000	0.322	416	647	231	0.000	-1.000	-0.008	1.000	0.012
74	709	635	0.003	1.000	0.082	0.462	1.169	416	683	267	-0.001	-1.000	-0.043	1.000	0.322
136	253	117	0.000	-1.000	-0.017	1.000	0.049	416	709	293	-0.003	-1.000	-0.071	1.000	0.865
136	369	233	0.013	0.525	0.122	0.152	2.563	490	503	13	0.000	-1.000	-0.008	1.000	0.012
136	416	280	0.000	-1.000	-0.017	1.000	0.049	490	528	38	-0.002	-1.000	-0.057	1.000	0.562
136	490	354	0.000	-1.000	-1.017	1.000	0.049	490	531	41	-0.001	-1.000	-0.031	1.000	0.170
136	503	367	-0.001	-1.000	-0.024	1.000	0.098	490	647	157	0.000	-1.000	-0.008	1.000	0.012
136	528	392	-0.017	-1.000	-0.165	0.052	4.685*	490	683	193	-0.001	-1.000	-0.043	1.000	0.322
136	531	395	-0.007	-1.000	-0.090	0.370	1.417	490	709	219	0.003	1.000	0.082	0.462	1.169
136	647	511	-0.001	-1.000	-0.024	1.000	0.098	503	528	25	0.002	0.221	0.032	1.000	0.176
136	683	547	-0.011	-1.000	-0.125	0.201	2.689	503	531	28	-0.002	-1.000	-0.044	1.000	0.342
136	709	573	0.013	0.535	0.127	0.146	2.790	503	647	144	0.000	-1.000	-0.012	1.000	0.024
253	369	116	-0.003	-1.000	-0.072	1.000	0.906	503	683	180	-0.003	-1.000	-0.061	1.000	0.649
253	416	163	0.000	-1.000	-0.006	1.000	0.006	503	709	206	-0.005	-1.000	-0.100	0.500	1.741
253	490	237	0.000	-1.000	-0.006	1.000	0.006	528	531	3	0.018	0.190	0.104	0.183	1.880
253	503	250	0.000	-1.000	-0.008	1.000	0.012	528	647	119	0.002	0.221	0.032	1.000	0.176
253	528	272	0.004	1.000	0.102	0.358	1.801	528	683	155	0.034	0.221	0.167	0.041*	4.838*
253	531	278	-0.001	-1.000	-0.031	1.000	0.170	528	709	181	-0.108	-0.651	-0.451	0.000***B	35.250***B
253	647	394	0.000	-1.000	-0.008	1.000	0.012	531	647	116	-0.002	-1.000	-0.044	1.000	0.342
253	683	430	-0.001	-1.000	-0.043	1.000	0.322	531	683	152	0.104	0.947	0.688	0.000***B	81.773***B
253	709	456	-0.003	-1.000	-0.071	1.000	0.865	531	709	178	0.026	0.330	0.146	0.081	3.707
369	416	47	-0.003	-1.000	-0.072	1.000	0906	647	683	36	-0.003	-1.000	-0.061	1.000	0.649
369	490	121	0.003	1.000	0.080	0.474	1.116	647	709	62	-0.005	-1.000	-0.100	0.500	1.741
369	503	134	0.006	1.000	0.114	0.223	2.245	683	709	26	0.044	0.336	0.205	0.008**	7.264

Key” Dist=Distance (bp)

Supplementary Figure S1. Trend of observed (Obs) and expected (Exp) haplotype heterozygosities



Effect of Different Proportions of Fig (*Ficus sur*) Fruits and Oats (*Avena sativa*) Grain Supplementation on Feed Intake, Digestibility, Nitrogen Retention and Live Weight Change of Hararghe Highland Sheep Fed Natural Pasture Hay Based Diets

Diriba Diba[a*], Yoseph Mekasha[b], Mengistu Urge[c] and Adugna Tolera[d]

[a]Wollega University, Department of Animal Science; P.O.Box 395, Nekemte, Ethiopi;
[b]International Livestock Research Institute, P.O.Box 5689, Addis Ababa, Ethiopia
[c]Haramaya University, School of Animal and Range Sciences; P.O.Box 138, Dire Dawa, Ethiopia
[d]Hawassa University, School of Animal and Range Sciences; P.O.Box 05; Hawassa, Ethiopia
[*]corresponding author: dnazerawi2010@gmail.com

Abstract

Energy and protein supplements are very scarce feed resources in Ethiopia. The present study was carried out to investigate the effect of inclusion of graded levels of dried and ground Ficus sur fruits (FSF) in the ration on feed intake, digestibility, growth performance and nitrogen (N) balance of Hararghe highland lambs. The experiment was laid out in a randomized complete block design (RCBD). A total of 30 yearling intact male lambs with similar body condition were grouped into six blocks based on their initial body weight and animals from each block were assigned to five treatment diets giving a replication of six animals per treatment. The dietary treatments used in the experiments were ad libitum natural pasture hay (control); 100% FSF and 0% oat grain (OG), which was represented as [100FSF]; 67%FSF:33%OG [67FSF]; 33%FSF:67%OG [33FSF]; 0%FSF:100% OG [0FSF]. Noug seed cake (NSC) was given to all animals to provide at least the maintenance requirement for protein at isonitrogenous level. The lambs fed the control diet achieved the least (p<0.0001) nutrient intake from the basal, concentrate and the total diet compared to those fed diets supplemented with FSF and OG. Lambs fed 100FSF consumed the highest (p<0.0001) amount of nutrients than the other treatment groups. The group fed 100FSF also achieved the highest apparent nutrient digestibility, average daily gain and N retention than the other groups. This implies that FSF is a better energy supplement than OG in the diets of growing lambs.

Keywords: *Digestibility, Feed intake, Fig fruits, Oat grain, Live weight change, Nitrogen balance, Sheep*

Introduction

Sheep are one of the very valuable livestock species reared in different agro-ecologies and production systems all over the world (Assen and Aklilu, 2012). They are particularly very important for the livelihood of the poor and economically disadvantaged members of the community. They are suitable for smallholder farmers as they serve as a means of survival, source of cash income and asset for the poor and the landless, and as a source of food and nutrition security.

Despite their relevance, sheep production in most sub-Saharan African countries is constrained mainly by feed shortage (Tessema et al., 2013). The natural pasture and crop residues, which serve as the main source of feed for livestock in Ethiopia, are characterized by low nutrient content, low digestibility and low voluntary intake resulting in poor animal performance (Adugna et al., 2012). Thus, in order to improve the nutritive value of the predominant feed resources to enhance productivity of livestock, including sheep, supplementation with energy and protein source diets is imperative (Dutta et al., 2009). Under smallholder farm conditions, different green herbages such as leguminous trees and herbaceous legumes grown on farm can be used as protein supplements (Getnet et al., 2008) whereas energy source concentrate supplements are very scarce in these production systems. Most of the energy rich supplements are costly cereal grains that are used as staple food by most farmers in the tropics. Other sources are agro-industrial by products that rural households cannot easily access due to inadequacy, localized availability and very high cost associated with their purchase and transport.

Therefore, searching for alternative and locally available energy supplements with minimal competition with human beings would be important to support the sector. *Ficus sur* fruit (FSF) could be hypothesized to be one of such locally available alternative energy supplements in rural areas. The genus *Ficus* comprises about 750 species, with about 100 species in Africa, 500 species in tropical Asia and Australia, and 150 species in tropical America (Lumbile and Mogotsi, 2008). One of the species found in Ethiopia is *Ficus sur* (Cv. Forssk.), which is commonly known as fig. It is widely distributed in different parts of the country and has been used as feed for many years by livestock (personal observation). During dry seasons of severe feed shortage, the ripen fruits drop from the trees by wind and all classes of livestock freely access with no restriction. During this time, these fruits become important natural concentrate supplements for livestock and many lambs and kids grow fast and reach market weight in short period of time (personal observation).

In spite of its nutritional merits and availability, this feed resource has not yet been studied. Thus, the objective of this study was to compare the effect of inclusion of graded levels of dried and ground FSF with levels of ground OG in the ration on feed intake, digestibility, nitrogen balance and live weight change of lambs.

Materials and Methods

Experimental site

The study was conducted at Haramaya University sheep farm, located at 9^o 26'N latitude and 42^{o}3'E longitude in eastern Ethiopia. The altitude of the area is about 1980 meters above sea level and the mean annual rainfall is about 910 mm with a range of 560-1260 mm. The mean maximum and minimum temperatures are 23.4^{o}C and 8.25^{o}C, respectively (summary report from Haramaya University Meteorological Station, 2012).

Animals, treatments and experimental design

A total of 35 yearling intact male Hararghe highland sheep with similar body condition were purchased from Kulubi open market. The animals were transported to Haramaya University and quarantined for 3-weeks during which they were sprayed with accaricides against external parasites, treated with *ivermectin* injection against internal parasites and *Penistrep* against Pneumonia disease. During this adaptation period, animals were fed natural pasture hay and noug seed cake (NSC) supplement which is the same size with the actual level of NSC in the experiment.

The different dietary treatments used in the experiments were natural pasture hay offered *ad libitum* at 20% refusal rate plus noug seed cake (NSC) supplement (control). The other four treatments received *ad libitum* natural pasture hay supplemented with different proportions of *Ficus sur* fruits (FSF) and oats gain (OG) in addition to NSC, and the treatments include 100% FSF and 0%OG [100FSF]; 67%FSF & 33%OG [67FSF]; 33%FSF & 67%OG [33FSF]; and 0%FSF & 100% OG [0FSF]. The amount of noug seed cake (NSC), as protein supplement, varied based on the nitrogen content of energy diets, FSF and OG, to keep all the treatments at isonitronegous level. This was to fulfill at least the maintenance requirement for protein of the control treatment (McDonalds et al, 2010).

At the end of the quarantine period, 30 animals that were healthy and in good condition were selected and ear-tagged. The experiment was laid out in a randomized complete block design (RCBD). The animals were grouped into 6 blocks based on their initial body weight and animals from each block were assigned to five treatment diets randomly giving a replication of six animals per treatment. The animals were then assigned to individual pens furnished with feeder and water trough in the experimental house. Both hay and concentrate diets were offered using separate containers in two equal meals at 08:00 and 16:00 hours.

Experimental feeds, feeding management and body weight measurement

Naturally ripen and dry FSF were collected as they drop on the ground from fig trees in Horro district, western Ethiopia, packed in clean sacks and taken to Haramaya University sheep farm. The fruits were further sun dried to ensure ease of grinding in conventional grain mill. The OG was purchased from Sheno town of North Shewa zone of Oromia National Regional State, ground in a similar mill, using the same speck size as FSF. These two feeds were used as energy supplements after being mixed in different proportions as shown in Table 1.

Prior to commencement of data collection, the animals were adapted to the experimental diets for two weeks. Clean tap water was provided in a bucket and changed whenever contaminated with feces or feed material. The hay basal diet and concentrate supplement were offered in separate feed troughs. The amount of feed offered and refused was measured and recorded every day. The basal diet offer was adjusted at interval of 3 days for *ad libitum* intake at 20% refusal rate. The live weight of the animals was recorded every fortnight before morning feeding using a balance that has sensitivity of 10 g and maximum weighing capacity of 100 kg. The total weight gain was calculated as the difference between the final and initial weights of the animals. The daily average weight gain of the animals was calculated by dividing the total weight gain (g) by the number of feeding days.

