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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.

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Genetic Distance and Differentiation among Cattle Breeds in Ethiopia: A Review

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ABSTRACT

Studies conducted on genetic diversity and population differentiation of Ethiopian cattle breeds have been reviewed. Out of 22-28 phenotypically and geographically recognized cattle breeds in Ethiopia about 17 breeds were characterized at molecular level. The population differentiation indicated that Ethiopian breeds significantly differ from taurine referenced Hanwoo and Friesian breeds. They exhibit lower observed heterozygosity than Hanwoo and Friesian breeds. Most studies in Ethiopia witnessed low between breeds and high within breed variations. The genetic distance among Ethiopian breeds did not necessarily depend on geographic distance. Zero genetic distance was estimated between Boran and Horro and between Abigar and Sheko populations based on mitochondrial cytochrome b gene analysis. From Ethiopian breeds, better genetic diversity was recorded in Abergelle (0.795) than Bagait (0.590), Afar (0.559), Raya (0.582), Arado (0.636), Fogera (0.541) and Irob (0.527) breeds. Similarly higher heterozygosity was found in Horro breed (0.387) followed by Ambo (0.386), Arsi (0.376), Boran (0.374) and Danakil (0.363). The low genetic diversity and heterozygosis observed in some populations could reflect inbreeding due to uncontrolled mating. Generally the high within breed variation observed in Ethiopia cattle population could create favorable condition for further improvement through selection. In addition, the previous studies might indicate higher admixture among Ethiopian cattle populations and absence of artificial selection pressure. The study conducted at molecular level so far were fragmented and not exhaustively included all breeds that were classified by phenotype or geography. Thus it is essential to carry out further studies which encompass all breeds in order to have full picture of breed diversity and differentiation among Ethiopian breeds. Furthermore, it will have an immense impact if the studies started on molecular characterization advanced to the level of utilizing genomic information for genetic improvement, resource conservation and disease resistance.

Keywords: Cattle, Heterozygosity, Genetic diversity

INTRODUCTION

Ethiopia has huge cattle population with an estimated 60.39 million heads (CSA, 2017). Of these the indigenous cattle population accounted for 98.5% of the national cattle population. The cattle production has been noticeably contributed for family nutrition, income generation, social values and overall livelihood improvement for several million farmers and pastoralists. According to Land O'lakes (2010), 83% of milk produced in Ethiopia comes from cattle. In pastoral area milk consumed by pastoral children accounted for 67% of the mean daily energy they require and 100% of their protein requirements during the wet season (Sadler and Catley, 2009). Furthermore, it was noted that adopter of crossbred cows technology generated 44% more income than non-adopters in some milk shed area of Ethiopia (Agajie Tesfaye *et al*., 2016).

The country is believed to have diverse cattle genetic resources distributed in different part of the country (Rege, 1999; EBI, 2016; Abreham Assefa and Abebe Hailu, 2018). It is indispensable to understand and document the diversity, genetic relationship and admixture of the available breeds for future genetic improvement, conservation and utilization of genetic resources. Most of breed classification and characterization in Ethiopia were conducted based on morphological features, historical evidence and geographical habitation. However, the animal movement, environmental factors and

admixture (exchange of genetic material) could limit the accuracy of phenotypic characteristics (Hanotte and Jianlin, 2005).

Application of genomic information is more advanced method and being practiced for evolutionary study, disease identification, genetic diversity, genetic conservation, genetic improvement and forensic studies (Kotze et al., 2000; Carlos et al., 2002; Shi et al., 2010; Gubta et al., 2015). The genomic evaluation methods have created favorable condition to study the genetic relationship between breeds and classification as compared to conventional method**.** Furthermore, the genetic improvement through progeny testing and application of best linear unbiased prediction (BLUP) has been significantly supported by molecular technique as it improves the accuracy of estimation of breeding value and genetic progress. Different studies were conducted on Ethiopian cattle population using molecular techniques. Having information on past research works in this regard will help to exploit the available potential and identify the gap for further studies. Hence, the aim of this paper was to review the genetic diversity and relationship among Ethiopian cattle breeds characterized at molecular level and suggest future research points for genetic improvement programs of cattle.

METHODOLOGY

All available published papers on genetic distance and differentiation among cattle populations in Ethiopia were used for this review. A total of 35 papers published from 1999 to 2018 were considered. Rege (1999) and EBI (2016) were used as references for population (breed) classification and then to identify population that was not studied at molecular level so far in Ethiopia. Parameters included in the reviewed papers are proportion of taurine alleles, expected and observed heterozygosity, genetic distance and population differentiation. Genetic markers used, locations studied, breeds considered and results of different studies were compared and discussed.

FINDINGS

Molecular markers used for genetic analysis

The most widely used markers for breed characterization in Ethiopia are single-nucleotide polymorphism (SNP), microsatellites, and dam and sire line markers (mtDNA and Y-chromosome). A single-nucleotide polymorphism (SNP) is a variation in a single nucleotide that occurs at a specific position of DNA sequence. Currently, analyses of SNP markers are used for diversity analysis and genome-wide studies. This is attributed to abundance in the genome, dispersed equally throughout the genome, genetically stable, and suitable to automated analysis to measure genetic variations found within and between populations (McKay et al., 2008; Lin et al., 2010; Zewdu Edea et al., 2012). Several authors used SNP markers to characterize different species and breeds in East African countries. For instance genetic diversity of 6 cattle populations in Ethiopia (Zewdu Edea et al., 2012), 3 local strain of Tanzanian Zebu, Boran and Friesian (Msalya et al., 2017) and 6 Ethiopian sheep population (Zewdu Edea et al., 2017) were conducted using SNP markers.

Microsatellites have been commonly used as a marker for DNA profiling in cancer diagnosis, kinship analysis and forensic identification over the past years. Similarly, it has been widely used to study the genetic diversity and other characterization of livestock species. They are highly informative, co-

dominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species (Mason, 2015). According to Vieira et al (2016) and Mburu and Hanotte (2005), it is a short sequence of 1 to 5 bp or more nucleotides which are randomly repeated. Microsatellites occur at thousands of locations within an organism's genome (Moniruzzaman et al., 2015; Ambreen et al, 2015, Anand et al, 2017).

Microsatellites markers were used for genetic analysis of cattle breeds in Ethiopia (Hailu Dadi et al., 2008), cattle breeds in East Africa (Adhiambo, 2002), Sheep breeds in China (Chen et al., 2016), Cattle breeds in Asia (Shi et al., 2010) and domestic cattle in African, Europe and Asia (Freeman et al., 2005). On the other hand Hanotte et al. (2000) studied the geographical distribution and the frequency of an indicine and a taurine y specific allele amongst African cattle breeds using Y chromosomes as it is transmitted paternally. In addition Li et al. (2007) explore the paternal gene pool and the mechanisms behind the genetic structure of 6 North Ethiopia breeds.

Mitochondrial DNA (mtDNA) is essential tool to study evolution and population genetics. The mode of maternal transmission of mtDNA makes uniform population of mtDNA transmitted through the female germ line from one generation to the next (Srirattana and John, 2017). As a result, mtDNA has been used as a marker for phylogenetic studies since control region of mtDNA has potential for high mutation rate, lack of recombination and maternal inheritance. Different studies were undertaken using mtDNA for phylogenetic analysis. For instance, Getinet Mekuriaw et al. (2018) and Hailu Dadi et al. (2009) studied the maternal ancestry and diversity of Ethiopian cattle population and Kim et al (2013) has also estimated genetic diversity and phylogenetic status for the Korean Chikso breed.

Proportion of taurine alleles in Ethiopian breeds

According to Rege (1999), there were 22 phenotypically characterized cattle breeds/populations in Ethiopia. However, this figure was escalated to 28 in the report of EBI (2016). Of these, about 17 cattle breeds were characterized at molecular level (Table 1). The group of breeds characterized and compared at molecular level at a time consist of ten breeds by Hanotte et al. (2000), three by Adhiambo, (2002), five by Fedlu Hassen et al. (2007), seven by Li et al. (2007), ten by Hailu Dadi et al. (2008), five by Zewdu Edea et al. (2013) and five by Getinet Mekuriaw et al. (2018).

Hanotte et al. (2000) had detected small proportion of the taurine alleles in Y chromosome of Sheko (10%), Abigar (7%) and Arsi breed (6%) of Ethiopia (Table 1). Several literatures classify Sheko breed into taurine type based on phenotypic characterization. The lower proportion of Y chromosome (10%) detected in Sheko breed may be justified in two ways. The first is, originally this breed could be a crossbred of taurine with zebu or sanga, and the other is, the indigenous taurine Y chromosome has been nearly eliminated from Sheko breed due to long term crossbreeding with zebu or sanga. Even though the cause of detection of taurine alleles in Y chromosome of Abigar and Arsi need further investigation, it could be related with sampling of individuals from the area where crossbreeding conducted or these breeds may have remnant of taurine alleles. Furthermore, studies on mtDNA revealed that there was no haplotype observed in Ethiopian cattle to cluster with the reference *Bos indicus* group (Hailu Dadi et al., 2008; Getinet Mekuriaw et al., 2018). The authors suggested that the zebu mtDNA of zebu cattle could be lost due to recurrent disease and rinderpest epidemics as mtDNA is more sensitive than nuclear genes to demographic processes such as bottleneck and fragmentation of population.

Table 1. List of genetically characterized cattle breed of Ethiopia

No.	Breed name	Breed group	Location	Proportion of Y allele % *		References (studied by)
				indicine	taurine	
1	Boran	Zebu	South Ethiopia	100	0	Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Zewdu Edea et al. (2012); Zewdu Edea et al. (2013); Hanotte et al. (2000); Getinet Mekuriaw et al. (2018)
2	Arsi	Zebu	Central highlands of Ethiopia	94	6	Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Zewdu Edea et al. (2012); Zewdu Edea et al. (2013); Hanotte et al. (2000); Fedlu Hassen et al. (2007)
3	Ambo	Zebu	Central highlands of Ethiopia			Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Zewdu Edea et al. (2012); Zewdu Edea et al. (2013)
4	Danakil	Sanga	East Ethiopia	100	0	Zewdu Edea et al. (2012); Zewdu Edea et al. (2013); Hanotte et al. (2000); Adhiambo (2002); Li et al. (2007); Hailu Dadi et al. (2008); Hailu Dadi et al. (2009)
5	Horro	Zenga	West Ethiopia	100	0	Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Zewdu Edea et al. (2012); Zewdu Edea et al. (2013); Hanotte et al. (2000): Fedlu Hassen et al. (2007); Getinet Mekuriaw et al. (2018)
6	Bale	Zebu	South East Ethiopia	100	0	Hanotte et al. (2000)
7	Fogera	Zenga	North Ethiopia	100	0	Hanotte et al. (2000); Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Li et al. (2007)
8	Abigar	Sanga	West Ethiopia	93	7	Hanotte et al. (2000); Adhiambo (2002); Fedlu Hassen et al. (2007); Getinet Mekuriaw et al. (2018)
9	Raya-Azebo	Sanga	North Ethiopia	100	0	Hanotte et al. (2000); Li et al. (2007); Hailu Dadi et al. (2008); Hailu Dadi et al. (2009)
10	Sheko	Taurine	West Ethiopia	90	10	Hanotte et al. (2000); Adhiambo (2002); Fedlu Hassen et al. (2007); Hailu Dadi et al. (2008); Hailu Dadi et al. (2009); Getinet Mekuriaw et al. (2018)
11	Ogaden	Zebu	East Ethiopia	100	0	Hanotte et al. (2000); Hailu Dadi et al. (2008)
12	Begait	Zebu	North Ethiopia			Li et al. (2007)
13	Arado	Zenga	North Ethiopia			Li et al. (2007)
14	Abergelle	Zenga	North Ethiopia			Li et al. (2007)
15	Irob	Zenga	North Ethiopia			Li et al. (2007)
16	Guraghe	Zebu	Central highlands			Fedlu Hassen et al. (2007); Getinet Mekuriaw et al. (2018)
17	Adwa	Zebu	North Ethiopia			Hailu Dadi et al. (2008); Hailu Dadi et al. (2009)

*(Proportion of Y allele %, Hanote et al., 2000)

Genetic diversity and differentiation of Ethiopian cattle populations

Genetic diversity is usually described by level of allelic polymorphism, expected and observed heterozygosity. Zewdu Edea et al. (2013) found that the Ethiopian cattle breeds (Horro, Danakil, Boran, Arsi and Ambo) were less polymorphic (mean MAF ≥ 0.05 = 83.96 %) than Hanwoo breed (95.21 %) which was considered as a reference of taurine breeds. Similarly the Hanwoo breed exhibit higher observed heterozygosity (0.410) as compared to Danakil (0.363), Horro (0.387), Borana (0.374), Arsi (0.376) and Ambo (0.385). However, the values estimated for observed heterozygosity by Zewdu Edea et al. (2013) are lower than that of Hailu Dadi et al. (2008) who found 7.03, 7.30, 7.47, 7.07, 7.17 and 7.30 for the same breeds, respectively. The difference could be attributed to molecular markers used, type of laboratory procedures followed, sample size and area coverage of each sample collected. On the other hand, about 10-20 percent of the loci from 10 studied cattle populations (Hailu Dadi et al., 2008) and 6 percent SNPs markers from 5 cattle breeds (Zewdu Edea et al., 2013) were significantly deviated from HWE.