Natural pasture hay was offered *ad libitum* whereas noug seed cake was given to make the diets of all animals isonitrogenous. Control= ad libitum natural pasture hay supplemented with noug cake; 100FSF = 100% FSF with 0% oats grain; 67FSF= 67% FSF with 33% oats grain; 33FSF= 33% FSF with 67% oats grain; 0FSF= 0% FSF with 100% oats grain

Table 1. Ingredient proportion and chemical composition of experimental diets (DM basis)*

	Dietary proportions (DM basis)				
Ingredients (g)	Control	100FSF	67FSF	33FSF	0FSF
Ficus sur fruits	0	300	201	99	0
Oats grain	0	0	99	201	300
Noug seed cake	225	210	190	170	150
Nutrient composition of diets (%)					
Dry matter	91.8	91.4	91.4	91.4	91.4
Ash	9.3	8.1	7.3	6.9	5.6
Crude protein	15.4	15.4	15.4	15.4	15.4
Neutral detergent fiber	58.3	33.7	35.2	36.8	38.6
Acid detergent fiber	41.1	22.6	23.1	23.7	24.3
Hemicelluloses	17.1	11.1	12.0	13.1	14.3
Cellulose	33.6	17.2	17.7	18.3	18.8
Acid detergent lignin	7.6	5.4	5.4	5.5	5.5
ME calculated (MJ/kg DM)	8.6	10.4	10.2	9.9	9.6

FSF= *Ficus sur* fruits; ME= metabolizable energy; *

Digestibility and nitrogen balance experiment

The digestibility and nitrogen balance experiments were conducted following the completion of the growth experiment. To determine the nutrient digestibility, total fecal collection method was employed using the same lambs used in the growth experiment. The fecal samples were collected using feces collection bags made of canvas (inner plastic sheet) fitted to each animal. Animals were adapted to carrying of fecal collection bags for three days followed by seven consecutive days of fecal collection. Every morning, the feces voided by each animal was emptied into plastic bucket, weighed and recorded for each animal, thoroughly mixed and about 20% was sampled each day to make seven days of composite samples. The feces samples were kept frozen at −20oC in deep freezer pending chemical analysis. The frozen feces were thawed, thoroughly mixed, sub-sampled and half of the samples were oven dried at 105^{o}C for 24 hours for DM determination. The rest of the samples were dried at 65^{o}C to constant weight in a forced draft oven and ground to pass through1mm sieve size for chemical analysis. Feed intake during the digestibility period was recorded following similar procedure as described for growth experiment. A sample of feed offer was collected every day, bulked, and sub-sampled at the end of the trial for later chemical analysis. Likewise, refusals by each animal was collected, weighed and pooled per treatment, thoroughly mixed and sub-sampled for chemical analysis per treatment. Apparent dry matter digestibility was calculated as:

$$\text{Apparent Digestibility (\%)} = \frac{\text{Nutrients intake} - \text{Nutrients voided in feces}}{\text{Nutrient intake}} * 100$$

The amount of urine excreted by individual animal, kept in metabolic cage, was collected into plastic bottles. About 10ml of (10%) H_2SO_4 was added to each urine collection bottle daily to trap the N that may escape as NH_3 from the urine. After recording the volume of urine excreted by each animal using a graduated cylinder, about 20% of the urine excreted daily per animal was sampled and stored in deep freeze at -20^{o}C, and pooled for the collection period for N analysis. Nitrogen retention was calculated as the difference between N consumed and N excreted in the feces and urine.

Chemical analysis of feeds, feces and urine

The chemical analysis of the experimental feeds, feces and urine were performed at Haramaya University Animal Nutrition laboratory. The chemical analysis for each sample was run in duplicates. The DM and ash contents of the feed and feces samples were determined following the procedure of AOAC (1995). The NDF, ADF, and ADL were determined according to Van Soest and Robertson (1985). Hemicelluloses and cellulose were calculated as NDF-ADF and ADF - (ADL+ADF ash), respectively.

The ME (MJ/kg) of the diets was estimated according to the procedure described by Moran (2005) as ME = 0.16DDM%-0.8. The N content of the samples was determined by the micro-Kjeldahl method and CP was calculated as N X 6.25.

Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure of the statistical analysis system (SAS, 2008). When the ANOVA declared difference among the dietary treatments, Tukey test was used to separate means. The model used in the analysis was: $Y_{ijk}=\mu +\tau_i +\beta_j + \varepsilon_{ijk}$ where, μ=overall mean of the population; τ_i= the i^{th} (1-5^{th}) dietary treatment effect; β_j= the j^{th} (1-6^{th}) block effect and ε_{ijk}=random error associated with yij.

Results

Nutrient composition of experimental diets

There were no differences in the DM and CP contents of the experimental diets (Table 1).The Ash content was numerically higher in the control diet and tended to decrease with decreasing level of FSF in the diet. The fiber (NDF, ADF, cellulose, hemicelluloses and lignin) contents were higher in the control than in the supplemented diets and tended to increase with decreasing level of FSF in the diet. On the other hand, the calculated ME content was lower in the control than in the supplemented diets and tended to decrease with decreasing level of FSF in the diet.

Voluntary feed intake

Basal diet DM intake was higher (P<0.05) in groups supplemented with different proportions of FSF and OG than the control group (Table 2). Basal diet DM intake was higher (P<0.05) in groups supplemented with different proportions of FSF and OG than the control group (Table 2). On the other hand, the concentrate DM intake was higher (P<0.05) in sheep fed 100FSF than in those fed 33FSF, 0FSF and the control diets. The total DM and OM intakes were significantly higher (P<0.05) in animals fed 100FSF than in those fed 33FSF, 0FSF and control diets. The NDF intake was also higher (P<0.05) in animals fed the 100FSF diet than the other treatments. Animals fed the control diet had the lowest intake of DM, OM and NDF. In general, DM, OM and NDF intake showed an increasing trend, at least in magnitude, with increasing level of FSF in the diet. However, no significant differences were detected in the CP intake among the different dietary treatments.

Table 2. Voluntary nutrient intake of sheep fed natural pasture hay supplemented with graded levels of FSF

Intake (g/day)	Treatments Control	100FSF	67FSF	33FSF	0FSF	SEM	P-level
Dry matter							
Basal hay	316.3[b]	356.5[a]	349.6[a]	345.3[a]	331.0[a]	2.98	0.001
Noug seed cake	168.8[d]	250.1[a]	237.1[ab]	223.7[bc]	211.4[c]	3.37	0.001
Total	480.0[d]	601.6[a]	580.7[ab]	564.0[b]	537.4[c]	5.08	0.001
Organic matter	475.0[d]	596.6[a]	575.7[ab]	559.0[b]	532.4[c]	5.08	0.001
Neutral detergent fiber	304.6[d]	374.7[a]	364.4[b]	357.8[b]	349.7[c]	1.74	0.001
Crude protein	71.3	71.3	70.7	71.0	70.6	0.24	0.062

[abc]Means with different superscript in the same row are significantly different. FSF=*Ficus sur* fruits; Control = fed natural pasture hay *ad libitum*; 100FSF = supplemented with 100% FSF and 0% oats grain (OG); 67FSF = supplemented with 67% FSF and 33% OG; 33FSF = supplemented with 33% FSF and 67% OG; 0FSF = supplemented with 0% FSF and 100% OG

Apparent digestibility

Apparent nutrient digestibility of the experimental diets is shown in Table 3.

Table 3. Nutrient digestibility of experimental diets in sheep fed natural pasture hay supplemented with different proportions of FSF

Digestibility (%)	Treatments Control	100FSF	67FSF	33FSF	0FSF	SEM	P-level
Dry matter	59.0[c]	69.9[a]	68.7[a]	67.4[ab]	65.5[b]	0.64	0.001
Organic matter	62.0[c]	73.0[a]	71.8[a]	70.4[ab]	68.5[b]	0.64	0.001
Neutral detergent fiber	57.2[d]	64.7[a]	62.7[b]	60.5[c]	59.8[c]	0.37	0.001
Crude protein	63.0	63.1	63.0	62.9	62.9	0.24	0.062

[abcd]Means with different superscript in the same row are significantly different. FSF=*Ficus sur* fruits; SEM= standard error of the mean; Control treatment = fed natural pasture hay *ad libitum*; 100FSF = supplemented with 100% FSF and 0% oats grain (OG); 67FSF = supplemented with 67% FSF and 33% OG; 33FSF = supplemented

The 100FSF and 67FSF diets had significantly higher (P<0.05) DM digestibility (DMD) and OM digestibility (OMD) values than the 0FSF diet, which in turn had higher (P<0.05) DMD and OMD values compared to the control diet. The NDF digestibility was highest (P<0.05) in animals fed 100FSF diet followed by 67FSF and lowest in the control diet. Nonetheless, no significant differences (P>0.05) were detected among the treatment groups in CP digestibility.

Nitrogen balance

Table 4 shows the nitrogen balance of the experimental sheep determined during the digestibility trial. Fecal N loss was higher (P<0.05) in control than in the supplemented animals. On the other hand, the urinary N loss was highest (P<0.05) in animals fed 0FSF diet followed by the 67FSF and 33FSF diets whereas the animals fed the control diet had the lowest (P<0.05) urinary N loss followed by those fed the 100FSF diet. N retention was higher (P<0.05) in animals fed 100FSF and 67FSF diets than those fed 0FSF and control diets. In general, there was an increasing trend of N retention with increasing level of FSF inclusion in the diet.

Table 4. Nitrogen balance of sheep fed natural pasture hay supplemented with different proportions of FSF and OG

	Treatments						
Variables	Control	100FSF	67FSF	33FSF	0FSF	SEM	P-level
N intake (g/day)							
Basal hay	3.36	3.51	3.44	3.41	3.37	0.07	0.076
Noug seed cake	8.05	8.05	8.03	8.03	7.99	0.03	0.061
Total	11.4	11.6	11.5	11.4	11.4	0.07	0.074
N excreted (g/day)							
Fecal	6.11[a]	5.26[b]	5.22[b]	5.25[b]	5.30[b]	0.02	0.001
Urinary	1.10[d]	1.39[c]	1.56[b]	1.57[b]	1.71[a]	0.02	0.001
Total	7.21[a]	6.65[c]	6.78[b]	6.82[b]	7.00[a]	0.03	0.001
N retention (g/day)	4.18[c]	4.89[a]	4.69[a]	4.60[ab]	4.34[bc]	0.07	0.001

[abcd]Means in the same column with different superscript are significantly different; N= nitrogen; FSF=Ficus sur fruits; SEM= standard error of the mean; Control treatment = fed natural pasture hay ad libitum; 100FSF = supplemented with 100% FSF and 0% oats grain (OG); 67FSF = supplemented with 67% FSF and 33% OG; 33FSF = supplemented with 33% FSF and 67% OG; 0FSF = supplemented with 0% FSF and 100% OG as energy sources. Noug seed cake (NSC) was supplemented to all animals at isonitrogenous level.

Live weight change

The body weight change of lambs fed the different treatment diets is presented in Table 5. The final body weight (FBW) was higher (P<0.05) in animals fed 100FSF diets than those fed 67FSF diet, which in turn had higher (P<0.05) final body weight than the animals fed 0FSF and control diets. The total body weight gain (TWG) and average daily gain (ADG) were highest in animals fed 100FSF diets followed by 67FSF diets and were lowest in animals fed the control diet.

Table 5. Growth performance of sheep fed natural pasture hay supplemented with different proportions of FSF

	Treatments						
Growth parameters	Control	100FSF	67FSF	33FSF	0FSF	SEM	P-level
Initial body weight (kg)	14.5	14.8	14.1	14.3	14.0	0.25	0.617
Final body weight (kg)	18.4^{c}	21.4^{a}	19.8^{b}	19.3^{bc}	18.5^{c}	0.29	0.001
Total weight gain (kg)	3.90^{d}	6.62^{a}	5.67^{b}	5.03^{bc}	4.50^{cd}	0.16	0.001
Average daily gain (g)	43.3^{d}	73.5^{a}	63.0^{b}	55.8^{bc}	49.9^{dc}	1.73	0.001

^{abcd}Means with different superscript in the same row are significantly different. FSF=*Ficus sur* fruits; SEM= standard error of the mean. Control treatment = fed natural pasture hay *ad libitum*; 100FSF = supplemented with 100% FSF and 0% oats grain (OG); 67FSF = supplemented with 67% FSF and 33% OG; 33FSF = supplemented with 33% FSF and 67% OG; 0FSF = supplemented with 0% FSF and 100% OG as energy sources

Discussion

Chemical composition and energy value of feeds

The similarity of the experimental diets in CP content is the reflection of the formulation of the diets on isonitrogenous basis. The tendency of having relatively higher ash content in the diets with higher FSF content may indicate contamination of the FSF with soil since the fruits were collected as they drop from the fig tree to the ground. The tendency of increasing fiber content and decreasing ME content with decreasing level of FSF in the diets, though not significant, indicates that the FSF was lower in fiber and higher in ME content than the oats grain. Thus, based on the chemical composition, FSF appears to be a better alternative source of energy supplement than oats grain.

Voluntary feed intake

Voluntary feed intake is among many other factors which affect animal performance and it depends, in one way or another, on the nature and type of the diet. The small feed intake in the control diet was due to lack of energy concentrate supplement that resulted in low rumen microbial activity and then low digestion rate in the rumen. At low digestion rate, passage rate is also low and this must have resulted in low voluntary feed intake (Nurfeta et al., 2008). This implies that rumen microbial growth needs both energy and protein supplements in a proportion that supports optimum ruminal ecology. Pittroffet al.(2006) noted the necessity to develop feeding regimes that optimize a balance of protein and energy supply in support of better animal performance. In similar way Dutta et al.(2009) suggested that balanced feeding in terms of energy and protein optimizes animal performance. The importance of meeting energy requirements as a primary goal in the control of voluntary feed intake was also highlighted by Adugna and Sundstøl (2000a).