Li et al (2007) noted that Y-chromosome haplotype diversity was generally low in north Ethiopian cattle (0.527–0.636) than that of European Holstein-Friesian (0.645) except Abergelle breeds (0.795) which show higher diversity than the other 6 breeds. This is in line with the finding of Hailu Dadi et al (2009) and Getinet Mekuriaw et al. (2018) who noted 100 and 95.45 % genetic variance accounted by within population variation respectively based on mtDNA analysis. Most studies in Ethiopia witnessed low between breeds and high within breed variations (Fedlu Hassen et al., 2007; Li et al., 2007; Hailu Dadi et al., 2008; Zewdu Edea et al., 2013). This might also show that most cattle populations in Ethiopia have common source of origin. The lower heterozygosity (diversity) in Ethiopian breeds than taurine counterparts could be attributed to higher inbreeding (due to uncontrolled mating) and recurrent drought which causes loss in genetic diversity.

Among Ethiopian cattle populations' better genetic diversity was recorded in Abergelle breed (0.795) than Bagait (0.590), Afar (0.559), Raya (0.582), Arado (0.636), Fogera (0.541) and Irob (0.527) (Li et al., 2007). Similarly Zewdu Edea et al. (2013) estimated higher heterozygosity for Horro breed (0.387) followed by Ambo (0.386), Arsi (0.376), Boran (0.374) and Danakil (0.363). Hailu Dadi et al (2008) estimated greater observed heterozygosity in Ambo (0.700), Horro (0.692) and Fogera (0.691) than other seven breeds (Boran, Arsi, Adwa, Ogaden,Raya, Danakil,and Sheko).

The analysis of population differentiation between Ethiopian and Hanwoo and with Friesian breeds revealed significant variation at molecular level (Adhiambo, 2002; Li et al., 2007; Hailu Dadi et al., 2008; Zewdu Edea et al., 2013). Fedlu Hassen et al. (2007) clustered Sheko differently from other 4 breeds (Horro, Sheko, Arsi, Abigar and Guraghe highland) and found the smallest genetic divergence between Guraghe highland and Abigar followed by between Guraghe highland and Arsi. Based on this, the authors pointed out that the genetic distance did not necessarily depend on geographic distance as Guraghe highland is more close to Arsi than Abigar breed geographically. Furthermore, zero genetic distance was estimated between Boran and Horro and between Abigar and Sheko populations based on mitochondrial cytochrome b gene analysis (Getinet Mekuriaw et al., 2018).

The pair-wise population differentiation (FST) and Reynolds' genetic distance analysis conducted by Zewdu Edea et al. (2013) also indicated lower genetic distance among Horro, Ambo and Arsi breeds than with Danakil and Boran populations (Figure1).

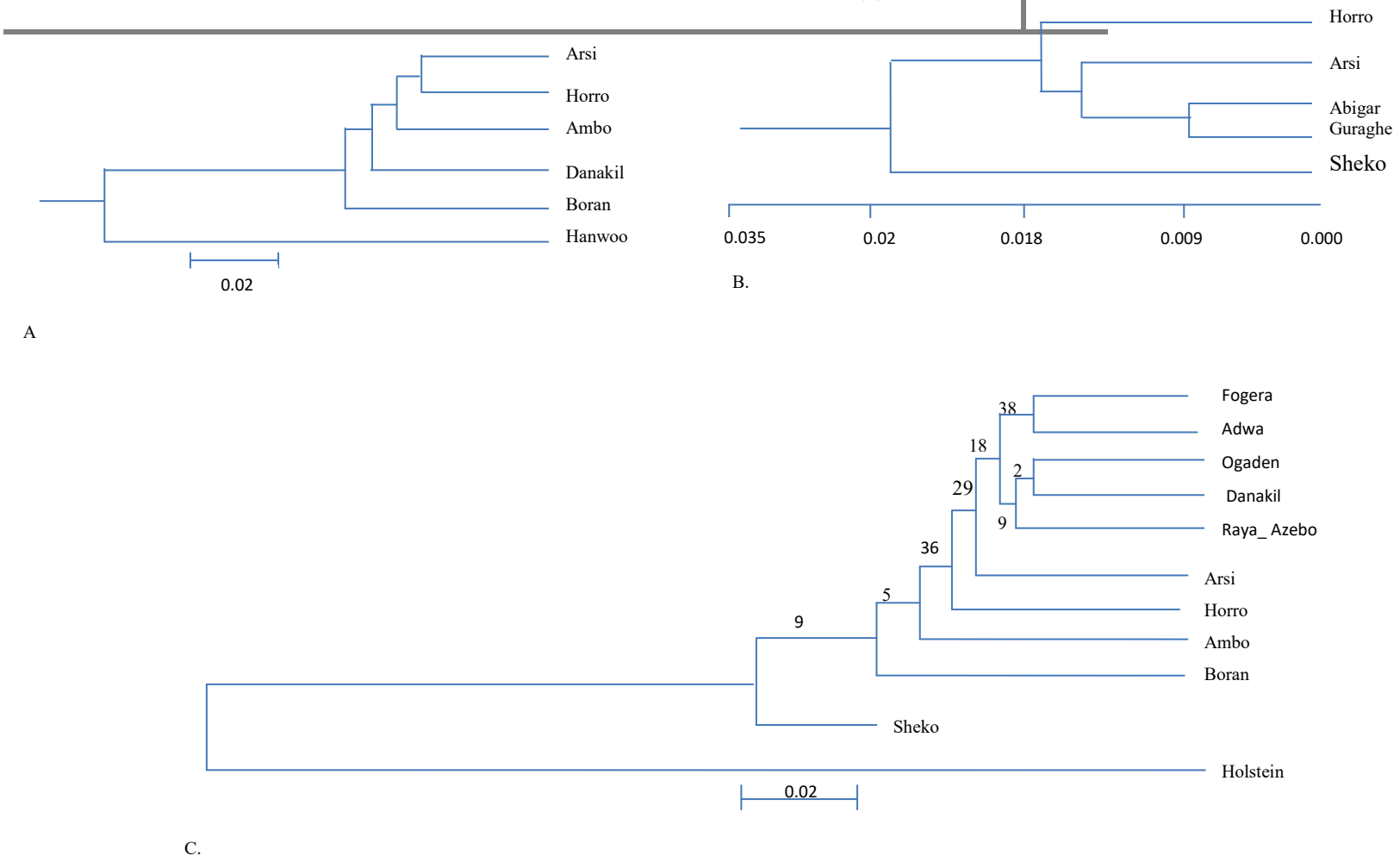

Figure 1. A. Phylogenict tree showing the genetic relationships among the six cattle populations using Reynold setal.(1983) genetic distance. Source Edea et al. (2013); B. Dendrogram generated by the UPGMA method for Nei (1978) genetic distance using NTSYS program. Source: Hassen et al (2007); C. Neighbour-joining dendrogram summarizing genetic relationships among 11 cattle populations using DA genetic distances based on 30 microsatellite loci. The numbers on the nodes indicate the percentage bootstrap values generated from 1000 replications. Source: Dadi et al (2008)

They clustered Arsi and Horro as one sub-cluster and Borana separately while Danakil and Ambo were intermediately positioned between Arsi and Horro sub-cluster and the Boran. Even though, Horro and Ambo have more geographical linkage, the Reynolds' genetic distance estimation placed Horro more related to Arsi than Ambo. Similarly Hailu Dadi et al. (2008) found lower genetic distance between Arsi and Ambo than other 8 breeds characterized and grouped, while Sheko breed clustered differently from other Ethiopian breeds.

CONCLUSION

The high within breed variation observed in Ethiopian cattle populations could create favorable condition for further improvement through selection. In addition, literatures might indicate the presence of higher admixture among cattle populations and absence of artificial selection pressure. However, the studies conducted so far at molecular level were fragmented and did not exhaustively include all breeds that were classified by phenotypic features and geographic location. Some of the populations like Mursi, Hammer, Gofa, Kereyu and other indigenous breeds/populations were not characterized at molecular level. Thus it is essential to carry out further studies which encompass all breeds in order to have full picture of breed diversity and differentiation among Ethiopian breeds. Furthermore, it will have an immense impact if the studies started on genetic diversity could be advanced to the level of utilizing genomic information for genetic improvement, genetic resource conservation, disease resistance and other economically important traits.

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Risk Factors and Possible Strategies to Mitigate Microbiological Hazards in Milk and Dairy Products in Ethiopia: A Review

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ABSTRACT

The quality and safety of milk and dairy products are global concerns, particularly in developing countries like Ethiopia. Poor animal health and unhygienic production environments often contribute to on-farm contamination with microbiological hazards throughout the milk value-chain. Sources of contamination include milk handling equipment with unsanitary design, improper milk storage conditions, and unhygienic milk transportation. Moreover, lack of knowledge and skills for hygienic production and processing of milk and dairy products are the major concerns for the dairy industry in the country. All of these challenges contribute to microbial contamination of milk and dairy products, which increases the risk of foodborne diseases. To protect the public health, improving the safety of milk and dairy products should be prioritized through interventions targeting improvements in hygienic and sanitary production practices.

Key words: Milk, Ethiopia, contamination, hygiene, prevention

INTRODUCTION

Milk is known for its high nutritional value and has been increasingly included in human diets (FAO, 2013; Mwambete & Nakembetwa, 2015; Walstra *et al.*, 2006). However, the nutritional content of milk, together with its high water activity (aw), provides suitable conditions for growth of a multitude of spoilage and pathogenic microorganisms (FAO, 2013; Paraffin *et al.*, 2018; Velázquez-Ordoñez *et al.*, 2019). Additionally, milk produced in unhygienic environments using methods that do not follow the principles of good hygienic practices is conducive to microbial contamination (Paraffin *et al.*, 2018). This may increase the exposure of consumers to foodborne pathogens, resulting in foodborne infections. Exposure to foodborne pathogens through consumption of contaminated milk and dairy products is a global problem, which is exacerbated in developing countries (Ahmedsham *et al.*, 2018; EL-Ziney & AL-Turki, 2007). It needs to be mitigated by improving the management of environmental and personal hygiene in the dairy supply chain. The first step towards mitigation includes effective educational interventions (Ahmedsham *et al.*, 2018; Kebede *et al.*, 2019; Yodit *et al.*, 2017; Velázquez-Ordoñez *et al.*, 2019).

Milk collected from healthy cows typically has low microbial load and deemed free of pathogenic microbial contamination (FAO, 2013; SNV, 2017). Microbial contaminants are most commonly introduced into milk during the milking practice and/or at subsequent milk processing steps (Fufa *et al.*, 2019; Asaminew & Eyassu, 2011). For example, the farm environment such as dirty udder exteriors, feces, bedding, and soil in the milking environment and contaminated surfaces of milk handling equipment and utensils (unsanitary design and insufficient cleaning) contribute heavily to contamination during milking (Amanuel & Ulfina, 2018; Hayes *et al.*, 2001; Makovec & Ruegg, 2003; McKinnon & Bramley, 1990; Oladipo *et al.*, 2016; Amanuel & Haftom, 2016; Velázquez-Ordoñez *et al.*, 2019). These problems are seeable in countries like Ethiopia where there are a number of challenges in acquiring appropriate milk handling equipment and limited access to clean water (Tadele*, et al.*, 2016; Solomon *et al.*, 2013; Weldegiorgis & Gebremariam, 2019). This compounding effect deteriorates the quality, safety, and quantity of milk produced in the country, ultimately jeopardizing food security, public health, and agriculture development (Fekadu, 1995; SNV, 2008).

The objective of this paper is to provide an overview of the potential sources of microbial contamination of milk and dairy products in Ethiopian dairy supply chain and to provide information on promising mitigation procedures through a review of previously published peer-reviewed literature. Moreover, the information provided in this paper can be used to inform future interventions areas in the dairy value chain of Ethiopia.

METHODOLOGY

This paper was prepared through a comprehensive literature review by searching scientific literature databases, including Semantic Scholar, African journals online, PubMed, Directory of Open Access Journals, Europe PMC, and Science Direct. Peer-reviewed studies reporting hygienic practices and microbial quality of milk and dairy products in Ethiopia were identified and reviewed. Additionally, articles that reported risk factors associated with microbial contamination of milk and dairy products and mitigation procedures were included in the review. Between January 2020 and June 2020 six (6) databases were searched using the keywords “Ethiopia” AND (“dairy” OR “milk”) AND (“risk factors” OR “microbiological contamination” OR “prevention” OR “mitigation”) AND/OR “quality/safety”. The results of each search were filtered based on the relevance of the title and abstract. Relevant papers were also reviewed to further identify relevant literature, which was included in this review paper.

FINDINGS

Microbial contamination of milk and dairy products can originate from various sources (Oumer *et al.*, 2017). Therefore, source attribution can be challenging and difficult to determine. However, there are several risk factors that, when examined, can provide insight into the root of the microbial contamination of milk and dairy products **(Figure 1).** The factors include animal health, farm management and environmental factor, milking and milk handling practice, milk handling equipment and sanitary practices, milk storage and transport, and water source. The following discussion provides further detail on these factors and possible mitigation procedures.

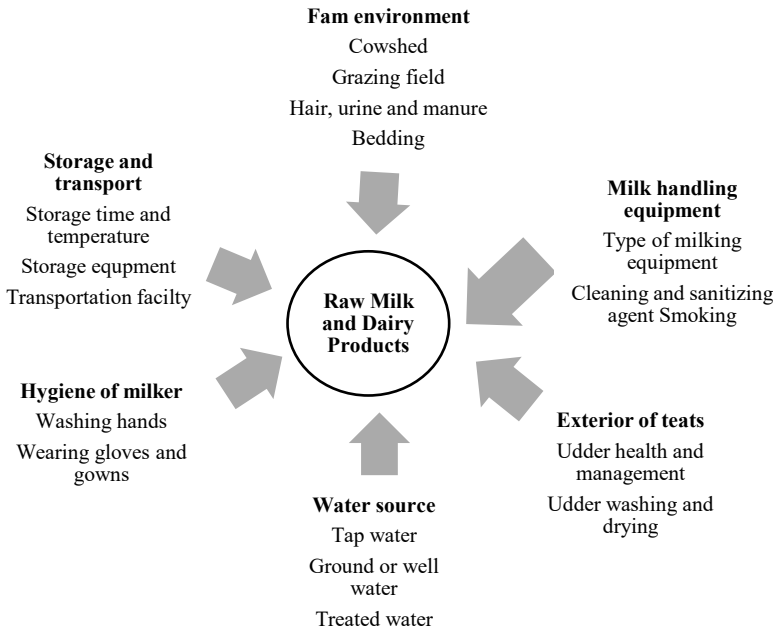

Figure 1. Factors contributing to microbial contamination of milk and dairy products

Animal Health

Animal health is an essential part of milk production as it influences the quantity, safety, and quality of milk being produced (Quinn *et al.*, 1994). Therefore, the health status of a dairy herd is the first indicator of the safety and quality of milk and dairy products. Unhealthy animals, particularly lactating cows can produce unsafe milk due to shedding of microorganisms that can cause infection in both animals and humans. Hence, poor animal health can have a negative public health impact by increasing the risk of foodborne illness (Alehegne *et al.,* 2004; Bekele & Molla, 2000; Jay, 2000; Quinn *et al.*, 1994; Radostits *et al.*, 1994). Bovine tuberculosis caused by *Mycobacterium bovis* and brucellosis caused by *Brucella* are among the major animal diseases that can impact public health through the consumption of raw milk produced by infected herds (Pedro Acha & Boris Szyfres, 2001). In addition to these two infectious diseases, mastitis is considered as one of the most concerning dairy cattle disease (Naqvi *et al.*, 2018; Tančin *et al.*, 2018). Mastitis is associated with a bacterial infection in one or more quarters of the mammary gland of dairy cows (Hamann, 2010; Pandey & Voskuil, 2011). *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, and coliforms are the most common contagious pathogens known to cause bovine mastitis (Acha & Szyfres, 2001; Molalegn *et al.*, 2010; Dufour *et al.*, 2019; Gao *et al.*, 2017; Idriss *et al.*, 2014; Naqvi *et al.*, 2018; Schaika *et al.*, 2005; Velázquez-Ordoñez *et al.*, 2019).

Although mastitis has been a serious challenge for the Ethiopian dairy industry, it is not given due attention (Fufa *et al.*, 2019; Lore *et al.*, 2006). Studies conducted across Ethiopia reported variable prevalence of mastitis **(Table 1)** with the overall prevalence of both clinical and sub-clinical mastitis exceeding 60% (Birhanu *et al.*, 2013; Demelash *et al.*, 2005; Yien et al., 2014; Nibret *et al.*, 2012; Bayush & Ataro, 2018; Mulugeta & Wassie, 2013). Non-isolation and milking of cows infected with mastitis were identified as major sources of pathogenic microbial contaminants in milk such as *Escherichia coli*,

Streptococcus uberis, *Streptococcus dysgalactiae*, and other *Gram-positive* and *catalase-negative cocci* (Dufour *et al.*, 2019). Furthermore, failure to maintain sanitary shelter with proper ventilation and lack of regular veterinary visits for early detection and treatment of disease has exacerbated the problem and resulted in widespread mastitis in Ethiopian cattle (Nibret *et al.*, 2012). To prevent the entrance of many pathogenic bacteria into the milk chain, therefore, compliance with good milking practices is extremely important. These practices include cleaning and removal of soil particles from the teats prior to milking, using sanitary bedding material that can facilitate ease of cleaning, and removal of manure from the teats, udder and adjacent parts (FSA, 2006; ICAR, 2011; Nangamso, 2006; SNV, 2017). In general, using proper milk handling equipment, regular physical or clinical examination, culling chronically infected cows, monitoring of udder health and maintaining appropriate environment are critical animal health management and mastitis control procedures (Murphy, 1996; O'Connor, 1995; Radostits *et al.*, 2006; Velázquez-Ordoñez *et al.*, 2019; Zelalem *et al.*, 2011).

Farm Management and Environmental Factors

The production of safe milk begins with the implementation of good hygienic practices on-farm, which is an effective first step in reducing milk contamination (Barbuddhe & Swain, 2008; Carloni *et al.*, 2016; Bekele & Molla, 2000; Ramírez-Rivera *et al.*, 2019). Farm management includes preventing cows from grazing in unhygienic pasture and living in sheds that are not cleaned on a regular basis (Carloni *et al.*, 2016; Pandey & Voskuil, 2011).

Unhygienic milking environments can facilitate the spread of microorganisms (Fuentes *et al.*, 2014; Zdanowicz *et al.*, 2004). Exposure of cow's udder to environment contaminated with feces or debris is a major source of microbial contamination of milk (Fufa *et al.*, 2019; Vacheyrou *et al.*, 2011). Additionally, irregular cleaning of the milking areas and animal sheds contributes to cross-contamination of milk in household dairy farms (Carloni *et al.*, 2016). This is a major challenge in Ethiopia, as on-farm infrastructure is commonly underdeveloped. And most of the cow sheds are built using trees while a few of them are made of blocks and iron sheets (Shija, 2013).

Table 1: Prevalence of mastitis across cow breed, age, lactation and parity in Ethiopia

Cows	*Breed type*			*Lactation stage*			*Parity No. (in calves)*		*Age (years)*			*Study location*	*Reference*
	Holstein-Friesian	*Holstein-Zebu*	*Zebu*	*Early*	*Mid*	*Late*	*1-3*	*>4*	*3-5 (young)*	*6-10 (adult)*	*>10 (old)*		
Examined	53	113	17	66	67	50	142	41	-	-	-	Hawassa; SNNPR	Nibret *et al.*, 2012
Infected	17	35	4	14	17	25	32	24	-	-	-		
Prevalence	32.1	30.9	23	21.2	25.3	50	22.5	58.5	-	-	-		
Examined	-	-	-	37	74	10	23	98	22	27	72	Gambella	Yien *et al.,* 2014
Infected	-	-	-	21	41	8	13	58	9	17	47		
Prevalence	-	-	-	56.7	55.4	80	56.5	59	40.9	62.96	65		
Examined	186	259	446	214	403	357	328	315	326	399	249	SNNPR	Demelash *et al.*, 2005
Infected	105	73	138	98	104	138	37	198	77	152	111		
Prevalence	56.5	28.2	30.9	45.8	25.8	38.7	11.3	62.9	23.6	38.1	44.6		
Examined	-	-	-	64	176	-	91	305	68	174	89	Addis Ababa	Fufa *et al.*, 2019
Infected	-	-	-	32	99	-	42	168	23	97	53		
Prevalence	-	-	-	50	56.3	-	46.2	56.3	33.8	55.75	59.5		
Examined	-	349	139	94	121	134	177	137	104	155	90	Wolayita, SNNPR	Mulugeta & Wassie, 2013
Infected	-	103	15	62	4	37	21	58	11	44	48		
Prevalence	-	29.5	10.5	65.9	3.3	27.6	11.9	42.3	10.6	28.4	53.3		
Examined	-	-	-	-	-	-	202	204	197	168	63	Holeta, Oromia	Berhanu *et al.*, 2010
Infected	-	-	-	-	-	-	68	112	66	94	32		
Prevalence	-	-	-	-	-	-	37.7	54.9	33.5	56.0	49		
Examined	499	-	-	133	132	234	94	108	323	176	-	Addis Ababa	Tesfaheyw et *et al.*, 2013
Infected	373	-	-	116	87	171	43	96	210	164	-		
Prevalence	74.1	-	-	87.2	65.9	73.1	45.7	88.9	65	93.2	-		
Examined	185	-	-	67	61	88	76	140	90	27	99	Jimma, Oromia	Bayush & Ataro, 2018
Infected	126	-	-	50	32	54	51	85	58	19	59		
Prevalence	92.6	-	-	36.8	23.5	39.7	37.5	62.5	42.6	13.97	43.4		
Examined	290	-	-	94	90	106	152	137	135	107	48	Sebeta, Oromia	Yomiyu *et al.*, 2017
Infected	164	-	-	35	45	57	70	67	60	51	26		
Prevalence	56.5	-	-	37.2	50	53.8	45.8	55.1	44.4	47.66	54.2		
Examined	91	-	293	115	108	161	198	186	202	122	60	Haramaya, Oromia	Bayan Amin *et al.*, 2017
Infected	40	-	149	74	33	85	85	104	86	63	40		
Prevalence	43.9	-	56.6	64.3	30.5	52.7	42.9	58.1	42.5	51.6	66.6		
Examined	125	-	26	74	-	77	119	32	107	44	-	Ambo, Oromia	Getachew & Edilu, 2016
Infected	56	-	4	34	-	29	37	26	30	31	-		
Prevalence	47.2	-	15.4	45.9	-	37.7	31.1	81.3	28.0	75	-		
Examined	327	-	57	152	80	152	280	104	153	195	36	Harrargh e, Eastern Ethiopia	Tesfaheyw et & Gerema, 2017
Infected	170	-	29	77	49	73	135	64	48	130	21		
Prevalence	52	-	50.9	50.7	61.3	48.0	53.0	61.9	31.4	66.7	58.3		
Examined	14	-	370	130	194	60	161	77	135	167	82	Benchi Maji, Western Ethiopia	Teshome *et al.*, 2019
Infected	10	-	106	18	71	27	21	35	25	51	40		
Prevalence	71.4	-	28.6	13.8	36.6	45	13	45.5	18.5	30.5	48.8		

A study conducted by Mitiku *et al.* (2019) in Haramaya district, reported that all cowsheds (100%) included in their study were not constructed in a way that would facilitate drainage of farm waste, including animal feces and urine. The report also indicated that cowsheds did not use proper bedding materials like sand bedding for the animals to prevent dairy cow udders from becoming soiled. Similar studies revealed that 81% and 83% of the evaluated households did not use any bedding material in Jimma and Sidama Zones Respectively (Abebaw & Ephrem, 2018; Mesfin *et al*., 2015). Moreover, the floors were not hygienically cleaned rather they were commonly covered with manure, and had improper drainage systems. In another study conducted in southern part of Ethiopia Abebe *et al.* (2012) reported that 67% of the households used straw or hay as bedding material. However, such bedding materials need to be changed frequently, to prevent the transmission of pathogenic bacteria potentially present in the environment to milk (Sanaa *et al.*, 1993). Microorganisms present in bedding material can also contaminate the surface of animal udder, resulting in mastitis (Vacheyrou *et al.*, 2011). Zdanowicz *et al.* (2004) indicated that coliform counts in milk samples is reduced when cows are housed in an environment with sand bedding as compared to straw or sawdust bedding. Thus, clean and dry bedding condition is important to reduce microbial contamination of milk (Abebe *et al.*, 2012; Gurmessa, 2015; Sanaa *et al.*, 1993).

Milking is conducted inside a confined shed on a majority of smallholder dairy farms in Ethiopia, where there is a high risk of contamination through the dusty air and insects (Abebe *et al.*, 2012). Lack of sufficient space, especially in urban areas, and irregular cleaning of milking rooms and cowsheds can create suitable conditions for the growth of insects like flies that can transmit pathogens (Pandey & Voskuil, 2011). Furthermore, most of the smallholder farmers, particularly in rural areas, share a common dwelling with their animals, and the close proximity can facilitate the spread of bacteria to the milk originating from human hair, cloth and other sources (Abebe *et al.*, 2012). Betelihem and Shimels (2017), reported that 52% of the farms included in their study did not have a separate milking cowshed. In this regard, lack of comprehensive and uniform hygienic procedures to be followed by producers has posed a challenge to implement and use new procedures and research findings in the dairy sector of Ethiopia (Tesfaye, 2019; SNV, 2017; Zelalem, 2003).

In general, cleanliness of the premises and the environment can significantly reduce risk factors contributing to poor quality milk production and mastitis as it results from unhygienic conditions (Abebe *et al.*, 2012; Buncic, 2006). Hence, a proper and clean housing environment, is a pre-requisite to produce milk of acceptable quality and safety as it can significantly reduce risk factor of mastitis and other pathogenic microbes like *Listeria monocytogenes* (Abebe *et al.*, 2012; Amanuel & Ulfina, 2018; Sanaa *et al.*, 1993).

Milking and Milk Handling Practices

Milking and milk handling practices have significant effects on the quality and safety of milk and milk products (Betelihem & Shimels, 2017). Hygienic milking practices aim to prevent the transmission of zoonotic and communicable diseases through milk to consumers. Hygienic milking practices include regular cleaning and washing of animal udder and milk handling equipment before and after milking, use of separate and clean drying towels between cows, the filtering of milk after milking and avoiding the feeding of cows during milking. Good hygienic practices can prevent the transmission of zoonotic diseases by reducing the risk of milk contamination with pathogenic bacteria (Barbuddhe & Swain, 2008; Lore *et al.*, 2006; Pandey & Voskuil, 2011).

Poor cleaning and disinfection of teat has repeatedly been identified as a risk factor for contamination of raw milk by certain pathogens like, *Listeria monocytogenes* (Sanaa *et al.*, 1993). In 2019, Fufa *et al.* (2019) reported that udder washing before milking is not widely practiced by Ethiopian dairy farmers. Of the 70 participants surveyed in their study, 26% did not wash udders prior to milking and only 30% of them used separate drying towels or cloths between milked cows to dry udders after washing. This data is based on selected sub-cities of the country's capital, Addis Ababa, and it is the authors' belief that this issue is magnified more in rural parts of the country where farmers typically do not avoid milking cows that show signs of infections, and where improper hand washing and handling of milk is common. In similar studies conducted in the cities of Gonder, Harrarghe and Dangila, 72, 99, and 94% of the participants, respectively, were not regularly washing cows' udders and teats before and after milking cows, unless the udder was contaminated with manure (Bekele *et al.*, 2015; Mitiku *et al.*, 2019; Betelihem & Shimels, 2017). Other studies also revealed that among the participants who practiced regular washing of cows' udders, more than 80% failed to dry the washed udder using a dry and clean towel or a cloth (Abebe *et al.*, 2012; Bayan Amin *et al.*, 2017; Bekele *et al.*, 2015; Gezu *et al.*, 2015).