The highest feed intake observed in lambs fed 100FSF could be an attribute of lower NDF content of FSF compared to OG. Higher fiber content of feeds lowers digesta passage rate and reduces feed intake as occurred in sheep fed with 0FSF diets in the present study. In accordance with the present study, forage diet with long particle cuts and having higher NDF content reduced passage rate and DM intake of animals (Yang and Beauchemin, 2006). Wang et al (2011) noted that higher NDF in the diet of dairy cows increased chewing activity resulting in lowered passage rate, thereby feed intake. Likewise, Almaz et al (2012) found lower dry matter intake in Ethiopian highland sheep when fed diets that contained the highest NDF. Consistent with the results of the current study, Lloyd et al.(1992) and Rowe and Coss (1994) revealed that supplementation of oats grain to roughage diets resulted in reduced feed intake. Moreover, Tadesse (2011) noted high fiber concentration in OG and suggested the importance of treatment to improve its nutritive value. Accordingly, malting OG was found to slightly increase CP and decrease fiber fractions, which resulted in improved digestibility and animal performance when supplemented with NSC (Hailu, 2012). This confirms that OG has less soluble components compared to FSF. The best energy supplement is that which support optimum intake of the basal diet to which it was supplemented. In this regard, higher levels of FSF supplementation resulted in higher voluntary feed intake compared to OG. The similarity in mean CP intake between the dietary treatments was due to supplementation of the diets at isonitrogenous levels.

Apparent nutrient digestibility

The least apparent DMD, OMD and NDF digestibility in lambs fed the control diet may be due to lack of adequate energy supplement. This was in agreement with ME values for control diets indicated under Table 1. Energy supplements in the form of soluble carbohydrates enhance rumen microbial growth which gradually increase in population and colonize the fibrous mats in the rumen. According to Lascano et al (2009) the amount of live rumen bacteria count increased with increased level of energy concentrate in the diet of dairy heifers while the dead count of the same bacteria decreased. Even in the presence of protein supplement, microbial protein production and N efficiency would most likely be affected by the relative degradability of carbohydrate source diets or energy concentrates (Eriksson et al., 2009). This may be a reason for the relatively low values of nutrient digestibility in the present study, except for CP digestibility which did not differ among the treatments.

The higher apparent digestibility of DM and OM for 100FSF diet compared to 0FSF (100 OG) diet was mainly due to less ADF and ADL contents of FSF compared to OG. The DM digestibility of diets containing high levels of FSF in the present study is comparable to the digestibility for pods of tropical legume (*A. pennatula;* 66.8%) in sheep (Chay-Canulb et al., 2012). The OM digestibility was also comparable to a diet of sheep fed maize stover supplemented with 450 g of *Desmodium intortum* hay per head per day (Adugna and Sundstøl, 2000a). But the digestibility in the former was higher than the apparent digestibility of perennial Pangola grass (*Digitaria decumbens)* pasture (62.8-65.7% %) harvested at 28, 42 and 56 days of re-growth and fed to sheep (Archimede et al 2000). It is also higher than cowpea haulms (54 - 60%) supplemented *P. purureum* grass fed to sheep (Anele et al., 2010).

The higher digestibility of NDF in lambs that consumed 100FSF is attributed to the higher nutrient intake in this group. This shows that FSF supplement is likely to improve rumen environment for more efficient microbial fermentation of ingested feeds. Hence, the higher nutrient digestibility in lambs fed higher levels of FSF is indicative of higher supplementary value of this feed resource. The similarity in CP digestibility among the different treatments is attributable to supplementation of Noug seed cake at isonitrogenous levels to all experimental animals.

Nitrogen balance

All the lambs achieved positive N balance indicating that the animals obtained the amount of N above their maintenance requirement. The similarity of N intake between groups of lambs under the different treatments was due to equal provision of N in their diet. However, the significantly higher N retention in lambs fed 100FSF was presumably due to higher N microbial activity and higher microbial N supply. In addition, FSF might have contributed to higher amount of microbial cells for digestion in lower gut and hence more N retained in the body of lambs supplemented with higher proportion of FSF. The relatively lower amount of N excreted in urine of lambs with higher levels of FSF supplementation has important implication for natural resources management and sustainability because of reduced ammonia emission to the environment (Kaitho et al., 1998; Adugna and Sundsøl, 2000b). In general, the level of N retention found in the present study was neither deficient nor in excess, rather it was indicative of just sufficient and optimum level of N supply for maintenance and growth of the lambs (McDonalds et al, 2010).

Growth performance of lambs

Higher performance of the lambs raised on FSF and OG supplements as compared to those fed only control diet indicates that protein supplement alone is not enough to optimize lambs performance and that additional energy concentrate supplement is necessary (Dutta et al., 2009; Pittroff et al., 2006; Adugna and Sundstøl, 2000a). Higher growth performance in sheep supplemented with higher proportion of FSF is a reflection of the higher feed intake, higher metabolizable energy intake, higher nutrient digestibility and higher N retention of diets with higher FSF inclusion.

The daily body weight gain of lambs fed 100FSF in the present study was 36.1% higher than that of local lambs fed maize stover at different maturity stages as basal diet supplemented with 450 g day^{-1} *Desmodium intortum* hay (Adugna and Sundstøl (2000a). This may, among others, be due to lack of energy diets supplementation in the later study. It was also about 16.1% higher than the average daily weight gain of lambs of the same breed, Haraghe highland sheep, fed urea treated maize stover supplemented with graded levels of concentrate mix (Hirut et al., 2011). However, the daily weight gain in the current study was about 12.9% lower than that of the same breed of lambs fed natural pasture hay basal diet supplemented with mixtures of onion leaves, noug seed cake, and wheat bran at different proportions (Tsehai, 2012). This may presumably due to more number of supplementation mix that might have helped the lambs obtained different nutrients in the later case. Generally the better growth performance of lambs fed 100FSF in the present study indicates FSF to be a promising energy supplement than OG in sheep nutrition.

Conclusion

The results in the present study showed that lambs offered control diet performed poorer in every metabolic parameter studied compared to those supplemented with FSF and OG. The voluntary feed intake, apparent digestibility, N retention and live weight changes were highest for sheep offered diet consisting maximum level of FSF, implying that FSF supplied better ME to growing sheep than OG. Hence, it can be concluded that FSF can be used as energy supplement in the diets of growing lambs with better efficiency than OG.

Acknowledgement

The authors are indebted to Swedish International Agency (SIDA) for the financial support and Haramaya University for allowing use of farm and laboratory facilities. We are grateful to Dr Henock Ayalew for his perseverance in taking care of the health of the experimental animals.

References

Adugna Tolera and Sundstøl F, 2000a. Supplementation of graded levels of *Desmodium intortum* hay to sheep feeding on maize stover harvested at three stages of maturity 1. Feed intake, digestibility and body weight change. Animal Feed Science and Technology 85: 239-257

Adugna Tolera and Sundstøl F, 2000b. Supplementation of graded levels of *Desmodium intortum* hay to sheep feeding on maize stover harvested at three stages of maturity; 2. Rumen fermentation and nitrogen metabolism. Animal Feed Science and Technology 87: 215-229.

Adugna Tolera, Alemu Yami, Alemayehu Mengistu., Dawit Alemu, , Diriba Geleti, Getinet Assefa, Lemma Gizachew, Seyoum Bediye and Yirdaw Weldesemayat, 2012. Livestock Feed Resources in Ethiopia: Challenges, Opportunities and the Need for Transformation: Main issues, Conclusions and Recommendations; PP: 1-135.

Ajebu Nurfeta, Adugna Tolera, Eik, L. O., and Sundstøl, F., 2008. The supplementary value of different parts of enset (Enseteventricosum) to sheep fed wheat straw and Desmodiumintortum hay. Livestock Science 119: 22–30.

Ayenew Almaz, BerhanTamir and Solomon Melaku, 2012. Feed Intake, digestibility and Live Weight change of Lambs Fed Finger Millet (Eleusinecoracana) Straw Supplemented With Atella, noug Seed (*Guizotia abyssinica*) cake and their Mixtures. Agricultura Tropica Et Subtropica 45: 105-111.

Anele, U.Y., Arigbede, O.M., Südekum, K.H., Ike, K.A., Oni, A.O., Olanite, J.A., Amole, G.A., Dele, P.A., Jolaosho, A.O., 2010. Effects of processed cowpea (Vignaunguiculata L. Walp) haulms as a feed supplement on voluntary intake, utilization and blood profile of West African dwarf sheep fed a basal diet of *Pennisetum purpureum* in the dry season. Animal Feed Science and Technology 159: 10-17

AOAC., 1995. Official Methods of Analysis of AOAC International, 16th edition. Standard compendium of laboratory methods for analyzing foods and related substances; AOAC, Gaithersburg, MD.

Archimede, H., Boval, M., Alexandre, G., Xande, A., Aumont, G., Poncet, C., 2000. Effect of re-growth age on intake and digestion of *Digitariadecumbens*consumed by Black-belly sheep. Animal Feed Science and Technology 87: 153-162

Getnet Assefa, Kijora C., AimiroK., SeyoumB., Peters K.J., 2008. Evaluation of tagasaste (Chamaecytisuspalmensis) forage as a substitute for concentrate in diets of sheep. Livestock Science 114 : 296–304

Assen Ebrahim and Aklilu Hailemichael, 2012: Sheep and goat production and utilization in different agro-ecological zones in Tigray, Ethiopia. Livestock Research for Rural Development. Volume 24, Article #16. Retrieved October 16, 2014, from http://www.lrrd.org/lrrd24/1/asse24016.htm

Chay-Canulb, A.J., Ayala-Burgosa, A.J., Aguilar-Péreza, C.F., Solorio-Sáncheza, F.J., Ku-Veraa J.C., 2012. Voluntary intake, apparent digestibility and prediction of methane production by rumen stoichiometry in sheep fed pods of tropical legumes _ E.G. Brice˜no-Poota, A. Ruiz-Gonzáleza. Animal Feed Science and Technology 176: 117-122.

Dutta, T.K., Agnihotrib, M.K., Sahooc, P.K., Rajkumara, V., and Das Arun, K., 2009. Effect of different protein–energy ratio in pulse by-products and residue based pelleted feeds on growth, rumen fermentation, carcass and sausage quality in Barbari kids. Small Ruminant Research 85: 34–41.

Eriksson, T., Ciszuk, P., Burstedt, E.2009. Proportions of potatoes and fodder beets selected by dairy cows and the effects of feed choice on nitrogen metabolism. Livestock Science 126: 168–175.

HirutYirga, SolomonMelaku and Mengistu Urge, 2011. Effect of concentrate supplementation on live weight change and carcass characteristics of Hararghe Highland sheep fed a basal diet of urea-treated maize stover. Livestock Research for Rural Development. Volume 23, Article #245. Retrieved October 16, 2014, from http://www.lrrd.org/lrrd23/12/hiru23245.htm.

Kaittho, R.J., Umunna, N.N., Nsahlai, I.V., Tamminga, S., Van Bruchem, V., 1998. Utilization of browse supplements with varying tannin levels by Ethiopian Menz sheep. 2. Nitrogen metabolism. Agroforestry System 39: 161-173.

Lascano, G.J., Zanton, G.I., Heinrichs, A.J. 2009. Concentrate levels and *Saccharomyces cerevisiae* affect rumen fluid-associated bacteria numbers in dairy heifers. Livestock Science 126: 189–194.

Lloyd, C.E. and Davies H L., 1992. Effect of supplementing sheep with oat grain on their intake, digestion, and nitrogen balance when fed chopped carpet grass (*Axonopusaffinis* Chase) hay, Australian Journal of Experimental Agriculture 32: 163 – 165.

Lumbile, A.U. and Mogotsi, K.K., 2008. *Ficus sur*Forssk. [Internet] Record from Protabase. In: Louppe, D., Oteng-Amoako, A.A. & Brink, M. (eds.). PROTA (Plant Resources of Tropical Africa / Ressourcesvégétales de l’Afriquetropicale), Wageningen, Netherlands.

McDonald, P, Edwards, R. A., Greenhalgh, J. F. D. and Morgan, C.A., 2002. Animal Nutrition. 6th eds. Prentice Hall, London.

McDonalds,P., Edwards, R.A., Greemhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. and Wilkinson, R.G. (2010). Animal Nutrition: Feeding Standards and Nutrient Requirements; 7th Editions; PP: 343-361.

Moran, J., 2005 How energy is measured. In: TROPICAL DAIRY FARMING. Feeding Management for Small Holder Dairy Farmers in the Humid Tropics. Landlinks Press, Australia, PP:30-33.

Pittroff, W., Keisler, D.H., and Blackburn, H.D., 2006. Effects of a high-protein, low-energy diet in finishing lambs: Feed intake, estimated nutrient uptake, and levels of plasma metabolites and metabolic hormones. Livestock Science 101: 262 – 277.