Ethiopian farmers may use a myriad of techniques to remove dirt from udders, including allowing a calf to suckle prior to milking or using a dry cloth to remove dirt from the teats and udder of the animal. On the other hand, covering of the udder by using dung or mud is practiced in some parts of the country to prevent calves from suckling while the cows are grazing. When calves are given the teats before milking to suckle, the unwashed teats and saliva left from calves can be sources of bacterial contamination during milking. Failure to thoroughly clean and dry the udder and teats is a common source of coliforms in milk (Alehegne *et al.,* 2004; Pandey & Voskuil, 2011). The above-outlined poor practices increase the risk for mastitis or similar diseases, which can result in a significant loss in both quantity and quality of milk produced (Alehegne *et al.,* 2004).

Hand milking is a common practice across the country and can contribute to milk contamination by the milker. In most parts of the country, all cows in a given farm are milked by a single milker (Zelalem *et al.,* 2011; Alehegne *et al.,* 2004). As the milkers' moves from one cow to the next, without washing and disinfecting their hands, they can potentially transfer pathogenic microorganisms between animals in the herd. Furthermore, if the milker is sick, s/he can transmit disease through milk handling (Abebe *et al.*, 2012; Mitiku *et al.*, 2019). Betelihem and Shimels (2017) reported that out of 60 randomly selected dairy farmers included in their study, 19 (32%) did not practice hand-washing prior to milking. In many instances, where hand-washing practices were in place, only water was used to wash hands (Mitiku *et al.*, 2019). This is not necessarily sufficient for the removal of all bacteria from hands and can compromise milk quality and safety (Pandey & Voskuil, 2011; Zelalem *et al.*, 2011). Hence, proper handwashing both before and after milking should be practiced among dairy farmers by using water and soap, which can significantly reduce the microbial load on hands and therefore reduce the risk of milk contamination (Sanaa *et al.*, 1993; Eyasu *et al.*, 2015).

Filtering of milk before further processing is an important step followed to avoid exposure of milk to physical hazards (Pandey & Voskuil, 2011; SNV, 2017). Tadele *et al.* (2016), reported that 80, 15, and 5% of the participants use bare hands, sticks, and spoons, respectively, to remove extraneous material from milk. It is evident that the use of filters may be the appropriate solution to minimize cross-contamination and prevent physical hazards (e.g., hair, soil, jewels, and other similar extraneous materials) from entering into the milk. Filtering of milk can result in good quality reducing physical hazards; and ensuring consumer's health (Schaika *et al.*, 2005). Moreover, inappropriate animal

husbandry practices like feeding roughage at the time of milking should be avoided, as the dust and/or smell easily contaminates the milk (Pandey & Voskuil, 2011).

In the dairy sector of developing countries, women have an important role, particularly in milking and milk handling practices (FAO, 2011; Berhanu *et al.*, 2006). In most parts of Ethiopia, particularly in rural areas, activities related to animal husbandry and milk production are responsibilities of women (Mushir & Mulugeta, 2012; Amanuel & Ulfina, 2011; Mitiku *et al.*, 2019; Amanuel & Haftom, 2016). Therefore, along with the implementation of hygienic milk handling procedures, empowering women with necessary skills and knowledge is one intervention area that has a potential to reduce the risk of milk contamination (FAO/IDF, 2011). In general, milk production and handling practices in Ethiopia are not carried out hygienically. Hence, in order to raise awareness among the dairy value chain actors and design effective and acceptable interventions to instigate behavior change in the milk production and handling, it is crucial to understand the local context of milk production, handling, and processing (Kebede *et al.*, 2019; Koome, 2016; Lore *et al.*, 2006)

Milk Handling Equipment and Water Source Used for Sanitation

Equipment used for milk handling, storage, and transportation has an effect on the safety and quality of milk and is a major source of microbial contamination (FSA, 2006; SNV, 2017). Microbiological contamination can result from equipment surfaces, especially joints, open seams, and dents that are difficult to clean properly and can harbor microorganisms such as spore-forming bacteria and *Listeria monocytogenes*, and can lead to microbial persistence within milk processing facilities (Chmielewski & Frank, 2004; Pauline & Karin, 2006; Simo˜es *et al.*, 2010; Vissers & Driehuis, 2008).

In Ethiopia, the majority of the farmers use plastic containers, clay pots, and bottle gourds to carry milk, which are difficult to thoroughly clean due to their shape and narrow opening (Abebe *et al.*, 2012; Aleme *et al.*, 2018; Habtamu & Adugnaw, 2018; Felleke, 2003). Donkor *et al.* (2007), indicated that the use of plastic milk containers was found to be one of the potential risk factor associated with coliform contamination in milk. The use of plastics should be avoided because the material may be easily scratched and that surface can serve as source of persistent contamination and cross-contamination. Hence, the surface of the materials should be smooth, with minimal joints or open seams, and should be free from dents (Buncic, 2006; Pandey & Voskuil, 2011). Stainless steel is recommended to use, as it is easy to clean, durable, does not absorb smells, is not corrosive and can resists detergents (Johanna *et al.*, 2003). However, small scale dairy farmers may not be able to afford stainless steel containers as they are a bit expensive, in such cases it is highly recommended to use other available milking and transportation containers like Mazzican (MTS), which is introduced by SNV. Mazzican is a durable 10 litre food-grade plastic container that has a wide opening and transparent plastic, which makes it easy to pour milk into it and enables the farmers to detect dirt easily (SNV, 2018). Therefore, milk handlers need to pay particular attention to the type of milk handling equipment used (Simo˜es *et al.*, 2010).

In Ethiopia, the main sources of water for sanitary activities associated with milk handling equipment include rivers or spring water, ponds, rain water, ground or well water and tap water (Abebe *et al.*, 2013; Mitiku *et al.*, 2019; Mesfin *et al.*, 2015). Water from these sources is typically used without further treatment (Fufa *et al.*, 2019; Aleme *et al.*, 2018; Dessalegn, 2017; Mitiku *et al.*, 2019; Shija, 2013). Furthermore, the use of poor-quality contaminated tap water can also lead to introduction of pathogenic bacteria into the milk production chain (Amanuel & Ulfina, 2018; Oladipo *et al.*, 2016). Eyasu

et al. (2015) and Sanaa *et al.* (1993) reported that the use of detergent together with clean and warm water reduced the risk of contamination of milk with *Staphylococcus aureus* and *Listeria monocytogenes*. Efficacy, safety and ease of removal are the selection criteria's for detergents and disinfectants to be used for cleaning and disinfection of milk handling equipment (Dosti *et al.*, 2005; Simo˜es *et al.*, 2010).

Smoking of milking and milk handling equipment after washing with tap water is well practiced in most parts of the country (Tadele *et al.*, 2016; Aleme *et al.*, 2018; Mitiku *et al.*, 2019; Tsadkan & Gurja, 2018). Mogessie (1996), reported smoking of milk handling equipment can influence the growth of pathogenic and spoilage microorganisms. The study indicated that smoking has an inhibitory effect on *Listeria monocytogenes*. It is evident that smoking can also contribute to milk quality by improving flavor, appearance and texture of fermented dairy products.

In conclusion, to reduce the risk of microbial contamination of milk during and after milking, milk handling equipment should be kept hygienic and washed regularly with clean tap water and then thoroughly scrubbed with warm water and detergent. In addition, it must be brushed properly with clean bristles used only for food contact surfaces, to reduce the level of contamination and minimize food safety risks (Fufa *et al.*, 2019; Kebede *et al.*, 2019; Lore *et al.*, 2006). Finally, after rinsing with clean water, the container should be left for drying turned upside down on a drying rack aiding fast drying and reducing exposure to environmental contaminants (Pandey & Voskuil, 2011; Yien, 2019; SNV, 2017).

Milk Storage, Transport and Cold chain

Poor storage and transportation conditions can further facilitate the contamination of milk from milk handing equipment. Raw milk can only be kept for hours without storage at an appropriate temperature (4°C) before it deteriorates in both quality and safety (SNV, 2008). Therefore, it must be stored and kept cool using proper refrigeration within two hours after milking, it maintains nearly its original quality and remains fresh for a reasonably longer time until processing and consumption (Pauline & Karin, 2006; SNV, 2008). However, such storage facilities are not readily available in Ethiopia, particularly in rural areas and cooling systems are not feasible due to lack of the required dairy infrastructure and unstable power supply (Mitiku *et al.*, 2019; O'Connell *et al.*, 2016). When available, there is a high cost associated with facilities maintaining refrigerators for small smallholder producers (Abebe *et al.*, 2013). Hence, the raw milk is easily spoiled, which results in significant losses in milk production. According to Forsbäck *et al.* (2011), milk quality, in terms of protein and fat deteriorates much faster during storage, owing to increased somatic cell count (SCC) and mastitis pathogens (e.g., other bacteria, mainly *psychrotrophs*).

Means of transportation used for the delivery of milk can also influence the quality and safety of milk. Animal-drawn carts, motor bicycles, three-wheel drive vehicles (Bajaj), four-wheel-drive vehicles, or public transportation are among the methods used as a means to deliver milk to collection centers or selling points by dairy farmers in Ethiopia (SNV, 2017). These forms of transportation are not appropriate, especially when important hygiene and food-safety considerations are not taken into account. Almost all means of transportation, particularly public transportation, are not safe as they do not provide facilities for cooling the milk (Wayua *et al.*, 2012).

The time it takes to transport or deliver milk to collection centers is another factor that affects its quality and safety. According to Eyasu *et al.* (2015), samples from dairy farmers that had more than a 30 min travel time to the collection center had a 5.6 times higher risk of contamination with *Staphylococcus aureus* when compared to farmers that had less than 30 min of travel time to the collection centers. The study also indicated that for every one-liter increase in milk delivered, the probability of contamination

with *Staphylococcus aureus* increased by 4%. The establishment of milk collection centers with cooling facilities near to the dairy farmers can be seen as one of the ways to minimize the milk waste due to improper storage and transportation conditions (Sintayehu *et al.*, 2013). In rural areas of the country, placing the milk in containers at cool (windy) places or in a cool water and electrical or solar operating bulk cooling tanks can be used to cool milk at the farm level (Alehegne *et al.,* 2004; Amanuel & Ulfina, 2018). These alternatives allow harvested milk to be stored longer and maintain its quality and safety. Even though not well known or practiced, the use of preservatives like lacto-peroxidase has been recently used to prolong milk shelf life (Pandey & Voskuil, 2011; SNV, 2017). In conclusion, milk should be cooled to a suitable temperature (4°C) and transported by means that maintain its quality and safety.

Hygienic Conditions at Market places

Milk and dairy products are marketed in formal and informal marketing systems (Mohamed *et al.*, 2004; Weldegiorgis & Gebremariam, 2019). In Ethiopia, the informal milk marketing system is dominant (Land O'Lakes, 2010). Ninety percent of the milk produced by smallholders is marketed in an informal marketing system; and only the remaining 10% is delivered to the formal market (SNV, 2008).

Informal marketing systems are widely observed in traditional open markets and at the household level, in which limitations on infrastructure, proper packaging, storage and transportation equipment are present (Aleme *et al.*, 2018; Eyassu & Asaminew, 2014). Market access in a pastoral production system is particularly limited, which has led to a majority of the produced milk to be sold through informal market settings (Kebede *et al.*, 2019; Dessalegn, 2017; Tsehay, 2001). The hygienic conditions of the informal markets are not monitored or sustainably maintained (SNV, 2008; Mohamed *et al.*, 2003; Kebede *et al.*, 2019; Tsehay, 2001; Welearegay *et al.*, 2012; Tsadkan & Gurja, 2018). According to the Central Statistics Agency (CSA), of the total urban milk production, 73% is sold, 10% is left for household consumption, 9.4% goes to calves and 7.6% is processed into butter and cheese (CSA, 2011).

Recently, efforts have been made to establish and expand dairy cooperatives in different parts of the country which is important for increasing and improving the formal milk marketing systems; leading to an improved infrastructure and frequent product quality monitoring (Tesfaye, 2019). According to Berhane and Workneh (2003), dairy marketing cooperatives could provide farmers with continuous milk outlets and easy access to essential inputs such as artificial insemination, veterinary services and formulated feeds. Thus, dairy cooperatives are needed to start a positive series of development in the milk production sub-sector and further improve the existing dairy cooperatives around the country.

Future perspectives

During the past few decades, many studies have been carried out showing the prevalence and risk factors associated with pathogenic and spoilage microorganisms in milk and dairy products in Ethiopia (Abdi *et al.*, 2020; Nibret *et al.*, 2012; Bayush & Ataro, 2018; Tesfaheywet & Gerema, 2017; Tesfaheywet *et al.*, 2013). However, the published results so far in this area are limited to the district or regional level. Thus, a more comprehensive study is required to show the overall prevalence and risk of contamination of milk across the country. To develop a more complete framework for the prevention and reduction of contamination of milk and dairy products, future studies should consider the development of hygienic practices and procedures guidelines based on identified sources of contamination. Moreover, future

studies should be supported with experiments to evaluate the efficacy of specific intervention that had not been well researched.

CONCLUSION

Milk contaminated with foodborne pathogens poses a threat to human health. The contamination may result from infected or sick animals, unhygienic conditions and practices in milking and milk handling, unhygienic milking equipment and poor quality of water. The safety and quality of milk is highly affected by unhygienic practices in different stages of milk production. Reduced quantity and quality of milk production has been a challenge for the dairy sector in Ethiopia, resulting in a significant economic and social impact. The elimination of pathogenic and spoilage microorganisms from human carriers and environmental sources is critical for the success and the production of high quality and safe milk. Improvement in animal husbandry and farm management, increasing the awareness of hygienic milking and milk handling practices among the dairy value chain actors, and the development and implementation of hygienic milk production procedures are identified as priority areas for intervention to improve the quality and safety of milk and dairy products produced in Ethiopia.

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Effects of Type of Starter Culture, Increase of Dry Matter and Microbial Transglutaminase on the Texture and Consumer Acceptability of Fermented Camel Milk

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ABSTRACT

Camel milk has been found to be difficult to process into different dairy products due to slower rate of acidification and other factors related to its composition. Hence, this study was aimed to evaluate effects of two different mesophilic starter cultures, addition of camel milk powder (CMP) and the use of microbial transglutaminase (MTGase) on the texture, viscosity, sensory and physicochemical properties of fermented camel milk. The two mesophilic starter cultures used were R-707 (Lactococcus lactis) and CHN-22 (contain multiple strains of Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. lactis biovar diacetylactis, Leuconostoc mesenteroides and Leuconostoc pseudomesenteroides). All milk samples were heat treated (90 ^{o}C, 10 min), cooled to inoculation and incubation temperature (27 ^{o}C) of starter cultures and incubated until pH reaches 4.6. Use of a single strain starter culture (R-707) resulted in fermented milk which was significantly ($P<0.05$) higher in cohesiveness and adhesiveness than a multi-strain (CHN-22) starter culture. But addition of CMP significantly ($P<0.05$) decreased the firmness, cohesiveness and adhesiveness of the fermented camel milk. Use of MTGase has improved the textural attributes of fermented camel milk samples and the effect depended on the starter culture used. The cohesiveness of the fermented camel milk was significantly ($P<0.05$) higher when made using R-707 starter culture with MTGase compared to using CHN-22 starter culture. Fermented camel milk produced using CHN-22 starter culture with MTGase was significantly ($P<0.05$) lower in titratable acidity as compared to that produced using CHN-22 starter culture without MTGase. Therefore, R-707 starter culture was found to be more preferable to improve the textural attributes of fermented camel milk samples, and MTGase can be used by dairy industries and smallholder farmers to improve the textural attributes of fermented camel milk.

Keywords: Camel milk powder, Fermented camel milk, Mesophilic starter cultures, Microbial transglutaminase, Texture.

INTRODUCTION

Camel milk is technically more difficult to process into different products than milk from other domestic animals (Mehaia, 1994; Ibrahim, 2009; Konuspayeva *et al.,* 2014). For instance, Jumah *et al.* (2001) reported that the viscosity of camel milk yoghurt does not change during gelation, and Mohammed *et al.* (1990) observed that camel milk failed to form gel like structure after 18 hours incubation with lactic acid culture. This was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin and immunoglobulin in camel milk (El Agamy *et al.,* 1992). However, a recent report by Tesfemariam Berhe *et al.* (2018) found that the slower speed of acidification in camel milk than bovine milk was due to difference in proteolysis rather than the presence of inhibitory substance

in camel milk. The authors concluded that the proteolytic systems of the starter cultures used are unable to support a growth rate in camel milk as fast as in bovine milk. Farah *et al.* (1990) reported that the *Suusa* (traditional fermented camel milk) can be improved by using selective mesophilic lactic acid cultures.

It has been reported that the compositional properties of camel milk attributed to the product quality during processing (Tesfemariam Berhe *et al.,* 2017). The content of heat-stable serum proteins of camel milk which make up 20-25% of the total protein (Desouky *et al.,* 2013) and the weak interaction between denatured serum proteins and casein due to lack of β-lactoglobulin (Shabo *et al.,* 2005), the lower amount of κ-casein (Farah, 1993), the high whey protein to casein ratio (Shamsia, 2009) in camel milk can be attributed to the weak texture and thin consistency of camel milk yoghurt (Tesfemariam Berhe *et al.,* 2017). Compared to bovine milk, camel milk casein has larger micelle size (Bornaz *et al.,* 2009). It has been reported that smaller casein micelles have improved the gelation properties of bovine milk (Glantz *et al.,* 2010). The lower amount of κ-casein, the high ratio of whey protein to casein, and the larger micelle size in camel milk also result in formation of a less firm coagulum and lower yield during cheese processing (Tesfemariam Berhe *et al.,* 2017).

Enzymatic cross-linking of milk proteins is a method that has received increasing attention during the last two decades (Faergemand *et al.,* 1998; Motoki and Seguro, 1998). One of the cross-linking enzymes available for catalysing covalent bond formation between protein molecules on a commercial scale is microbial transglutaminase (MTGase) (Dickinson, 1997). MTGase is a transferase which catalyzes the acyl-transfer reaction between γ-carboxamide groups of peptide or protein bound glutamyl residues and primary amines (Dickinson and Yamamoto, 1996; Bonisch *et al.,* 2007). Cross-linking of milk proteins by MTGase modifies functionality such as hydration ability and rheological as well as emulsifying properties (Motoki and Seguro, 1998; Lorenzen, 2000). MTGase is effective in reducing syneresis in acid milk gels and has been reported as a method of improving the texture and shelf-life of yoghurt (Motoki and Seguro, 1998).

Some attempts have been made to improve the texture and sensory properties of fermented camel milk by increasing the total solids through addition of milk powder (Mortada and Omer, 2013). The commonly used dry dairy ingredients to increase the solids content of yoghurt mix are skim milk powder, whey protein concentrate and sodium caseinate. Milk supplements with milk proteins can affect the texture and the physical properties of the yoghurt (Ibrahim, 2015). For instance, the addition of skim milk powder assisted in increasing the viscosity and gel strength of yoghurt as compared to the unfortified yoghurt (Peng *et al.*, 2009).

The use of selected commercial mesophilic starter cultures for the fermentation of camel milk combined with addition of camel milk powder (CMP) and use of MTGase to improve the texture of fermented camel milk has not previously been investigated. Therefore, the objectives of the present study were to evaluate the effects of mesophilic starter cultures, MTGase and addition of CMP on the texture, viscosity, sensory and physicochemical properties of fermented camel milk.

MATERIALS AND METHODS

Materials

Pooled fresh camel milk used in this study was collected from Errer Valley, Babille district, Eastern Ethiopia. The milk samples were collected from about 10 lactating camels in the early morning. After collection, the milk samples were brought to the Dairy Technology Laboratory of Haramaya

University within two hours of milking. A total of about 20 litres of camel milk was collected on three different occasions.

The mesophilic starter cultures (CHN-22 and R-707) were obtained as freeze-dried multiple and pure cultures from Christian Hansen A/S (Hørsholm, Denmark A/S). CHN-22 contains multiple strains of *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*, whereas R-707 contains a single strain of *Lactococcus lactis* without biovar *diacetylactis*. The MTGase (ACTIVA® MP) was obtained from Ajinomoto Foods Europe S.A.S (Paris, France). The ingredients of the enzyme include Lactose, Maltodextrin and Transglutaminase. Camel milk powder (CMP) was obtained from *Camelicious* Company in Dubai. The compositions (100 g) of the CMP were 25 g fat, 40 g carbohydrates, 38 g lactose, 25 g protein, 1.6 g salt; different vitamins [vitamin A (87.2 µg), vitamin B1 (0.4 mg), vitamin B2 (0.3 mg), vitamin C (22.6 mg), vitamin D (0.7 µg) and vitamin E (100 µg)]; and 1100 mg calcium.

Inoculum Preparation

Inoculums were prepared according to Tesfemariam Berhe *et al.* (2018). A 50-unit sachet of culture was added in 500 ml autoclaved bovine milk. The cultures were distributed into 100 ml bottles, capped tightly and frozen at -20 °C. During fermented camel milk preparation, 1 ml of the thawed inoculums was added to 400 ml milk.

Treatments and Experimental Design

The experiment was designed as factorial experiment (2*2*2=8) with two starter cultures (R-707 and CHN-22), two levels of camel milk powder [with (5%) and without] and two levels of MTGase [with (0.2 g L^{-1}) and without]. Eight different fermented camel milk samples were prepared as shown in Table 1. The experiment was done in three replications.

Table 1. Fermented camel milk samples

	Starter cultures			
Sample (S)	R-707	CHN-22	CMP	MTGase
S1	+	-	-	-
S2	+	-	+	-
S3	+	-	-	+
S4	+	-	+	+
S5	-	+	-	-
S6	-	+	+	-
S7	-	+	-	+
S8	-	+	+	+

Note: + = added (with); - = not added (without); CHN-22 =starter culture containing mixed strains of *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *lactis* biovar *diacetylactis, Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*; R-707 = starter culture containing a single strain of *Lactococcus lactis* without biovar *diacetylactis*; CMP = camel milk powder; MTGase = microbial transglutaminase.

Fermented Camel Milk Production

The flow diagram of the fermented camel milk production is shown in Figure 1. The pooled fresh camel milk brought to Haramaya University Dairy Technology Laboratory was sieved using a muslin

cloth and immediately divided into eight portions (each portion was used as individual fermented camel milk sample (S)). The experiment was done in triplicates with the milk collected on three different occasions according to the procedures outlined in Figure 1.

For texture profile analysis, 80 ml of inoculated milk samples was added to three 100 ml beakers, from each treatment. Then, all the milk samples were incubated in a thermostatically controlled water-bath (model WNB 45, D-91126, Memmert GmBH, Büchenbach, Germany) at 27 °C until the pH reaches 4.6. The acidification progress of the samples was checked by measuring the pH using a pH-meter during the incubation period. After 24 h storage time, the fermented milk samples were analysed for different parameters.

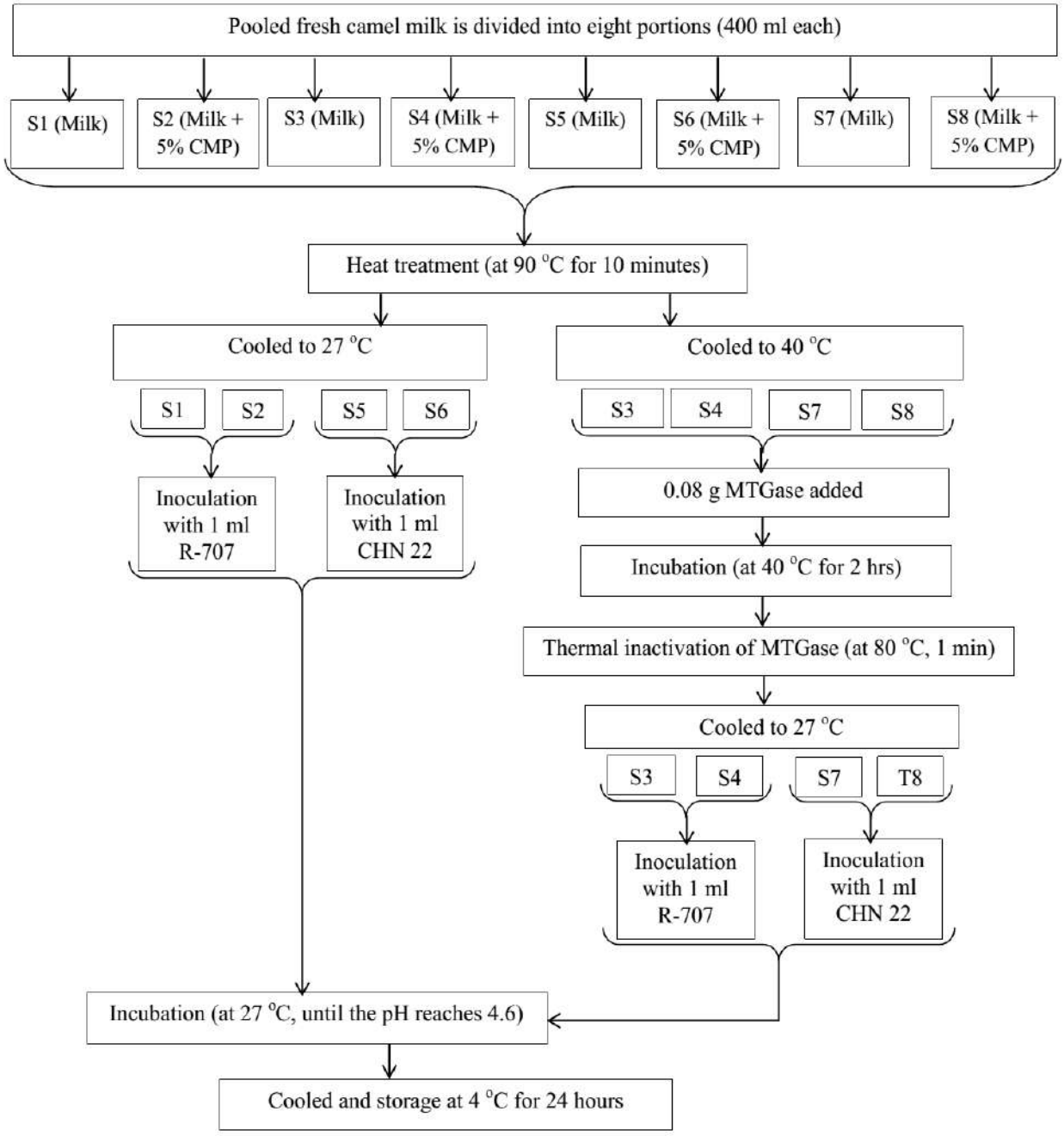

Figure 1. Flow diagram for fermented camel milk production

Physicochemical Analysis of Raw and Fermented Camel Milk

The pH of raw and different fermented camel milk samples was measured using a calibrated digital pH-meter. For determination of titratable acidity, 9 ml of raw or fermented camel milk sample was measured into a beaker and three drops of 0.1% phenolphthalein indicator was added into a sample and then titrated with 0.1N sodium hydroxide (NaOH) solution until faint pink colour persisted. The titratable acidity was expressed as percent lactic acid (Richardson, 1985). Thus, percent lactic acid was calculated as:

$$\% \text{ Lactic acid} = \left(\frac{(ml \text{ of } 0.1\text{N NaOH} \times 0.009 \times 100)}{ml \text{ of milk sample used}}\right)$$

Fat, protein, lactose, total solids (TS), and solids not fat (SNF) contents of raw and different fermented camel milk samples were determined using a MilkoScan FT1 (FOSS Analytical A/S, Hilleroed, Denmark). Eighty millilitres of the raw and fermented milk samples were used for the analysis of fat, protein, lactose, TS, and SNF. Just before analysis, the fermented milk samples in the beakers were thoroughly homogenized using an Ultra-Turrax T18 homogenizer (IKA-Labortechnik, Staufen, Germany). The ash content was determined according to AOAC (1995). The ash content of the raw and different fermented camel milk samples was determined gravimetrically by igniting in a muffle furnace (Fisher Scientific, Model 650-58, Canada). Five grams of the samples was measured into crucibles using sensitive balance and oven dried at 102 °C for 18 hrs. Then, the samples were transferred to the muffle furnace and ignited at a temperature of 550 °C for 3hrs. The samples were taken out of the muffle furnace and put in desiccators for 30 min and then measured on a sensitive balance. Finally, the percentage ash content was calculated as:

$$\text{Percentage Ash} = \left(\frac{\text{Residue weight}}{\text{Sample weight}}\right) * 100$$

The experiment was replicated three times and the measurements were done two times per replication for all physicochemical parameters.