Rowe, J.B. and L.R. Coss, 1994. Lignin content of oat grain - does it affect animal performance when sheep are given oats as a supplementary feed. Proceedings of Australian Scociety of Animal Production 20: 421-425.

SAS (Statistical Analysis System), 2008 The Little SAS® Book: A Primer, Fourth Edition, version 9.1.3. Statistical analysis system institute Inc., NC. USA.

Tadesse, G., 2011. Effects of supplementation with graded Levels of concentrate mix of oats grain and lentil screening on the performance of Menz sheep fed hay, a thesis submitted to the school of graduate studies, in partial fulfillment of the requirement for the degree of Master of Science in Agriculture Haramaya University, Ethiopia

Berhanu Tesema, Getachew Animut and Mengistu Urge, 2013. Effect of green *Prosopis juliflora p*ods and noug seed (*Guizotia abissynica*) cake supplementation on digestibility and performance of Blackhead Ogaden sheep fed hay as a basal diet. Science, Technology and Arts Research Journal 2: 38-47.

Tsehai Regassa, 2012. Effect of graded levels of onion leaves as a substitute for wheat bran in concentrate mixture on performance and carcass characteristics of Hararghe highland sheep; A Thesis Submitted to the School of Graduate Studies (School of Animal and Range Sciences); Haramaya University; In Partial Fulfilment of the Requirement for the Degree of Master of Science In Agriculture (Animal Nutrition), Ethiopia

Van Soest, P.J. and Robertson, J.B., 1985. Analysis of forage and fibrous feeds. A laboratory manual for Animal Science 613 Cornell University, Ithaca, New York, USA.

Yang, W.Z. and Beauchemin, K.A., 2006. Increasing the physically effective fiber content of dairy cows diet may lower efficiency of feed use. Journal Dairy Science 89: 2694-2704.

Wang Min, Xiao Gang Zhao, Hai Yan Liao, Zhi Liang Tan, Shao Xun Tang, Zhi Hong Sun, Chuan She Zhou and Xue Feng Han, 2011. Effects of rice straw particle size on digesta particle size distribution, nitrogen metabolism, blood biochemical parameters, microbial amino acid composition and intestinal amino acid digestibility in goats. Animal Science Journal 82:78–85.

Yoseph Mekasha., Azage Tegegn., Alemu Yami and Umunna, N.N., 2002. Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants: *in vitro* and metabolism study with sheep. Small Ruminant Research 44: 25–35.

Feed Availability, Conservation Practices and Utilization in Selected Milk-Shed Areas in the Central Highlands of Ethiopia

Fekede Feyissa* and Gezahegn Kebede

Holetta Agricultural Research Center, P.O.Box 31, Holetta, Ethiopia
*Corresponding Author: ffeyissa@yahoo.com

Abstract

This study was conducted to assess feed availability, conservation practices and utilization of stored feeds for feeding dairy cattle in three milk-shed areas (Ejere, Sululta and G/Jarso) in the central highlands of Ethiopia. Data were collected from 147 dairy farmers using a structured questionnaire, observations, and sampling and measurements of pasture productivity from representative sites. Data was analyzed using SPSS and descriptive statistics. About 11 different feed types or categories were available in the study areas of which natural pasture hay followed by crop residues and pasture grazing constituted the dominant sources of basal feed to dairy cattle. Farmers produce hay on their own pasture land and/or by contracting standing hay on a total average area of 1.1ha with an estimated average production of 6.85 tons per household. Different crop residues dominantly of cereal straws were produced and used for feeding dairy cattle mainly in the peri-urban dairy production systems at Ejere and Sululta. Moreover, 36.7% of the sample households have adopted the production of improved fodder crops on an average area of 0.19ha for feeding dairy cattle. Farmers also use different agro-industrial and locally available by-products to supplement dairy cattle. Natural pasture hay was the dominant feed conserved followed by crop residues, while few farmers also make hay using oats/vetch mixture. Hay was stored in loose form and in open air by majority (≥70%) of the dairy farmers. Baling crop residues was totally uncommon in the study areas. However, crop residues were stored under shelter shade by 70% of the surveyed farmers. Farmers reported to feed dairy cattle with hay, crop residues and all stored feeds for 8, 5.8 and 10.4 months, respectively per annum. The study showed that both the form and method of feed storage practiced in the study areas were less efficient to ensure quality basal feed supply to dairy cattle. Therefore, regular trainings and other advisory services should be provided to farmers and development agents on efficient feed conservation and utilization to curb feed shortage. Moreover, introduction of baling machines and promotions of baling hay and crop residues are essential for efficient handling and utilization of available feeds. Baling will also allow mobilizations of seasonally excess feeds available in different areas for use in deficit areas.

Key words: *- natural pasture hay, crop residues, storage method, dairy cattle, central highlands of Ethiopia*

Introduction

Ethiopia is known for its large cattle resource base and suitable climatic conditions for livestock production in general and dairy production in particular. However, milk production and the per capita consumption in the country has been one of the least in the world (Azage *et al.,* 2006; Staal *et al.,* 2008; FAOSTAT, 2010; LMP, 2014). Ethiopia is spending considerable amount of foreign currency for importing dairy products mainly powder milk in order to augment the milk deficit. According to FAOSTAT (2015) the country has spent about 1.6 billion USD for the import of dairy products from 2002 to 2010.

Livestock production in general and dairy cattle productivity in particular has been mainly constrained by inadequate supply and poor quality of available feed resources in Ethiopia (EIAR, 2017). This could be further emphasized by the fact that feed accounts for 60-70% of the costs associated with livestock production. Dairy production is a function of genetics, feeding, health care and other management practices. Evidences indicate that genetic improvement will lead to an improvement in milk productivity of cattle in the range of 60 to 300% only if accompanied by better feeding regimes (McDermott *et al.,* 2010). As dairying is a routine venture which requires continuous and adequate supply of the required nutrients, no improvement in dairy production is possible without adequate understanding of the requirements and associated improvement in feed quantity and quality.

Natural pasture hay and crop residues which provide the bulk of livestock/dairy feed in Ethiopia are seasonally produced during particular periods of the year (October-January) following the main rainy season, and their extended use and quality will depend on proper harvesting, collection, storage, feeding and other management practices. Smallholder dairy producers in the central highlands of the country either partially or totally rely on conserved feed (hay and/or crop residues) for varying periods in a year. However, up-to-date information is lacking on production/availability and overall management practices such as forms and methods of storage, storage durations and utilizations of conserved feeds. Understanding these aspects would help to design appropriate interventions and extension guidelines for ensuring year round supply of better quality feed, and hence improve dairy production in potential milk shed areas. Therefore, the objective of this study was to assess feed availability, conservation practices and utilization of conserved feed for feeding dairy cattle in three selected milk shed areas in the central highlands of Ethiopia.

Materials and Methods

The study areas

The study was conducted in three selected milk-shed areas (Sululta and Girar Jarso from North Shewa zone, and Ejere from West Shewa zone) in the central highlands of Ethiopia. Girar Jarso is located between 9°38'47"N to 9°59'49"N and 38°34'17"E to 38°49'41"E and the zonal town Fiche, is situated 113 km northwest of Addis Ababa. Sululta is located between 9°4'30"N to 9°30'59"N and 38°31'26"E to

38°58'49"E and the woreda town Chancho, is situated 40 km northwest of Addis Ababa. Although it is geographically located in North Shewa zone of the Oromia Regional State, Sululta woreda has been administratively placed under the Oromia Special Zone Surrounding Finfinne since 2007. Ejere is located between 8°51'16"N to 9°14'53"N and 38°15'2"E to 38°28'45"E, about 40 km west of Addis Ababa. Both Sululta and Girar Jarso specifically the study sites are located at an altitude of above 2500 m above sea level (a.s.l), while Ejere is located at an altitude of 2400 m above sea level (a.s.l). Map of the study areas is shown in Figure 1.

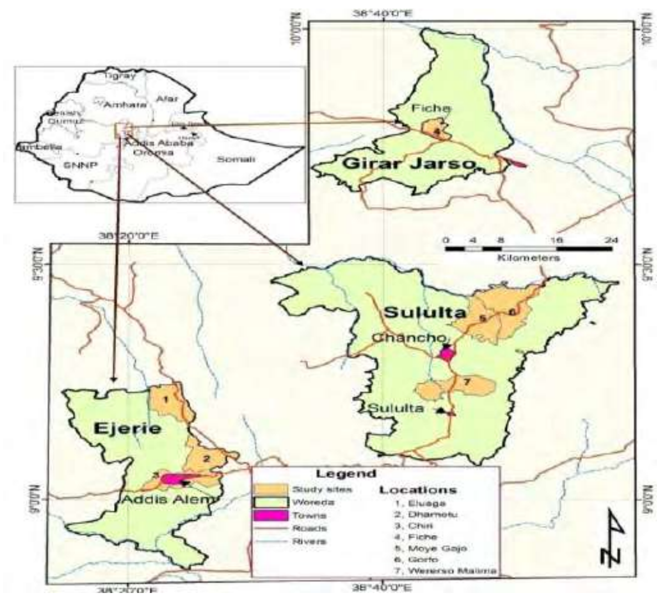


Figure 1. Map of the study areas

Girar Jarso (with Fiche town as focal study site) was selected to represent urban dairy production where dairying is practiced to support family income in addition to other off-farm activities. Dairy production in this system is relatively intensive and mainly based on stall-feeding using purchased feeds. Moreover, the exotic blood level in the herd could be high, but very few cows are kept per household.

Both Sululta and Ejere woredas represented peri-urban dairy production system where crop and livestock production are closely integrated, and agricultural activities other than milk production form additional source of income. The major difference between Sululta and Ejere is that in Sululta, cropping is mainly subsistent and livestock production particularly dairying is the major source of livelihood. Hence, it was selected to represent intensified dairy/crop livestock sub-system where some form of intensive dairy production is practiced and farmers had experiences with different dairy development projects which have influenced the production system (Azage *et al.,* 2005). Ejere is characterized by intensive cropping where both crops and livestock production have comparable contributions to livelihoods. Hence, it was selected to represent intensified crop/livestock (dairy) production sub-system where the cropping system is more intensive. Therefore, the three woredas were assumed to represent three segments of market-oriented smallholder dairy production systems/sub-systems *viz.* urban (Girar Jarso-Fiche), peri-urban intensified dairy/crop-livestock sub-system (Sululta) and peri-urban intensified crop/crop livestock sub-system (Ejere).

Sampling procedures and data collection

First hand information was gathered on the overall picture of dairy production, feed resources and other related issues via a quick survey and discussions with agricultural extension offices and available dairy cooperatives/unions in the three woredas. The information was used to identify focal villages (Kebeles) and individual farmers using multi-stage purposive sampling technique. Two to three villages were selected from each woreda on the basis of dairy production potential, linkage to milk market, experience in feed production and conservation, and accessibility. Subsequently, a total of 147 dairy farmers (60 from Sululta, 39 from Girar Jarso and 48 from Ejere) were selected with the help of village development agents. A pre-tested questionnaire was used to collect data by interviewing the farmers at their farm gates. The questionnaire covered various topics including household characteristics, dairy cows herd size and composition, major types of available feed resources, land area used for hay and improved forage production, feed conservation practices and methods of conservation, and contribution of stored feeds for feeding dairy cattle in terms of the duration of feeding period in a year.

Estimation of hay production from natural pasture

Sampling for herbage yield determination was made during the peak hay harvesting period (mid – late October) in all the study areas. Three to five representative pasture fields were selected in each of the villages included in the study at Sululta and Ejere woredas. At Girar Jarso, sampling was made from pastures grown in government compounds like the military camp and Farmers` Training Centers (FTC`s) found around Fiche town as these compounds were reported to be major sources of hay for dairy farmers in Fiche town. Sampling was made by placing a $0.5m^2$ quadrate at five randomly selected plots within the pasture field. After measuring the fresh weight of the biomass harvested from the five $0.5m^2$ plots, a sample of 350 gram was taken using a sensitive field balance for further DM determination through oven drying (65°C for 72h). The quantity of hay produced per household was estimated by multiplying the average DM yield per hectare with the corresponding areas of pasture land reported to be used for hay production by the households.

Estimation of crop residue production

Crop residue yield was estimated from the corresponding grain yields of the crops reported to be grown by the surveyed farmers in the three woredas. Information on grain yields of the different crops grown on the respective areas (as reported by the farmers) was first collected by interviewing the farm households. The information was used for estimating average grain productivity of the respective crops per ha in the three woredas. Moreover, national, Oromia regional and zonal average grain yields of the different crops were collected from the reports of central statistical agency (CSA, 2012). Then, the average grain yields estimated using farmers` information and the CSA reports was considered for estimating crop residue yields from the different crops grown by the surveyed farmers. Crop residue produced by the surveyed households was estimated by multiplying grain yield data with conversion factors established for each crop (Kossila, 1984; FAO, 1987). A multiplier of 1.5 was used for wheat, barley and tef (*Eragrostis tef*); 1.7 for oats, 2.0 for maize and 1.2 for the different pulse crops. This estimation did not consider the amount of feed that could be obtained from crop aftermath, the inevitable field losses and other alternative uses which may account for about 30% of the total crop residues.

Data analysis

The three woredas (representing the three dairy production systems/sub-systems) were used as fixed factors for the various dependent variables assessed in the study. The survey data was coded and analyzed using the statistical package for social sciences (SPSS, 2007). Where applicable, the significance of differences in mean values of the quantitative variables between the woredas were tested using Duncan`s Multiple Range Test (Duncan, 1955) at a probability level of 0.05. Qualitative variables were also described using descriptive statistics.

Results and Discussion

Available feed resources

Table 1 indicates the different types of feeds available for feeding dairy cattle in the study areas. About 11 feed types or feed categories have been reported to be used for feeding dairy cattle in the areas. Grass hay was the dominant basal feed resource followed by crop residues and pasture grazing. Crop aftermath (stubble) grazing was also reported as other important source of basal feed to dairy cattle in the peri-urban dairy production systems at Sululta and Ejere woredas. About 98.6% of all the sample households reported to use different agro-industrial by-products as supplementary feeds to dairy cattle. Considerable proportion of the sample households also reported to use local beverage residues, oats grain and/or hull and grain screenings to supplement dairy cattle. Improved fodder crops, tree leaves and weeds from crop fields were the other feed resources used for feeding dairy cattle in the study areas. The production and use of improved fodder crops for feeding dairy cattle was relatively better in Ejere as compared to the other two woredas. The tree leaves reported to be used as feed mainly belong to Tree lucerne *(Chamaecytisus palmensis)* and Sesbania *(Sesbania sesban)*, the promising fodder trees introduced to the study areas and established as live fences on the farmers backyards. The fodder trees remain green throughout the dry season and their leaves have been used for feeding dairy cattle in mixture with low quality roughages like crop residues.

The types and relative importance of the different feed resources reported in this study were in agreement with previous findings in various parts of Ethiopia (Agajie *et al*., 2002; Tessema *et al*., 2003; Yoseph *et al*., 2003; Zewdie, 2010). This suggests that these feed resources should be focused for targeted interventions to improve livestock feed supply in the country.

Table 1. Major types of feed resources available for feeding dairy cattle in the study areas

Type of feed resource	Proportion of respondents (%)			
	Sululta (n=60)	Girar Jarso (n=39)	Ejere (n=48)	Total (N=147)
Pasture grazing	98.3	5.1	95.8	72.8
Crop residues	100	64.1	97.9	89.8
Grass hay	100	100	100	100
Crop aftermath (stubble) grazing	96.7	2.6	93.8	70.8
Agro-industrial by-products	98.3	100	97.9	98.6
Improved fodder crops	30.0	30.8	68.8	42.9
Oats grain and/or hull	88.3	5.1	56.3	55.8
Grain screenings	55.0	7.7	47.9	40.1
Local beverage residue (`Attela`)	68.3	61.5	91.7	74.2
Tree leaves	15.0	-	47.9	21.8
Weeds from crop fields	38.3	-	12.5	19.7

Natural pasture hay production

Land area used for hay production, estimated pasture productivity and quantities of hay produced per household in the study areas are shown in Table 2. Dairy farmers produce hay on their own holding and/or by contracting standing hay. The landless dairy farmers in Fiche town (G/Jaro) mainly produce hay by contracting standing hay grown within the government compounds such as the military camp located in the vicinity of the town. The overall average total area of pasture land used for hay production per household was 1.10 ha (1.15 ha in Sululta, 1.76 ha in G/Jarso and 0.83 ha in Ejere). The average pasture land areas used for hay production per household in this study were greater than the earlier figures reported at Selale (Kelay, 2002) and Debre Birhan area (Zewdie, 2010). In all the study areas, dairy farmers opt to produce more hay by contracting standing hay which implies the better access and well established culture of renting pasture land in the areas. Moreover, the higher cattle herd size with increased proportion of crossbred cows might have necessitated more hay production by contracting pasture land on top of own holding. Most dairy producers in G/Jarso have also reported to secure the roughage feed supply by purchasing readily available hay (heaps, bales, donkey loads, etc) from the surrounding areas.

Pasture productivity was significantly higher ($p<0.05$) in G/Jarso followed by Ejere and was lower in Sululta, with the overall average yield of 6.38 t DM/ha. Dairy farmers in G/Jarso (Fiche town) reported to make hay by contracting the pasture grown in the surrounding government compounds like the military camp and FTC`s which are well protected from livestock. This could be the reason for the higher productivity of pasture sampled from these areas. In Sululta, hay is mainly produced on waterlogged lands which may retard pasture growth resulting in low yield. On average, the quantity of hay estimated to be produced per household in a season was 6.85 tones (ranging from 0.76-31.88 tones) on dry matter basis (Table 2). Higher ($p<0.05$) amount of hay was estimated to be produced per household in G/Jarso, followed by Sululta and Ejere. This shows that the dairy producers in G/Jarso are striving to secure as much hay as possible to ensure adequate year round roughage feed supply as stall feeding is the sole feeding management of dairy cows in the urban setting.

Table 2. Estimated pasture productivity and quantity of hay produced per household in the study areas

District	Variable		
	Total land area used for hay (ha)*	Estimated pasture productivity (t DM/ha)	Estimated quantity of hay produced per HH (t DM)
Sululta (n=58)	1.15^b	5.63^c	6.48^b (1.41-22.52)[@]
Girar Jarso (n=15)	1.76^a	7.97^a	14.01^a (0.88-31.88)
Ejere (n=47)	0.83^b	6.04^b	5.01^b (0.76-12.08)
Overall (N=120)	1.10	6.38	6.85 (0.76-31.88)

[a-c]Means with different superscripts within a row differ significantly ($P<0.05$)
**Includes both own holding and rented/contracted land*
[@]Figures in the brackets indicate ranges in the quantities of hay produced per household

Crop residue production

Table 3 shows the estimated quantities of different crop residues produced per household in a season. The types of crop residues produced by farmers were similar in Sululta and Ejere except maize stover which was produced by some households in Ejere. In Girar Jarso, only few households reported to produce some crop residues. The estimated total quantity of crop residues produced per household was higher ($p<0.05$) in Ejere (8.07 tones DM) followed by Sululta (5.28 tones DM), and was lower in Girar Jarso (3.04 tones DM). This could be attributed to differences in the intensities of cropping which in turn determines the availability of crop residues for feeding dairy cattle in the study areas. Higher quantities of barley and tef straws were produced per household in Sululta and Ejere, respectively. Although the quantity of tef straw produced per household was higher than wheat and oats straws in Sululta, its actual availability for feeding was reported to be lower than the other cereal straws due to its various alternative uses. At Ejere, the availabilities of both wheat and tef straws for dairy cattle feeding were comparable, while the availabilities of both barley and oats straws were comparatively lower. Among the pulses, higher amounts of faba bean and grass pea straws were respectively produced at Sululta and Ejere.

The quantities of crop residues produced by farm households depend on the land area allocated to each crops, grain productivities and harvest indexes of the different crops. In this study, cereals account for the major share in terms of both land allocation and crop residue production. This was in line with the national scenario in which cereals account for 79.34% of the total cropped land and about 86.06% of the total grain production in the country (CSA, 2012).

Table 3. Quantities of different crop residues produced per household in the study areas (t DM)

Type of crop residue	Sululta	Girar Jarso	Ejere
Barley straw	1.96 (0.57-5.68)*	1.42 (0.57-2.28)	1.36 (0.33-2.62)
Teff straw	1.87 (0.83-3.33)	1.97 (0.84-2.53)	3.88 (0.94-11.25)
Wheat straw	1.76 (0.48-10.53)	0.49	2.94 (0.59-7.03)
Oats straw	1.07 (0.48-2.40)	0.46	0.89 (0.28-2.31)
Maize stover	-	-	1.08 (0.61-1.21)
Faba bean straw	0.60 (0.16-1.33)	0.66 (0.33-1.00)	0.49 (0.22-1.10)
Field pea straw	0.47 (0.13-1.06)	-	0.41 (0.31-0.62)
Chick pea straw	0.42 (0.16-1.25)	-	0.40
Grass pea straw	0.48 (0.32-0.64)	0.65	0.62 (0.03-1.23)
Total	5.28^{ab} (0.79-15.89)	3.04^{b} (2.24-4.64)	8.07^{a} (0.66-23.45)

$^{a-b}$*Means with different superscripts within a row differ significantly (P<0.05)*

**Figures in the brackets indicate ranges of the different crop residues produced on the farm*

Improved forage production

The status of improved forage production in the study areas is shown in Table 4. Overall, 36.7% of the sample households reported to produce improved fodder crops. About 70.8 and 33.3% of the respondents in Ejere and Sululta, respectively, have reported to produce improved forages, while all the sample households in Girar Jarso (urban setting) had no experience in improved fodder production due to lack of access to farm land. The proximity of Ejere to Holetta Agricultural Research Center and the better access to improved forage technologies coupled with better extension efforts could be the major reason for the better adoption of improved forages in the area. A recent study has shown that adoption rate of improved forage crops in Oromia Region was 10% with comparatively higher adoption rates in North Shewa (23%) followed by Southwest Shewa (16%), Arsi (14%) and West Shewa (10%) (Agajie, *et al.,* 2016).

Table 4. Status of improved forage production in the study areas

Variable	Status	Sululta (n=60)		Girar Jarso (n=39)		Ejere (n=48)		Overall (N=147)	
		n	%	n	%	n	%	n	%
Production of improved forage crops	Yes	20	33.3	-	-	34	70.8	54	36.7
	No	40	66.7	39	100	14	29.2	93	63.3
Types of forage crops produced									
Napier grass (*Pennisetum purpureum*)		6	30.0	-	-	28	82.4	34	63.0
Treelucerne (*Chamaecytisus palmensis*)		13	65.0	-	-	24	70.6	38	70.4
Sesbania (*Sesbania sesban*)		3	15.0	-	-	14	41.2	18	33.3
Oats/vetch mixture		18	90.0	-	-	29	85.3	47	87.0
Fodder beet (*Beta vulgaris*)		4	20.0	-	-	1	2.9	5	9.3
Land area allocated for improved forage (ha)									
0.01 – 0.08		5	25.0	-	-	12	35.5	17	31.5
0.10 – 0.50		13	65.0	-	-	19	55.9	32	59.3
>0.50		2	10.0	-	-	3	8.8	5	9.3
Total		20	100	-	-	34	100	54	100
Mean±SE		0.16±0.03[b] (0.01–0.75)*				0.21±0.04[a] (0.01–1.13)		0.19±0.03 (0.01–1.13)	

[a-b] Means with different superscript letters within a row are significantly different (P<0.05)
**Indicate total range of land area covered by fodder crops in the three woredas*

The proportion of improved fodder producing farmers observed in this study was higher than the figures reported for crop-livestock mixed farms in different parts of Ethiopia *viz.*, Debre Birhan, Sebeta, Ziway and Jimma (Zewdie, 2010); in Benishangul-Gumuz (Beyene, *et al*., 2011); in Alaba area (Yeshitila, 2008); in Wolayta Soddo (Irvin, 2000) and in north-western Ethiopia (Yitaye, 2008). This may be attributed to the better exposure and access by farmers in the study areas to improved fodder production and utilization technologies than smallholder farmers in the other areas. The major types of improved fodder species produced in the study areas were Napier grass (*Pennisetum purpureum*), Tree lucerne

(*Chamaecytisus palmensis)*, Sesbania (*Sesbania sesban*), Oats/vetch mixture and Fodder beet (*Beta vulgaris*). Among the improved forage producing households, the majority (87%) have reported to adopt oats/vetch mixture followed by Tree lucerne (70.4%), Napier grass (63%), Sesbania (33.3%) and Fodder beet (9.3%). In Ejere, oats/vetch mixture and Napier grass were equally adopted followed by Tree lucerne, whereas in Sululta, Tree lucerne was the second important fodder produced after oats/vetch. This could be due to the difference in climatic conditions and soil types of the areas. Sululta is characterized by seasonal frost, waterlogged and poorly fertile soils which may limit the production of Napier grass which is highly susceptible to frost and low soil fertility than the other fodder crops.