Texture Profile Analysis

The texture profile of the different fermented camel milk samples were measured using a Texture Analyzer (TA.XT plus Stable Micro Systems, Godalming, Surrey, UK) fitted with 30 kg load cell. The 80 ml fermented camel milk samples prepared in 100 ml beakers of 45 mm diameter were individually fitted under the probe and the tests were carried out. After analysis, the following parameters were extracted from the force verses time curves: peak positive force, peak negative force, positive area and negative area as shown in Figure 2 were taken as measurement of firmness (g), elasticity (g), cohesiveness (g.sec) and adhesiveness (g.sec), respectively. The tests were done using 40 mm diameter back extrusion rig with the following settings: pre-test speed = 1 mm/sec; test speed = 2 mm/sec; post-test speed = 10 mm/sec; distance = 20 mm. The experiment was carried out three times and the analysis was done three times per replication. A typical graph for a measurement of fermented camel milk is shown in Figure 2.

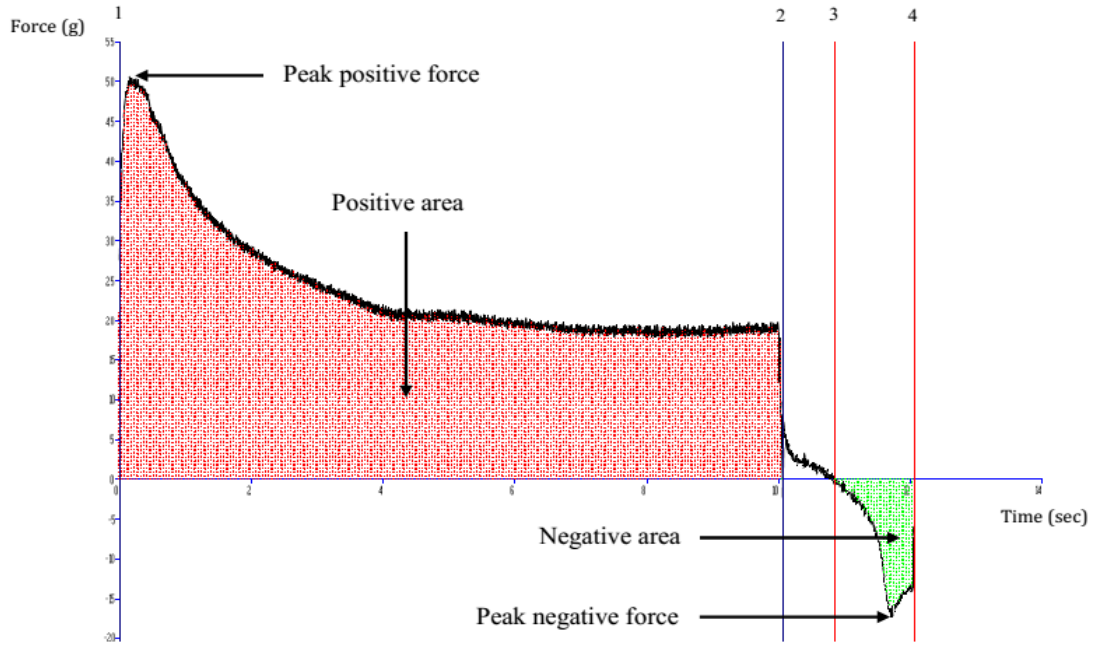

Figure 2. Graphic representation of measurements of textural attributes of fermented camel milk using texture analyser

Consumer Acceptability Test

For sensory analysis, 10 panellists were chosen to evaluate consumer acceptability of the different fermented camel milk samples. The panel members were selected based on the previous experience of evaluating sensory properties of different fermented dairy products and other processed foods. They evaluated all the fermented camel milk samples for sensory parameters such as color, appearance, aroma, taste, flavour, texture and overall acceptability using 7-point hedonic rating scale (7 = like very much; 6 = like moderately; 5 = like slightly; 4 = neither like nor dislike; 3 = dislike slightly; 2 = dislike moderately; 1 = dislike very much). About 40 ml of fermented camel milk samples were served in plastic cups. Pure bottled water was provided for panellists for cleansing palate between samples (Chen *et al.,* 1996).

Viscosity Analysis

Viscosity of fermented camel milk samples was measured using a post-humus funnel. The description of the post-humus funnel used is shown in Figure 3. Before the analysis, the fermented milk samples prepared in the bottles were thoroughly homogenized using an Ultra-Turrax T18 homogenizer (IKA-Labortechnik, Staufen, Germany). During viscosity measurement, the bottom outlet of the post-humus funnel was blocked by a finger and the homogenized fermented milk samples were poured into the post-humus funnel until it reaches the upper mark of the funnel. The bottom outlet was then opened and stop-watch started at the same time of opening the outlet. The time, in seconds, until the metal pin on the lower mark of the post-humus funnel visible was taken as viscosity values.

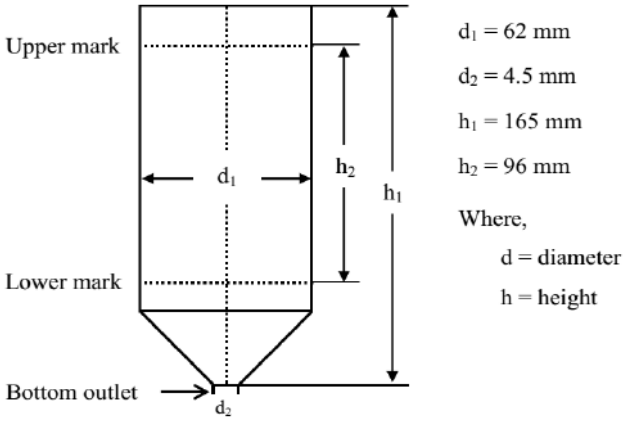

Figure 3. The diagram of a post-humus funnel used for measurements of viscosity of fermented camel milk

Statistical Analysis

The data were analysed with factorial analysis of variance (ANOVA) and the differences between means were assessed with Least Significant Difference (LSD) method. Statistical analysis was performed using SAS (2002) version 9.0. The level of significance for all analysis was done at *P<0.05.*

The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijk}$$

Where,

Y_{ijk} = the response variable

μ = overall mean; α_i = effect of starter culture; β_j = effect of camel milk powder

γ_k = effect of MTGase; $(\alpha\beta)_{ij}$ = interaction effects of starter culture and camel milk powder; $(\alpha\gamma)_{ik}$ = interaction effects of starter culture and MTGase; $(\beta\gamma)_{jk}$ = interaction effects of camel milk powder and MTGase; $(\alpha\beta\gamma)_{ijk}$ = interaction effects of starter culture, camel milk powder and MTGase; and ε_{ijk} = random error

RESULTS

Physicochemical Properties of Raw and Fermented Camel Milk

There was no significant difference (*P>0.05*) in the chemical composition (fat, protein, lactose, total solids and ash) of fermented camel milk due to the different starter cultures used or by the addition of MTGase (Table 2). However, fermented camel milk produced by the addition of CMP was resulted in significantly (*P*<0.05) higher content of protein, lactose, TS, SNF, ash and titratable acidity (% lactic acid).

Table 2. Effects of starter cultures, CMP and MTGase on the physicochemical properties (%) of fermented camel milk (Mean ± SE)

		Starter culture		CMP		MTGase	
Parameters	Raw camel milk	R-707	CHN-22	With	Without	With	Without
Fat	3.46	3.79 ± 0.31	3.70 ± 0.33	4.19 ± 0.29	3.30 ± 0.29	3.75 ± 0.32	3.74 ± 0.32
Protein	2.71	3.11 ± 0.22	3.15 ± 0.22	3.58 ± 0.18[a]	2.68 ± 0.17[b]	3.14 ± 0.22	3.13 ± 0.22
Lactose	4.51	3.75 ± 0.18	3.55 ± 0.12	4.01 ± 0.12[a]	3.29 ± 0.11[b]	3.70 ± 0.16	3.60 ± 0.16
TS	11.54	12.60 ± 0.66	12.44 ± 0.69	14.16 ± 0.46[a]	10.88 ± 0.46[b]	12.55 ± 0.67	12.49 ± 0.68
SNF	7.87	8.78 ± 0.40	8.67 ± 0.40	9.92 ± 0.17[a]	7.53 ± 0.17[b]	8.74 ± 0.40	8.71 ± 0.39
Ash	0.83	0.97 ± 0.05	0.98 ± 0.05	1.13 ± 0.02[a]	0.81 ± 0.03[b]	0.97 ± 0.06	0.98 ± 0.05
TA	0.14	0.90 ± 0.04	0.89 ± 0.05	1.00 ± 0.027[a]	0.79 ± 0.03[b]	0.86 ± 0.04	0.93 ± 0.04
pH	6.55						

Means with different superscripts in the same row are significantly different at $P < 0.05$; CMP = camel milk powder; MTGase = microbial transglutaminase; TS = total solid; SNF = solid not fat; TA = titratable acidity.

The titratable acidity of the fermented camel milk was significantly (*P<0.05*) higher when MTGase was used with R-707 starter culture than when used with CHN-22 starter culture (Table 3). The use MTGase with CHN-22 starter culture significantly (*P<0.05*) lower the titratable acidity of the fermented camel milk than that of without MTGase for the same starter culture. Therefore, the use of MTGase with CHN-22 starter culture can be an alternative method for reducing excess acid production.

Table 3. The interaction effects of starter cultures and MTGase on the titratable acidity (%) of fermented camel milk (Mean ± SE)

Starter culture	MTGase	
	With	Without
R-707	0.91 ± 0.07^{a}	0.90 ± 0.05^{b}
CHN-22	0.82 ± 0.05^{bB}	0.97 ± 0.06^{aA}
$LSD_{(0.05)}$	0.0782	

MTGase = microbial transglutaminase; LSD = least significant difference; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P< 0.05*.

Textural Properties of Fermented Camel Milk

Firmness

Addition of MTGase significantly increased gel hardness (Table 4). But addition of CMP, irrespective of the addition of MTGase, resulted in a significantly (*P<0.05*) lower firmness in the fermented camel milk (Table 4 and 5). In addition, when MTGase was applied, the resulting decrease upon addition of CMP was much more pronounced, illustrating that CMP, and hence extra camel milk protein, interfered with the positive effect of MTGase.

Table 4. The interaction effects of CMP and MTGase on the firmness (g) of fermented camel milk (Mean ± SE)

CMP	MTGase	
	With	Without
With	32.98 ± 4.46^{b}	21.54 ± 2.45^{b}
Without	86.77 ± 10.21^{aA}	36.29 ± 5.10^{aB}
$LSD_{(0.05)}$	14.202	

CMP = camel milk powder; MTGase = microbial transglutaminase; LSD = least significant difference; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P<0.05*.

Table 5. Effects of starter cultures, CMP and MTGase on the textural attributes of fermented camel milk (Mean ± SE)

Parameters	Starter culture		CMP		MTGase	
	R-707	CHN-22	With	Without	With	Without
Firmness (g)	46.54 ± 7.81	42.25 ± 9.37	27.26 ± 2.98^{b}	61.53 ± 9.35^{a}	59.88 ± 9.69^{a}	28.91 ± 3.50^{b}
Elasticity (g)	-15.49 ± 0.52	-16.00 ± 0.75	-15.27 ± 0.65	-16.23 ± 0.61	-17.32 ± 0.33^{a}	-14.17 ± 0.53^{b}
Cohesiveness (g.sec)	207.63 ± 9.07^{a}	136.21 ± 7.97^{b}	154.54 ± 11.56^{b}	189.31 ± 13.75^{a}	178.46 ± 15.72	165.38 ± 11.08
Adhesiveness (g.sec)	-7.54 ± 0.60^{a}	-3.68 ± 0.48^{b}	-4.72 ± 0.69^{b}	-6.51 ± 0.80^{a}	-6.59 ± 0.83^{a}	-4.64 ± 0.64^{b}

Means with different superscripts in the same row are significantly different at $P<0.05$; CMP = camel milk powder; MTGase = microbial transglutaminase.