The average area of land used for improved fodder production per household was 0.16 ha at Sululta and 0.21 ha at Ejere, with an overall average of 0.19 ha (Table 4). Majority of the improved fodder producing farmers (65% in Sululta, 55.9% in Ejere and 59.3% of the respondents) allocated 0.10-0.50 ha land for fodder production. Whereas, 25% in Sululta, 35.3% in Ejere, and 31.5% of the total fodder producing farmers reported to allocate only 0.01-0.08 ha land for fodder production. Small proportion (10% in Sululta, 8.8% in Ejere, and 9.3% of the total fodder producing farmers) reported to allocate more than 0.50 ha land for fodder production. The result showed that the average land area used for improved fodder production was estimated to account only for 5.9% of the total land owned by a household in the study areas. This is in line with the national scenario in which smallholder farmers are usually reluctant to take land away from food crop production and use for fodder production. Getnet *et al*., (2002) indicated that farmers are mainly willing to allocate their arable land for fodder production provided that they own crossbred dairy cows, and if the income from milk and milk products would be more rewarding than crop production.

Supplementary feed resources

The different types of supplementary feeds available for feeding dairy cattle in the study areas are presented in Table 5. Ten different concentrates and feed ingredients are used for feeding dairy cattle; the majority of which are agro-industrial by-products, while oats grain/hull and grass pea hull are locally produced. The majority of the dairy farmers reported to use wheat bran followed by noug (*Guizotia abyssinica)* seed cake and oats grain to supplement dairy cattle. Considerable proportion of the sample households also reported to supplement dairy cattle with molasses, compound dairy ration and brewery residues. Linseed and cotton seed cakes were mostly used for fattening and less commonly used for dairy cows except in Ejere where about 45.8% of the respondents reported to supplement dairy cattle with linseed cake especially when the animals lose condition.

The types of concentrate feeds/ingredients used to supplement dairy cattle were similar across the study areas except the variations in the degree of utilization of some ingredients like oats grain, grass pea hull and brewery residues. Oats grain was reported to be used to supplement dairy cattle by 85 and 37.5% of the sample households at Sululta and Ejere, respectively. About 45.8% of the sample households in Ejere also reported to use grass pea hull, which is commonly available in the area. Similarly, 58.3% of the sample households in Sululta reported to supplement dairy cattle with brewery residue (industrial), while it was uncommon in Ejere. However, local brewery residues were reported to be commonly used to supplement dairy cows in all the study areas.

Table 5. Types of supplementary feeds used for feeding dairy cattle in the study areas (% of respondents)

Type of supplementary feed	Sululta (n=60)	Girar Jarso (n=39)	Ejere (n=48)	Total (N=147)
Wheat bran	96.7	100	81.3	92.5
Wheat middling	30.0	15.4	14.6	21.1
Noug cake (*Guizotia abyssinica)*	75.0	82.1	77.1	77.6
Linseed cake	5.0	-	45.8	17.1
Cotton seed cake	3.3	2.6	-	2.1
Molasses	50.0	12.8	50.0	40.1
Brewery residues (industrial)	58.3	2.6	-	24.5
Compound dairy ration	18.3	33.3	31.3	26.5
Oats grain and/or hull	85.0	2.6	37.5	47.6
Grass pea hull	-	-	45.8	15.0

Feed conservation practices and utilization

Table 6 indicates the major types of conserved feeds in the study areas. Natural pasture hay was the dominant feed conserved followed by crop residues. Oats/vetch mixture hay was also reported to be conserved by some dairy farmers. Although oats is widely produced in Sululta than in Ejere, it is mainly used as a food grain than for hay production and this was the reason why oats/vetch hay conservation was reported by small proportion of farmers in Sululta than in Ejere. In both Sululta and Girar Jarso, natural pasture hay was ranked first as conserved feed followed by crop residues, whereas in Ejere, crop residues were the dominant conserved feeds. This is due to the better cropping intensity and higher crop residue production in Ejere than in the other two sites. Overall, natural pasture hay was ranked first as conserved feed for feeding dairy cattle. This shows that native hay is the major source of roughage feed for dairy cattle in the study areas and an improvement in feed supply for dairy cattle will depend on improvement in both productivity and quality of the available pasture lands. Moreover, development of oats/vetch mixture and conservation as hay can contribute to improve feed supply in the areas.

Table 6. Types of feeds conserved for feeding dairy cattle in the study areas

District	Type of feed conserved	% of respondents	Priority (1=highest; 3=lowest)			Rank
			1	2	3	
Sululta (n=60)	Natural pasture hay	100	90.0	10.0	-	1
	Crop residues	98.3	10.2	89.8	-	2
	Improved fodder hay	11.7	-	-	100	3
Girar Jarso (n=39)	Natural pasture hay	100	97.4	2.6	-	1
	Crop residues	64.1	4.0	96.0	-	2
	Improved fodder hay	2.6	-	-	100	3
Ejere (n=48)	Crop residues	100	78.7	19.2	2.1	1
	Natural pasture hay	97.9	20.8	72.9	6.3	2
	Improved fodder hay	25.0	8.3	25.0	66.7	3
Overall (N=147)	Natural pasture hay	99.3	69.4	28.6	2.0	1
	Crop residues	89.1	33.6	65.7	0.8	2
	Improved fodder hay	13.6	5.0	15.0	80.0	3

The forms and methods of storage of hay and crop residues practiced in the study areas are shown in Table 7. The majority of the sample households reported to store loose hay. Baling hay was totally uncommon in Ejere, while 31.7% and 35.9% of the respondents reported to make baled hay at Suluta and Girar Jarso, respectively. The sources of baler were private owners and the average baling costs ranged from 3.00-5.00 Birr/bale during this study (2012). Based on this, a farmer has to pay at least 1500 Birr for baling hay produced on 1 ha of land. Such a high baling cost was the major reason why most farmers store loose hay. Baled hay is advantageous over loose hay in terms of reducing field losses and facilitating the overall management practices such as collection, transportation, storage and feeding. Therefore, dairy development projects and government extension programs should consider introduction of balers to organized groups of farmers in potential hay producing areas.

About 57.8% of the sample households store hay in open air, 29.3% under shelter shade (corrugated iron roofed or grass thatched roof), and 12.9% using plastic covering on the hay stored outside (Table 7). It can be deduced that about 70% of the sample households store hay in open air as the protection provided by plastic sheets is only partial and does not equate to shelter shades. Most farmers believe that hay quality can be deteriorated when stored outside than under shade, and mentioned lack of resource capacity as the major limitation to construct shades. According to the farmers, mold growth, change in colour (black or brown-reddish), bad smell, low animal preference and high refusal are some indicators of spoiled hay. On the other hand, about 30% of the respondents perceived that hay can be stored outside without any problem if well thatched and piled properly.

A study at Holetta showed that CP content of natural pasture hay was reduced by 23.3 and 36.7% between the pre-storage period and eight months after storage when stored under shelter shade and in open air, respectively (Fekede *et al.,* 2014). This shows that storing hay outside for a long period will result in substantial loss in feed quality to the level detrimental to the nutrition of dairy cattle. Exposure to adverse weather conditions is the major factor responsible for the loss in hay quality when stored outside. Hay that is stored outside and subjected to wetting and drying cycles will develop a fibrous, weathered layer and this process is referred as "weathering". Weathering in hay refers to the wet, discolored, and moldy layer on the exterior and bottom surfaces of baled and/or loose hay (Lemus, 2009). The process will decrease digestibility, increases fiber concentration and reduces the overall hay quality. The highest nutrient loss in hay due to weathering is caused by leaching which refers to the dissolving and removal of nutrients by the passage of rain water over the surface of the hay. In this process, the more soluble nutrients (carbohydrates, lipids, fatty acids, etc) are washed out of the forage. The loss of nutrients in this way causes the fiber component to represent a larger proportion of the dry matter with the consequent reduction in total digestible nutrients. Hence, there is a likelihood of substantial loss in hay quality when stored in open air as practiced by majority of the respondents in this study.

The forms and methods of storage of crop residues practiced by farmers in the study areas are also shown in Table 7. Baling crop residues was uncommon in the study areas and only 5.1% of the households in Sululta reported to have ever used baled crop residues by purchasing from the local market as it was brought from other areas like Bishoftu. Farmers usually store mixtures of different crop residues together and some of them also practice re-threshing crop residues both to reduce the fiber length and thoroughly mixing the different straws prior to storage. The major reasons for storing mixtures of crop residues were to save storage space, to improve animal intake of the less preferred crop residues such as wheat straw by mixing it with the most preferred ones like barley and oats straws, and to improve the nutritive value of straws by mixing pulse straws with cereal straws. On the other hand, the farmers reported that tef (*Eragrostis tef*) straw, which has various alternative uses other than feed is usually stored in pure form in order to ease its management and utilization for the different purposes.

About 69.5% of the sample households store crop residues under shelter shade, 27.5% in open air, and the rest (3%) use plastic covering (Table 7). This was in contrary to the storage method practiced for natural pasture hay. According to the farmers, crop residues except tef straw are thick stemmed and less suitable for firm piling in a way to protect percolation of rain into the inside of the heap and the consequent spoilage. Moreover, farmers reported that crop residues are mainly fed during the rainy season in combination with some green feeds, as it is perceived that feeding crop residues alone during the dry season could lead to loss in animal condition and reduced milk production. In views of these, crop residues are stored for a long period necessitating storage under shelter shade to avoid spoilage resulting from exposure to rain.

In a study conducted to assess the effects of storage method and storage duration on the dynamics in nutritional qualities of tef and wheat straws sampled from Ejere area, the CP, IVOMD and ME contents showed consistently decreasing trends with prolonged storage durations, with higher nutrient losses in straws stored in open air than those stored under shelter (Fekede *et al.,* 2015). The result showed that the estimated losses in CP contents during the six months storage period when stored under shelter and in open air, respectively, were 30.2 and 41% in tef straw; and 22.3 and 46.9% in wheat straw. Similarly, IVOMD was reduced by 35.8 and 41.1% in tef straw and by 33.3 and 42.6% in wheat straw when stored under shelter and in open air, respectively, during the six months storage period. This shows that storing crop residues under shelter would help to maintain better nutritional quality in the straws.

The contribution of crop residues as livestock feed depends on proper collection, handling, processing, and storage. There was a well established practice of crop residue conservation for livestock/dairy cattle feeding in the study areas. However, baling crop residues was totally unknown and stacking loose straws into heaps was the sole traditionally adopted handling and storage of the crop residues to extend their use as feed. Such a practice may lead to losses in both biomass and nutritional quality during collection, transportation, storage and feeding. Moreover, it is difficult to handle and transport loose crop residues over long distances due to bulkiness. These factors could result in inefficient management and utilization of crop residues, not only in the study areas, but also throughout the country. For instance, there are many areas in the country where ample quantities of cereal straws are produced and left in the field for in situ feeding instead of being collected and conserved for long term feeding. When left on the field, the residues rapidly deteriorate, and the considerable proportion is usually trampled upon and wasted, while there is critical feed shortage especially in the urban and peri-urban dairy production systems in the highlands of Ethiopia. There could be a possibility of using the potentially wasted crop residues in excess producing areas by transporting into the feed deficit areas provided that they are properly collected and baled. Baling crop residues is not only convenient and reduces cost of transportation, but also helps to reduce the space required for storage and facilitates feed budgeting as compared to the traditional handling of crop residues in loose form (Massawe and Mruttu, 2001). Hence, possible introduction of balers and crop residues baling technologies in potential grain producing areas not only helps to tackle feed shortage via efficient utilization of crop residues, but also enables farmers to generate substantial income by selling crop residues.

The duration of feeding stored feeds to dairy cattle in the study areas is shown in Table 8. Stored hay is used to feed dairy cattle for about 8 months (range, 2-12 months) per year. The duration of hay feeding period was significantly ($p<0.05$) longer in Girar Jarso (10.5 months) followed by Sululta (8.5 months), and was shorter at Ejere (5.4 months). Most dairy farmers in Girar Jarso and Sululta use hay as a major source of basal diet to dairy cattle for 8 months or more per year. On the other hand, over 80% of the respondents in Ejere use hay for 2-7 months per year. Overall, 42.9% of the sample households feed dairy cattle with hay for 2-7 months per year, while the rest (57.1%) use hay for 8 months or more per year. Moreover, 46.1 and 25% of the respondents in Girar Jarso and Sululta, respectively, reported to feed hay for more than 10 months per year. Hay is considered as better quality roughage by the dairy farmers and is preferably fed to lactating cows, while the other groups of cattle are fed with crop residues especially in Ejere where the supply of hay is very limited as compared to the other two sites.

Table 7. Form of storage and method of storage of hay and crop residues in the study areas (% of respondents)

Feed type	Form and method of storage		Sululta (n=60)	Girar Jarso (n=39)	Ejere (n=48)	Overall (N=147)
Hay	Form of storage	Loose hay	68.3	64.1	100	77.5
		Baled hay	31.7	35.9	-	22.5
		Total	***100***	***100***	***100***	***100***
	Method of storage	Under shelter shade	38.3	25.6	20.8	29.3
		In open air	55.0	61.5	58.3	57.8
		Using plastic cover	6.7	12.8	20.8	12.9
		Total	***100***	***100***	***100***	***100***
Crop residues	Form of storage	Loose hay	94.9	100	100	97.7
		Baled hay	5.1	-	-	2.3
		Total	***100***	***100***	***100***	***100***
	Method of storage	Under shelter shade	71.2	60.0	72.3	69.5
		In open air	25.4	32.0	27.7	27.5
		Using plastic cover	3.4	8.0	-	3.0
		Total	***100***	***100***	***100***	***100***

The overall average duration of feeding crop residues to dairy cattle in the study areas was 5.8 months (range, 1 to 12 months) per year (Table 8). Crop residues were fed to dairy cattle for a longer ($p<0.05$) duration in Ejere (7.5 months) followed by Sululta (5.4 months), and the shortest at Girar Jarso (3.5 months). About 61.7% of the sample households in Ejere reported to use crop residues as a major source of basal feed to dairy cattle for 7 months or more per year. On the other hand, majority of the dairy farmers in Sululta and Girar Jarso use crop residues as basal feed to dairy cattle for 1-6 months per year. Overall, 67.2% of the total respondents in the study areas feed crop residues to dairy cattle for a period of 1-6 months, 23.7% for 7-9 months, and the rest 9.2% for more than 9 months per year. The study showed that the major share of annual basal feed supply to dairy cattle was obtained from crop residues in Ejere, and from natural pasture hay in Sululta and Girar Jarso. This implies that both technological and development interventions to improve basal livestock feed supply should mainly target crop residues in Ejere, and hay in Sululta and Girar Jarso.

As shown in Table 8, the overall average duration of feeding all stored feeds (natural pasture hay plus crop residues) to dairy cattle in the study areas was 10.4 months (range, 5-12 months) per year. Basal feed supply to dairy cattle was contributed by stored feeds the whole year in Girar Jarso (urban setting), for 10 months in Sululta, and for 9.7 months at Ejere. Dairy cattle were maintained on stored feeds for about 8-10 months per year according to 51.7% of the sample households in Sululta, 50% in Ejere and

37.4% of the total respondents. The remaining (55.1%)sample households (40% in Sululta, 100% in Girar Jarso and 37.5% in Ejere) also reported to maintain dairy cattle on stored feeds for 11-12 months per year. The findings showed that peri-urban dairy farmers in Sululta and Ejere areas maintain their cows on other feed resources mainly on grazing for about 2 months (range, 1-7 months) per year. On the other hand, in the urban production system at Girar Jarso, dairy cattle are totally confined and maintained on stored feeds the year round.

Table 8. Duration of feeding stored feeds to dairy cattle in a year (% of respondents)

Type of feed	Feeding period (months)	Sululta (n=60)	Girar Jarso (n=39)	Ejere (n=48)	Overall (N=147)
Hay	2 – 4	3.3	-	39.6	14.3
	5 – 7	33.3	2.6	43.7	28.6
	8 – 10	38.3	51.3	14.6	34.0
	>10	25.0	46.1	2.1	23.1
	Total	100	100	100	100
	Mean (months)	8.5^{b} (3-12)*	10.5^{a} (5-12)	5.4^{c} (2-12)	8.0 (2-12)
Crop residues	1 – 3	11.9	60.0	-	16.8
	4 – 6	67.8	32.0	38.3	50.4
	7 – 9	15.2	8.0	42.5	23.7
	>9	5.2	-	19.2	9.2
	Total	100	100	100	100
	Mean (months)	5.4^{b} (2-12)	3.5^{c} (1-8)	7.5^{a} (4-12)	5.8 (1-12)
All stored feed	5 – 7	8.3	-	12.5	7.5
	8 – 10	51.7	-	50.0	37.4
	11 – 12	40.0	100	37.5	55.1
	Total	100	100	100	100
	Mean (months)	10.0^{b} (6-12)	11.9^{c} (11-12)	9.7^{b} (5-12)	10.4 (5-12)

$^{a-c}$ Means with different superscripts within a row differ significantly (P<0.05)
**Figures in the brackets indicate total range in the duration of feeding period of stored feeds*

Conclusion

Natural pasture hay followed by crop residues and grazing constituted the dominant sources of basal feed used for feeding dairy cattle in the study areas. Dairy farmers produce hay on their own pasture land and/or by contracting standing hay. Very small percentage of farmers also make oats/vetch mixed hay. Different crop residues are used for feeding dairy cattle mainly in the peri-urban dairy production systems at Ejere and Sululta. About 36.7% of the farmers included in the study have adopted the production of improved fodder crops on an average land area of 0.19ha. Different agro-industrial and locally available by-products are also used to supplement dairy cattle.

Most farmers tore hay in loose form and in open air. Although baling crop residues is uncommon, about 70% of the dairy farmers store the crop residues under shelter. Dairy cattle are maintained on a basal diet of hay, crop residues and all stored feeds, respectively for 8, 5.8 and 10.4 months per annum. The study revealed that both the form and method of feed storage practiced by farmers were less efficient to ensure adequate supply of better quality basal feed to dairy cattle. Hence, frequent trainings and advisory services should be provided to farmers and development agents on efficient feed conservation and utilization. Moreover, introduction of baling machines and promotions of baling bulky feeds are essential for efficient handling and utilization of available feeds. Baling will also helps to facilitate mobilizations of seasonally excess feeds available in some areas for use in deficit areas.

Acknowledgments

This work was funded by Ethiopian Institute of Agricultural Research. We also thank the technical team (Mr. Getu Kitaw, Mr. Tadesse Bekele, Mr. Tadesse T/Tsadik, Mr.G/Medihin Hagos and Mr. Fekadu Mosisa) for their keen support in field data collection.

References

Agajie Tesfaye, Tadele Mamo, Tesfaye Solomon, Yared Deribe, Wudineh Getahun, Tolesa Alemu, Diriba Hunde, Tamirat Fikadu and Seyoum Bediye. 2016. Adoption Analysis of Smallholder Dairy Production Technologies in Oromia Region. Research Report 115, Ethiopian Institute of Agricultural Research (EIAR), 90 pp.

Agajie Tesfaye, Chilot Yirga, Mengistu Alemayehu, Elias Zerfu and Aster Yohannes. 2002. Smallholder livestock production systems and constraints in the highlands of North and West Shewa zones. In: Proceedings of 9th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 30-31, 2001. ESAP (Ethiopian Society of Animal Production) 433pp.

Azage Tegegne, Million Tadesse, Alemu Yami and Yoseph Mekasha. 2005. Market-oriented Urban and Peri-Urban Dairy Systems. In: UA Magazine 2 – Livestock in and around cities, ILRI, Addis Ababa, Ethiopia.

Azage Tegenge, Berhanu Gebremedhin and Hoekstra, D., 2006. Input supply system and services for market-oriented livestock production in Ethiopia. In: Dessie, T. (Ed.), Proceedings of the 14th Annual Conference of Ethiopian Society of Animal Production (ESAP), held in Addis Ababa, Ethiopia, pp. 1-19.

Beyene Teklu, Tegene Negesse and Ayana Angassa. 2011. Effect of farming systems on livestock feed resources and feeding systems in Benishangul-Gumuz region, western Ethiopia. International Research Journal of Agricultural Science Vol. 1(1) pp. 020-028. Available online@ http://www.interesjournals.org/IRJAS.

CSA (Central Statistical Agency). 2012. Agricultural Sample Survey Report on Area and Productivity of Major Crops. Statistical Bulletin, Addis Ababa, Ethiopia.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

EIAR. 2017. Livestock Research Strategies (2016-2030): Feeds and Nutrition, Rangelands and Animals Health, Ethiopian Institute of Agricultural Research, Addis Ababa. 239pp.

FAO (Food and Agriculture Organization of the United Nations). 1987. Master Land Use Plan, Ethiopia: Range/Livestock Consultancy. Report Prepared for the Government of the People's Democratic Republic of Ethiopia based on the work of L. Mayer, AG/ETH/82/010 Technical Report, FAO, Rome

FAOSTAT. 2015. Faostat.fao.org.

FAOSTAT (Food and Agriculture Organization Statistics), 2010. FAOSTAT Livestock primary, cow whole milk production.

Fekede Feyissa, Shiv Prasad, Getnet Assefa, Seyoum Bediye, Getu Kitaw, Aemiro Kahaliew and Gezahegne Kebede. 2014. Dynamics in nutritional characteristics of natural pasture hay as affected by harvesting stage, storage method and storage duration in the cooler tropical highlands. African Journal of Agricultural Research, Vol. 9(43), pp 3233-3244, DOI:10.5897/AJAR2014.8652. http://www.academicjournals.org/AJAR.

Fekede Feyissa, Gezahegne Kebede and Getnet Assefa. 2015. Dynamics in nutritional qualities of tef and wheat straws as affected by storage method and storage duration in the central highlands of Ethiopia. African Journal of Agricultural Research, Vol. 10(38), pp 3718-3725, DOI: 10.5897/AJAR2015.9903. http://www.academicjournals.org/AJAR.

Getnet Assefa, Abreham Gebeyehu, Fekede Fekede Feyissa and Tadesse Tekletsadik. 2002. On-farm performance of forage crops and assessment of natural pasture productivity in West Shoa Zone. In Gemechu Keneni, Yohannes Gojjam, Kiflu Bedane, Chilot Yirga and Asgelil Dibabe (eds.). 2002. Towards Farmer`s Participatory Research: Attempts and achievements in the Central Highlands of Ethiopia. Proceedings of Client-Oriented Research Evaluation Workshop, 16-18 October 2001, Holetta Agricultural Research Center, Holetta, Ethiopia. 371pp.

Irvin, S.A. 2000. An assessment of the demand for forages by smallholder farmers Wolayta Soddo, Ethiopia, and the constraints to their adoption. Masters thesis. Imperial College at Wye, London, UK. 58pp.

Kelay Belihu. 2002. Analyses of Dairy Cattle Breeding Practices in Selected Areas of Ethiopia. Ph.D. thesis, Humboldt University of Berlin, Germany.

Kossila, V.L. 1984. Location and potential feed use. In: Sundstøl, F. and Owen, E. (eds.): Straw and other fibrous by-products as feed, Elsevier, volume 14, 604pp.

Lemus, R. 2009. Hay Storage: Dry Matter Losses and Quality Changes. Mississippi State University Extension Service, USA.

LMP (Livestock Master Plan). 2014. Roadmaps for Growth and Transformation. Ministry of Agriculture, Addis Ababa, Ethiopia.

Massawe, N.F. and Mruttu, H.A. 2001. Dissemination of low-cost technology for handling crop residues and dry forages for dry-season feeding in Northern Tanzania. Research Project Report. Selian Agricultural Research Institute, Arusha, Tanzania.

McDermott J.J., Staal S.J., Freeman H.A., Herrero M. and Van de Steeg J.A., 2010. Sustaining intensification of smallholder livestock systems in the tropics. Livestock Science 130: 95–109.

SPSS, 2007. Statistical Package for Social Sciences. Version 16, Chicago, Illinois, USA.

Staal, S.J., Alejandro, N.P. and Mohammad, J., 2008. Dairy Development for the Resource Poor Part 2: Kenya and Ethiopia Dairy Development Case Studies. PPLPI working paper No. 44-2, 26-44.

Tessema Zewdu, Aklilu Agidie and Ameha Sebsibe. 2003. Assessment of the Livestock Production System, Available Feed Resources and Marketing Situation in Belesa Woreda: A Case Study in Drought Prone Areas of Amhara Region. Proceedings of the 10th annual conference of the Ethiopian society of animal production, Addis Ababa, Ethiopia, August 22-24, 2002, 407pp.

Yeshitila Admasu. 2008. Assessment of livestock feed resources utilization in Alaba woreda, southern Ethiopia. M.Sc thesis, School of Graduate Studies, Haramaya University, Ethiopia.

Yitaye Alemayehu. 2008. Characterization and analysis of the urban and peri-urban dairy production systems in the North western Ethiopian highlands. Ph.D. thesis, University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Yoseph Mekasha, Azage Tegegne, Alemu Yami and Ummuna, N.N. 2003. Variations in nutrient intake of dairy cows and feed balance in urban and peri-urban dairy production systems in Ethiopia. Proceedings of the 10th annual conference of the Ethiopian society of animal production, Addis Ababa, Ethiopia, August 22-24, 2002, 407pp.

Zewdie Wondatir. 2010. Livestock production systems in relation with feed availability in the highlands and central rift valley of Ethiopia. M.Sc Thesis, Haramaya Univesity, Ethiopia.