Elasticity

There was no significant difference (*P>0.05*) in elasticity between fermented camel milk prepared using R-707 and CHN-22 starter cultures (Table 5). The addition of CMP also did not significantly (*P>0.05*) affect the elasticity of the fermented camel milk. However, significantly (*P<0.05*) higher elasticity was observed for the fermented camel milk produced by the addition of MTGase (Table 5).

Cohesiveness

The cohesiveness of the fermented camel milk produced by R-707 starter culture was significantly higher than that produced by CHN-22 starter culture while the addition of CMP significantly reduced the cohesiveness of the fermented camel milk as compared to that prepared without CMP (Table 5). On the other hand, the interaction of starter culture and MTGase had a significant effect (*P<0.05*) on the cohesiveness of fermented camel milk (Table 6). The use of MTGase with R-707 starter culture significantly (*P<0.05*) improved the cohesiveness of the fermented camel milk as compared to the R-707 starter culture without MTGase (Table 6). Generally, the use of MTGase with R-707 starter culture has improved the cohesiveness of the fermented camel milk. However, MTGase did not improve the cohesiveness when it is used with CHN-22 starter culture.

Table 6. The interaction effects of starter cultures and MTGase on the cohesiveness (g.sec) of fermented camel milk (Mean ± SE)

	MTGase	
Starter culture	With	Without
R-707	224.08 ± 13.18^{aA}	191.18 ± 8.96^{aB}
CHN-22	132.84 ± 9.00^{b}	139.58 ± 13.92^{b}
$LSD_{(0.05)}$	19.547	

MTGase = microbial transglutaminase; LSD = least significant difference; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P<0.05*.

Adhesiveness

Fermented camel milk prepared with R-707 starter culture showed significantly (*P<0.05*) higher adhesiveness than that prepared with CHN-22 starter culture (Table 5). The addition of CMP significantly decreased the adhesiveness of fermented camel milk. Significantly (*P<0.05*) higher adhesiveness was observed for the fermented camel milk samples treated with MTGase as compared to the samples without MTGase. In general, in the present study, the addition of MTGase improved the textural attributes of fermented camel milk.

Consumer Acceptability of Fermented Camel Milk

Fermented camel milk samples produced with the addition of CMP and R-707 starter culture had significantly (*P<0.05*) lowered likeability of aroma as compared to that produced from R-707 starter culture without CMP (Table 7). Moreover, fermented camel milk produced using R-707 starter culture without CMP had significantly (*P<0.05*) better aroma score than that of CHN-22 starter culture without CMP.

Table 7. The interaction effects of starter cultures and CMP on the aroma of fermented camel milk (Mean ± SE) (n = 10)

Starter culture	CMP	
	With	Without
R-707	5.40 ± 0.31^{B}	6.25 ± 0.31^{aA}
CHN-22	5.80 ± 0.30	5.25 ± 0.34^{b}
$LSD_{(0.05)}$	0.6374	

LSD = least significant difference; CMP = camel milk powder; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P<0.05*.

Table 8. The interaction effects of starter cultures, CMP and MTGase on the sensory properties of fermented camel milk (Mean ± SE) (n = 10)

Parameters	Starter culture	CMP	MTGase		$LSD_{(0.05)}$	P-Value
			With	Without		
Color	R-707	With	6.5 ± 0.22	6.3 ± 0.21	0.4196	0.2389
		Without	6.5 ± 0.31	6.1 ± 0.28		
	CHN-22	With	6.4 ± 0.22	6.5 ± 0.17		
		Without	5.6 ± 0.56	6.5 ± 0.22		
Appearance	R-707	With	5.9 ± 0.35	6.1 ± 0.23	0.4116	0.1506
		Without	6.4 ± 0.22	6.1 ± 0.23		
	CHN-22	With	6.3 ± 0.15	6.3 ± 0.21		
		Without	5.5 ± 0.52	6.2 ± 0.25		
Aroma	R-707	With	5.1 ± 0.50	5.7 ± 0.37	0.6374	0.8762
		Without	5.9 ± 0.59	6.6 ± 0.16		
	CHN-22	With	5.8 ± 0.39	5.8 ± 0.49		
		Without	5.3 ± 0.47	5.2 ± 0.51		
Taste	R-707	With	5.1 ± 0.61	5.9 ± 0.43	0.6481	0.2853
		Without	5.8 ± 0.47	5.6 ± 0.22		
	CHN-22	With	5.5 ± 0.54	5.1 ± 0.50		
		Without	5.5 ± 0.45	5.5 ± 0.34		
Flavor	R-707	With	5.1 ± 0.62	5.4 ± 0.64	0.7166	0.3004
		Without	6.0 ± 0.26	5.7 ± 0.56		
	CHN-22	With	5.7 ± 0.47	5.0 ± 0.54		
		Without	5.3 ± 0.47	5.5 ± 0.40		
Texture	R-707	With	5.8 ± 0.25	6.0 ± 0.26	0.4869	0.4760
		Without	6.2 ± 0.25	5.7 ± 0.42		
	CHN-22	With	5.7 ± 0.50	5.8 ± 0.39		
		Without	6.0 ± 0.37	6.1 ± 0.23		
Overall acceptability	R-707	With	5.7 ± 0.50	5.9 ± 0.48	0.5562	0.3276
		Without	6.2 ± 0.33	6.3 ± 0.15		
	CHN-22	With	6.1 ± 0.41	5.5 ± 0.45		
		Without	5.4 ± 0.43	5.8 ± 0.29		

CMP = camel milk powder; MTGase = microbial transglutaminase; LSD = least significant difference.

The interaction of the three factors (starter cultures, CMP and MTGase) did not significantly (*P>0.05*) affect the sensory attributes such as color, appearance, aroma, taste, flavour, texture and overall acceptability of the fermented camel milk samples (Table 8). However, from the comments given by the panellists, none of the fermented camel milk samples was considered as unacceptable.

Viscosity of Fermented Camel Milk

Significant difference (*P<0.05*) was observed in viscosity between the fermented camel milk produced with starter cultures and CMP (Table 9). The significantly higher viscosity was recorded for fermented camel milk prepared using CHN-22 starter culture with CMP than R-707 starter culture with CMP. The viscosity was significantly (*P<0.05*) higher when CHN-22 starter culture interacts with CMP than when R-707 starter culture interacts with CMP. Moreover, the use of CMP with CHN-22 starter culture has significantly (*P<0.05*) improved the viscosity of the fermented camel milk than CHN-22 starter culture without CMP (Table 9).

Table 9. The interaction effects of starter cultures and CMP on the viscosity (seconds) of fermented camel milk (Mean ± SE)

	CMP	
Starter culture	With	Without
R-707	18.26 ± 0.14^{b}	18.02 ± 0.13
CHN-22	18.74 ± 0.17^{aA}	17.97 ± 0.06^{B}
$LSD_{(0.05)}$	0.245	

CMP = camel milk powder; LSD = least significant difference; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P<0.05*.

The use of MTGase with CHN-22 starter culture had significantly (*P<0.05*) improved the viscosity of the fermented camel milk than R-707 starter culture with MTGase (Table 10). Fermented camel milk produced using CHN-22 starter culture with MTGase was significantly (*P<0.05*) higher in viscosity as compared to that of CHN-22 starter culture without MTGase (Table 10).

Table 10. The interaction effects of starter culture and MTGase on the viscosity (seconds) of fermented camel milk (Mean ± SE)

	MTGase	
Starter culture	With	Without
R-707	18.03 ± 0.13^{b}	18.24 ± 0.15
CHN-22	18.54 ± 0.24^{aA}	18.17 ± 0.14^{B}
$LSD_{(0.05)}$	0.245	

MTGase = microbial transglutaminase; LSD = least significant difference; Means in the same row having different capital letter superscripts are significantly different at *P<0.05*; Means in the same column having different small letter superscripts are significantly different at *P<0.05*.

There was significant difference (*P<0.05*) in viscosity between fermented camel milk samples produced by the addition of CMP and without CMP (Table 11). The viscosity of the fermented camel milk produced by the addition of CMP was significantly (*P<0.05*) higher than that of without CMP (Table 11).

Table 11. The main effects of starter cultures, CMP and MTGase on the viscosity (seconds) of fermented camel milk (Mean ± SE)

Starter culture	R-707	18.14 ± 0.10
	CHN-22	18.35 ± 0.15
CMP	With	18.50 ± 0.13[a]
	Without	17.99 ± 0.07[b]
MTGase	With	18.29 ± 0.15
	Without	18.20 ± 0.10

Means with different superscripts in the same column are significantly different at *P<0.05*; CMP = camel milk powder; MTGase = microbial transglutaminase.

DISCUSSIONS

Physicochemical Properties of Raw and Fermented Camel Milk

The average fat content of raw camel milk used in the present study is higher than 2.95% that was reported by Haddadin *et al.* (2008) and in agreement with that of El Zubeir *et al.* (2012) (3.5%). The average protein content of raw camel milk in the present study is higher than 2.54% that was reported by Khaskheli *et al.* (2005), and lower than that of Shamsia (2009) (3.46%) and El Zubeir *et al.* (2012) (3.7%). These variations could be attributed to various factors such as analytical measurement procedures, camel breed, stage of lactation, age, health status, parity, herd management practices, environmental conditions, geographical origin and seasonal variations (Al Haj and Al Kanhal, 2010; Khaskheli *et al.,* 2005; Konuspayeva *et al.,* 2009).

It was expected and also reported by Farnsworth *et al.* (2006), there was no significant difference in the chemical composition of fermented camel milk as a result of the different starter cultures used or by addition of MTGase. The higher titratable acidity of the fermented camel milk produced with CMP is perhaps due to the increased buffering capacity of the additional proteins, phosphates, citrates, lactates and other milk constituents (Walstra and Jenness, 1984). The titratable acidity of the fermented camel milk was significantly ($P< 0.05$) higher when MTGase was used with R-707 starter culture which might be attributed to the acid producing strain of homo-fermentative *Lactococcus lactis* (Walstra *et al.,* 2006) of R-707. Tesfemariam Berhe *et al.* (2018) found that R-707 starter culture acidified camel milk faster than CHN-22. Use of MTGase with CHN-22 starter culture significantly lowered titratable acidity of the fermented camel milk which might be due to the inter- or intra-molecular cross-linking of milk proteins by transglutaminase between a γ-carboxyamide group of glutamine residues and an ε-amino group of lysine residues which leads to the formation of an ε-(γ-glutamyl) lysine iso-peptide bond with generation of one molecule of ammonia per crosslink (Folk and Finlayson, 1977). On the contrary, Jooyandeh *et al.* (2015) reported that there was no significant difference in acidity between MTGase-treated yoghurts and control sample.

Therefore, the use of MTGase with CHN-22 starter culture can be an alternative method for reducing excess acid production. The cross-linking of low molecular weight peptides and amino acids required for the growth of starter bacteria was a possible reason of slow growth of starter bacteria and this causes slower acidity development in yoghurt products (Ozer *et al.,* 2007).

Textural Properties of Fermented Camel Milk

Firmness

Addition of MTGase has significantly increased gel hardness and similar results were found for camel milk yoghurt reported by Abou-Soliman *et al.* (2017). But addition of CMP resulted in a significantly lower firmness which is contra-intuitive as addition of skim milk powder to bovine milk is well known to increase hardness and enhance texture (Lucey, 2002); and addition of bovine skim milk powder has indeed been shown to have this effect in camel milk yoghurt (Abou-Soliman *et al.,* 2017). However, Attia *et al.* (2000) studied the glucono delta-lactone induced acidification of dromedary milk and they found that the casein micelles show a very marked initial drop in hydration (to approx. 50% of the initial value, compared to a drop of only 10-20% for bovine milk). These authors also noted that the solvation of minerals proceeded somewhat differently compared to bovine milk and found initial higher amounts of soluble calcium at neutral pH (~15%) compared to bovine (~7%). The demineralization of micelles in dromedary milk started at around pH 5.8 whereas in bovine milk demineralization this initiated at the onset of acidification, and it exhibits a more pronounced, sharper drop. Formation of hydrogen as well as electrostatic bonds, which are important in providing structure to acid coagula from bovine milk (Lucey, 2002) could be restricted in dromedary milk, resulting in the more fragile curd observed which appears to be formed from disassociated casein micelles (Attia *et al.,* 2000). Addition of CMP could possibly further aggravate this phenomenon by supplying additional soluble calcium and increased casein concentration, resulting in casein aggregates less prone to interact with each other.

In addition, when MTGase was applied, the resulting decrease upon addition of CMP was much more pronounced, illustrating that CMP, and hence extra camel milk protein, interfered with the positive effect of MTGase. This is also in stark contrast to the results of Abou-Soliman *et al.* (2017) who found that addition of bovine skim milk powder together with MTGase treatment markedly improved the texture of camel milk yoghurt. Our observed result could possibly be due to the MTGase enzyme preferentially acting within the micelle, binding casein molecules together and consequently changing the internal structure of the micelle, instead of binding micelles together and form aggregates (Mounsey *et al.,* 2005).

Elasticity

Elasticity (springiness) is a measure of ability of food to return to its original form after being compressed (Prakasan *et al.,* 2015). In the present study higher elasticity was observed for the fermented camel milk produced by the addition of MTGase, and Dinkcei (2012) reported that the addition of 1.85 U MTGase g^{-1} of protein significantly increased the cohesiveness of strained yogurt as compared to control sample. On the contrary, Prakasan *et al.* (2015) reported that there was no significant change observed in elasticity characteristic of MTGase treated *paneer*.