Guidelines for Authors

General

The Ethiopian Journal of Animal Production (EJAP) publishes original articles of high scientific standard dealing with livestock and livestock related issues. Reviews on selected topics on livestock research and development appropriate to Ethiopia and other similar countries in the tropics and subtropics will also be considered for publication. Short communication and technical notes are also welcome.

Manuscripts should be written in English. Authors are advised to strictly stick to the format of the journal. Submit an electronic form of the manuscript in Word format using Times New Roman font. Use 13 point font size for titles and 11 point font size for the text with line spacing of 1.15.. Manuscripts submitted to the Editorial Office will be duly acknowledged. All articles will be sent to at least two reviewers (within or outside the country) selected by the Editorial Board and will be reviewed for relevance to the journal, scientific value and technicality. Rejected papers will be returned to the author(s) immediately. Accepted papers will be returned to the author with the comments of the reviewer(s) for further improvement of the manuscript. EJAP has no page charge.

Proofs will be sent to the author. Typeset proofs are not checked for errors. Thus, it is the responsibility of the primary author of each paper to review page proofs carefully for accuracy of citations, formulae, etc. and to check for omissions in the text. It is imperative that the authors do a prompt, thorough job of reviewing the returned proofs to ensure timely publication. Authors are instructed to return the proofs to the Editorial Office within three (three) days of receipt. Senior or corresponding authors will be provided with electronic copy of the published article to be shared with the co-authors.

Format for Manuscripts

Research paper should be as concise as possible and should not exceed 6000 words or about 10 to 12 pages including illustrations and tables. Papers should be partitioned into sections including abstract, introduction, materials and methods, results, discussion, acknowledgements and references. Main text headings should be centered and typed in capitals. Sub-headings are typed in capitals and small letters starting from left hand margin.

Headings: Title of the paper should be in upper and lower case. Main headings should be in upper and lower case, left justified.

Sub-headings: First sub-headings, flush left, separate line, capitalize main words; second sub-headings- flush left, same line as text, capitalize first word, followed by period; third sub-heading – flush left, same line as text, capitalize first word, italics followed by a dash.

Title: The title should be concise, specific and descriptive enough to contain key words or phrases including the contents of the article. A short running title of less than 50 characters should also be suggested.

Author and institution: The name(s) of author(s) and the institution(s) with which they are affiliated, along with the addresses, should be provided. Corresponding author should be identified in case of more than one author.

Abstract: Research or applied articles should have an abstract of no more than 300 words. The abstract should state concisely the goals, methods, principal results and major conclusions of the paper. Incomplete and uninformative descriptions should not be used. The use of acronyms is discouraged. Keywords of up to five words should be included.

Introduction: This part should be brief and limited to the statement of the problem or the aim of the experiment, justification and a review of the literature pertinent to the problem.

Materials and methods: The techniques and procedures of the research, the conditions under which the study was conducted and the experimental design are described under this heading. Relevant details about the animal should be given and the statistical design should be described briefly and clearly. Data should be analyzed and summarized by appropriate statistical methods; authors should examine closely their use of multiple comparison procedures. A measure of variability, e.g., standard deviation or standard error must be provided when reporting quantitative data. If standard methods of investigation and analysis are employed appropriate citation suffice.

Results: The summary of major findings and assessments of the investigation are given in this section. The results can be presented using tables, illustrations and diagrams.

Tables: Tables are numbered consecutively in arabic numerals (e.g., Table 1) and should bear a short, yet adequately descriptive caption. Avoid using vertical and/or horizontal grid lines to separate columns and/or rows. Metric units are clearly to be shown, abbreviated in accordance with international procedure. Footnotes to tables are designated by lower case which appear as superscripts in appropriate entries. Tables should be compatible with column width viz. 140 mm, and should be presented on separate sheets, and grouped together at the end of the manuscript. Their appropriate position in the text should be indicated and all tables should be referenced to in the text.

Illustrations and diagrams: These should be inserted into the text using any suitable graphics programmes. Freehand or typewritten lettering and lines are not acceptable. Authors are requested to pay attention to the proportions of the illustrations so that they can be accommodated in the paper without wastage of space.

Figures: Figures should be restricted to the display of results where a large number of values are presented and interpretation would be more difficult in a Table. Figures may not reproduce the same data as Tables. Originals of figures should preferably be A4 size, of good quality, drawn or produced on good quality printer and saved in a separate file. There should be no numbering or lettering on the originals. Numbering and lettering, which must be kept to an absolute minimum, should be legibly inserted on the copies. Vertical axes should be labelled vertically. A full legend, describing the figure and giving a key to all the symbols on it, should be typed on a separate sheet. The symbols preferred are: ▲,■ ○ ■, but + and x signs should be avoided. Figures should be numbered consecutively in arabic numerals (e.g., Figure 1), and refer to all figures in the text.

Photographs: Should be original prints and suitable for reproduction. They should be unmounted with lettering clearly indicated on overlays or photocopies. For composites, photographs should be unmounted and a photocopy enclosed to indicate the required measurement. Magnification should be given in the legend or indicated by a scale or bar. They should be numbered as part of the sequence of Figures. If several plates or coloured photographs are submitted, the authors may be asked to the cost of reproducing them.

Discussion: The reliability of evidence (result), comparison with already recorded observations and the possible practical implication is discussed.

Conclusion: Authors are encouraged to forward conclusion (two to three brief statements) from the study summarising the main findings and indicating the practical implications of the findings.

Acknowledgements: Should be briefly stated following the conclusion.

References: Cite references by name and date. The abbreviation et al should be used in the text where more than two authors are quoted. Personal communications and unpublished work should be cited in the text only, giving the initials, name and date. They should not appear in the list of references. All references should be listed alphabetically. References should be selected based on their relevance and the numbers should be kept to a minimum. Journal names should be abbreviated according to the World list of Scientific Periodicals.

Examples

Journal article:

Zerbini, E., Gemeda, D., Tegegne, A., Gebrewold, A. and Franceschini, R. 1993. The effects of work and nutritional supplementation on postpartum reproductive activities and progesterone secretion in F1 crossbred dairy cows in Ethiopia. Theriogenology 40(3):571-584.

Crosse, S., Umunna, N.N., Osuji, P.O., Tegegne, A., Khalili, H. and Tedla, A.. 1998. Comparative yield and nutritive value of forages from two cereal-legume based cropping systems: 2. Milk production and reproductive performance of crossbred (Bos taurus x Bos indicus) cows. Tropical Agriculture 75 (4):415-421.

Article by DOI

Negewo, T., Melaku, S., Asmare, B. and Tolera, T. 2018. Performance of Arsi-Bale sheep fed urea treated maize cob as basal dietand supplemented with graded levels of concentrate mixture. *Tropical Animal Health and Production*. https://doi.org/10.1007/s11250-018-1544-4

Book

Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York.

Chapter in a Book

Zerbini, E., Gemeda, T., Gebre Wold, A. and Tegegne, A. 1995. Effect of draught work on the metabolism and reproduction of dairy cows. In: Philips, C.J.C. (ed.), Progress in Dairy Science. Chapter 8. CAB International. pp. 145-168.

Paper in Proceedings

Gebre Wold, A., Alemayhu, M., Tegegne, A., Zerbini,E. and Larsen, C. 1998. On-farm performance of crossbred cows used as dairy-draught in Holetta area. Proceedings of the 6th National Conference of the Ethiopian Society of Animal Production (ESAP), May 14-15, 1998, Addis Ababa, Ethiopia, pp. 232-240.

Thesis/Dissertation

Trent, J.W. 1975. Experimental acute renal failure. Dissertation, University of California

Online document

Tekle, D., Gebru, G. and Redae, M. 2018. Growth performance of Abergelle goats fed grass hay supplemented with pigeon pea (*Cajanus cajun* (L.) Millsp) leaves. *Livestock Research for Rural Development. Volume 30, Article #149.* Retrieved August 2, 2018, from http://www.lrrd.org/lrrd30/8/desta30149.html

Cartwright, J. 2007. Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Abbreviations

Follow standard procedures.

Units

All measurements should be reported in SI units. (e.g., g, kg, m, cm)

Table 1. The following are examples of SI units for use in *EJAP*

Quantity	Application	Unit	Symbol or expression of unit
Absorption	Balance trials	Grams per day	g d^{-1}
Activity	Enzyme	Micromoles per minute per gram	µmol min^{-1} g^{-1}
Area	Land	Hectare	ha
	Carcass	Square centimetre	cm^2
Backfat	Carcass	Millimetres	mm
Concentration	Diet	Percent	%
	Blood	Gram per kilogram	g kg^{-1}
		International unites per kilogram	IU kg^{-1}
			mg dL-^{1}
		Milligram per 100 mL	Mequiv L^{-1}
		Milliequivalents per litre	
Density	Feeds	Kilogram per hectolitre	kg hL^{-1}
Flow	Digesta	Grams per day	g d^{-1}
	Blood	Milligrams per minute	mg min^{-1}
Growth rate	Animal	Kilogram per day	kg d^{-1}
		Grams per day	g d^{-1}
Intake	Animal	Kilograms per day	Kg d^{-1}
		Grams per day	g d^{-1}
		Grams per day per kg bodyweight0.75	g d^{-1} $kg^{-0.75}$
Metabolic rate	Animal	Megajoules per day	MJ d^{-1}
		Watts per kg bodyweight	W kg^{-1}
Pressure	Atmosphere	Kilopascal	KPa
Temperature	Animal	Kelvin or degree Celsius	K or ^{o}C
Volume	Solutions	Litre	L
		Millilitre	ML
Yield	Milk production	Litres per day	L d^{-1}
Radioactivity	Metabolism	Curie or Becquerel	Ci (=37 GBq)

Units with two divisors should be written with negative indices (e.g., kg ha^{-1} yr^{-1}). The use of solidus (/) should be reserved for units written in full (e.g., mole/kilogram) or to separate a physical quantity and unit (e.g., yield/ha). Units should be chosen so that the numeric component falls between 1 and 10 or 1 and 100 when using one or two significant figures, respectively (e.g., use 31.2 mg than 0.0312 g).

Membership to the Ethiopian Society of Animal Production (ESAP)

Membership advantages

Some of the personal benefits afforded to active members of the Ethiopian Society of Animal Production (ESAP) include the following:

- A convenient means of keeping up-to-date on current scientific and production developments;
- An avenue for personal involvement in fostering high standards and professional developments in Animal Science;
- To receive a printed copy of the Ethiopian Journal of Animal Production (EJAP);
- Receiving copies of the Society's newsletter, Membership Directory, and advanced registration information for national meetings;
- Eligibility to present abstracts at national meetings and to submit manuscripts for publication in the Ethiopian Journal of Animal Production (EJAP);
- Eligibility to provide personal leadership to the field of animal science by serving on the Executive Committee of the society or by accepting other society assignments; and
- Eligibility to be selected for prestigious society-sponsored awards

Eligibility for membership

Membership is open to individuals interested in research, instruction or extension in Animal Science or associated with the production, processing, marketing and distribution of livestock and livestock products.

Application form for Membership

Application form for Membership

Ethiopian Society of Animal Production (ESAP)

Name ________________ ________________ ________________

First Middle Last

Mailing Address: ________________________________

Current Employment: ________________________________

Company/Institution: ________________________________

Phone: ________________________________

Fax: ________________________________

E-mail: ________________________________

Type:

- ☐ Professional
- ☐ Student

Other__________ Specify:____________________

Signature: ________________ Date:________________

Bank Account: Commercial Bank of Ethiopia
Andinet Branch
Account Number 0171810076800
Addis Ababa, Ethiopia

Manuscript Submission Address

Manuscripts can be submitted by email addressed to The Editorial Office of the Ethiopian Journal of Animal Production (EJAP) at esapeth@yahoo.com or can be directly submitted to the Editor-in-Chief at adugnatolera2@gmail.com. The main address of EJAP is as follows.

The Editorial Office
Ethiopian Journal of Animal Production (EJAP)
C/o Ethiopian Society of Animal Production (ESAP)
P.O.Box 62863
Addis Ababa
Ethiopia
Tel: (+251-1) 15547498
Email: esapeth@yahoo.com

Subscription and Communications

Information to Subscribers

Subscription rates for one year (one issue), including airmail, are as follows:

	Local	Foreign
Institution:	50 (Birr)	25 (US$)
Individuals:	25 (Birr)	10 (US$)

All business correspondences about subscriptions, back issues, single copies, change of address and claims for missing issues should be sent to:

The Editor-in-Chief
EJAP Editorial Office
C/o ESAP Office
P.O.Box 62863
Addis Ababa; Ethiopia;
Tel: (+251-1) 15547498