Cohesiveness

Cohesiveness indicates the strength of internal bonds making up the body of food and the degree to which a food can be deformed before it breaks (Radocaj, 2011). In the present study the cohesiveness of the fermented camel milk produced using R-707 starter culture with MTGase was significantly higher while the addition of CMP significantly reduced its cohesiveness. This is in line with the report by Iličić *et al.* (2013) who elucidated the cohesiveness of fermented milk samples were improved by the addition of MTGase as compared to control sample.

Adhesiveness

Fermented camel milk prepared with R-707 starter culture and MTGase showed significantly higher adhesiveness and the increased values in adhesiveness for camel milk yogurt treated with MTGase. In general, in the present study, the addition of MTGase improved the textural attributes of fermented camel milk. This could be attributed to the strengthening of the network structure by the enzyme as a result of forming inter- and intra-molecular isopeptide bonds in and between all types of milk proteins (Romeih *et al.,* 2014). It was unexpected that the addition of CMP did not improve the textural attributes of the fermented camel milk samples. Jooyandeh *et al.* (2015) concluded that the cross-linking of milk proteins by means of MTGase seems to be an acceptable alternative instead of addition of extra protein or stabilizer in yogurt production.

Consumer Acceptability of Fermented Camel Milk

The interaction of the three factors (starter cultures, CMP and MTGase) did not significantly affect the sensory attributes. However, from the comments given by the panellists, none of the fermented camel milk samples was considered as unacceptable. Farah *et al.* (1990) reported that fermented camel milk samples made with mesophilic lactic cultures was clearly preferred by panellists. The treatment with MTGase did not have a negative effect on aroma and flavour (Şanli, 2015). Similarly, Prakasan *et al.* (2015) suggested that MTGase treatment did not lead to any objectionable change in odor and appearance of the products, which could lead to rejection of products by the consumer.

Viscosity of Fermented Camel Milk

The use of MTGase with CHN-22 starter culture has significantly improved the viscosity of the fermented camel milk which might be attributed to MTGase cross-linking reaction that improved the viscosity of skimmed milk yoghurt (Aprodu *et al.,* 2012). Significantly higher viscosity values were also obtained for MTGase-treated yoghurt (Ozer *et al.,* 2007). The production of polysaccharides by lactic acid bacteria can greatly enhance the viscosity of fermented dairy products (Hati *et al.,* 2013).

CONCLUSION

We found that R-707 starter culture was better than CHN-22 starter culture in improving the texture of fermented camel milk particularly the cohesiveness and adhesiveness. Addition of R-707 starter culture with MTGase has improved the cohesiveness of fermented camel milk. The addition of CMP had a negative effect on the firmness, cohesiveness, adhesiveness and/or it decreased the textural attributes. The use of MTGase has improved the textural attributes of the fermented camel milk. Therefore, MTGase can be used by dairy industries and smallholder farmers to improve the textural attributes of fermented camel milk.

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Development of Yogurt from Camel Milk Using Exopolysaccharide Producing Lactic Acid Bacteria

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ABSTRACT

This research was conducted to develop and evaluate improved camel milk yogurt using a combination of exopolysaccharide (EPS) producing lactic acid bacteria (LAB), starch (S) and camel milk powder (CMP). The experiment had five treatments with YF-L904 culture used as EPS producing LAB and YC-X 11 culture as non-EPS producing LAB. Both strains of LAB composed of Streptococcus thermophilus (ST) and Lactobacillus, delbrueckii ssp bulgaricus (LB). About 400ml of camel milk sample was used for each treatment. The results show that both additives (starch and CMP) had significantly increased (P<0.05) protein, lactose, total solid, and solid not fat. The level of syneresis of yoghurt with EPS and starch was significantly (P<0.05) lower than the other yoghurt samples. Yogurt with EPS and starch had the highest viscosity and the lowest syneresis value (36.17s and 36.67%, respectively) among the treatments considered. The highest level of syneresis was observed on the yoghurt produced with CMP (68.33%) as compared to yogurt produced with starch alone (36.67%) and starch with CMP (54.67%). The level of starch inclusion in the yogurt had significant effect (P<0.05) on reducing syneresis and increasing viscosity. The texture value of yogurts produced with starch had significantly (P<0.05) higher firmness, elasticity, cohesiveness and adhesiveness in both camel and cow milk yogurt. However, CMP addition did not improve the textural properties of both camel and cow milk yogurt. In conclusion, using EPS producing LAB and addition of starch in yogurt making from camel milk could improve viscosity and textural properties and reduce the syneresis of the product.

Key words: Camel milk, Starch, Syneresis, Texture, Viscosity, Yogurt

INTRODUCTION

Earlier studies indicated that camel milk could not be processed in to different dairy products, but used only for drinking (Yagil *et al*., 1984). The difficulties might be related to the absence of the whey protein (β-LG) and a low proportion of κ-casein in camel milk that cause differences in dairy processing (Yonas Hailu *et al*., 2016). Even though there are difficulties of processing camel milk, some researchers reported that various products are produced from camel milk including yogurt (El-Zubeir and Jabreel, 2008; Rüegg and Farah, 1991; Aleme Asrasie *et al*., 2013).

Camel milk whey protein doesn't contain β-LG and has lower amount of k-casein (Shamsia, 2009) which could lead camel milk to coagulate slowly and have poor texture. Yogurt texture is a very important characteristic that affects its quality such as appearance, mouth feel and overall acceptability. The most common sensory attributes related to yogurt texture are thickness /viscosity, smoothness and sliminess (or ropiness). Many quality problems, such as low viscosity or high syneresis, which occur during milk product manufacturing, are often solved by increasing the total solid or adding stabilizers, such as milk powder, modified starch, carrageenan, guar gum, pectin, gelatin and sodium caseinate. Yoghurt from camel milk stabilized with gelatin and corn starch was

acceptable and comparable with cow milk yoghurt (Muliro, 2007). Stabilizers and polysaccharide-producing cultures have also been used to improve texture and prevent syneresis (Escalante *et al*., 1998).

Lactic acid bacteria (LAB) are used in many fermented foods particularly fermented dairy products such as cheese, buttermilk, and fermented milks. Some LAB produces lactic acid and carbon dioxide that contributes to texture and shelf life of fermented foods some also produces acetic acid, diacetyl, and acetaldehyde for flavour. In addition, certain strains of LAB are able to synthesize exopolysaccharides (EPS) that play a major role as natural texturizer in industrial production of yoghurt, cheese, and milk-based desserts. In general, EPS are known to have highly significant effects on the texture properties of many types of fermented milk products (Cerning, 1990). Moreover, they are considered as natural bio thickeners since they are produced *in situ* by LAB starter culture and helps to avoid the use of some other stabilizers which are prohibited or restricted (Amatayakul *et al*., 2005). A protein gel (mainly casein) interacts with EPS that formed in the protein matrix, can reduce the amount of free water and minimize syneresis (Tamime *et al*., 1984).

The bovine milk products fermented with EPS-producing cultures obtain high viscosity, high creaminess as well as an increased water-binding capacity (Rawson and Marshall, 1997). However, camel milk fermented products have not been developed using EPS producing LAB and starch as additive so far. Therefore, this study was conducted with the objective of developing yogurt from camel milk using EPS producing thermophilic lactic acid bacteria, tapioca starch and camel milk powder.

MATERIALS AND METHODS

Milk Sample Collection

Fresh camel milk samples were collected from camel rearing pastoralists in Erer valley of Babile district, eastern Hararghe Zone, Ethiopia. Milk was sampled by directly milking into clean containers. Throughout the experiment, around 18 litres of camel milk samples were collected from eight different camels and was brought to Haramaya University Dairy Technology Laboratory using clean plastic containers (Jerry-cans) within two hours. Cow milk, which was used for comparison, was collected from Haramaya University dairy farm. Both milk samples were collected early in the morning.

Materials

Additives (stabilizers) such as camel milk powder (CMP) (which constituents 25g fat, 40g carbohydrates, 25g protein and 1.6g salt per 100g) (*Camelicious*, Dubai, United Arab Emirates), Starter cultures (YF-L904, used as EPS (+ve) and YC-X11 used as EPS (-ve)) (Chr Hansen A/S, Denmark), and Tapioca Starch (Cream Tex® 75720 (Sino-Thai Starch Co., Ltd, Thailand) were donated from Denmark.

Physicochemical Analysis of Milk and Yogurt

The chemical composition of milk and yogurt that include fat, protein, lactose, TS and SNF was analysed at Haramaya University Dairy Technology laboratory using MilkoScan (MilkoScan™ FT1 FOSS, Hillerød, Denmark). The pH values of raw milk as well as yogurt were, however, analysed using digital pH meter (pH-016 PH METER).

Procedure for Yogurt Production

Yoghurt samples were prepared according to Lee and Lucey (2010) and Dirar (1993) methods with slight modifications. Fresh camel milk sample was first filtered using sterile cheese clothes to remove impurities. The raw milk was heated to 40^{o}C for 1 minute before adding tapioca starch and/or CMP. Then, tapioca starch and camel milk powder were immediately added to the camel milk followed by mixing at 5.0x10^{3} rpm for 2 minutes using an Ultra-Turrax T18 homogenizer (IKA-Labortechnik, Staufen, Germany) to evenly disperse and thoroughly mix tapioca starch and CMP. After proper mixing, the milk was pasteurized at 85^{o}C for 30 min as described by Dirar (1993) and rapidly cooled to 43^{o}C. Then, starter cultures (YF-L904 as EPS (+ve) and YC-X11 as EPS (-ve) were inoculated at a concentration rate of 0.8ml for 400ml of milk sample (Tesfamariam Berhe *et al.,* 2018). The inoculated milk samples were incubated at 43^{o}C. The pH of the milk was monitored until it reached pH 4.6 using digital PH meter. The same procedure was used to produce yogurt from cow milk for comparison.

Treatments of Yogurt

The experiment had five treatments. In addition to starch and CMP, two types of starter cultures: YF-L904 as EPS producing LAB (YEPS) and YC-X 11 as non-EPS producing LAB (YNEPS) were used in treatment setups as follows:

Treatment 1 (YNEPS): Camel milk + EPS (-) LAB

Treatment 2 (YEPS): Camel milk + EPS (+) LAB

Treatment 3 (YEPS+S): Camel milk + EPS (+) LAB+ Starch (5%)

Treatment 4 (YEPS+CMP): Camel milk + EPS (+) LAB+ Camel milk powder (5%)

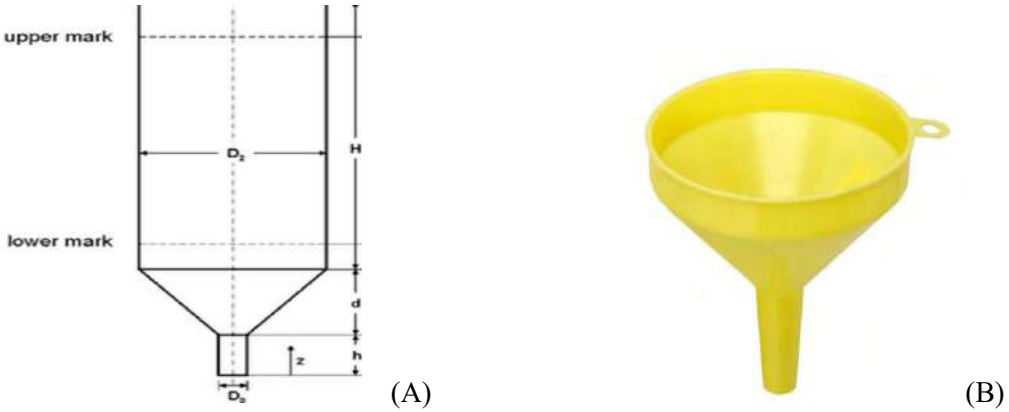
Treatment 5 (YEPS+S+CMP): Camel milk + EPS (+) LAB+ Starch (2.5%) + Camel milk powder (2.5%). A 400ml of camel milk sample was used in blue cap reagent bottles for each treatment.

The concentration level of starch and CMP was based on the trial done for Paneer type cheese at Copenhagen University Dairy Technology Pilot Test, Denmark; and the concentration level for both EPS-producing thermophilic LAB and non-EPS producing LAB was used according to Tesfamariam Berhe *et al*. (2018). Both strains of LAB composed of *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii ssp bulgaricus* (LB). The same procedure was followed for cow milk yogurt and all treatments were done in triplicates. T1 yogurt (YNEPS) was comparable with T2 yogurt (YEPS) whereas T3 (YEPS+S), T4 (YEPS+CMP) and T5 (YEPS+S+CMP) yogurts were compared with each other due to the presence of additives (CMP and starch) in addition to exopolysaccharides producing LAB.

Viscosity Analysis

Viscosity of yogurt was analysed and evaluated by simple posthumous funnel test. The Posthumous funnel test is an empirical and fast method used to evaluate the viscosity of yoghurts and other fermented dairy products. It is based on the time needed to the yoghurt to pass through the posthumous-funnel. The procedure was filling the funnel with yoghurt to the upper mark (on the inside), while keeping the hole at the bottom of the funnel closed with a finger. Then the finger

removed and the stopwatch started at the same time. And then the time it takes is measured until the lower mark or the metal pin sticking out is visible. The flow time is an indication of the viscosity and mouth-feel of the yoghurt. This method was applied for camel milk yogurt, however, it was not applied for cow milk since cow milk yogurt had higher viscosity especially for the yogurt that produced using starch. The viscosity of cow milk yogurt was therefore measured using plastic funnel. Yoghurt samples of 200ml were poured in to plastic funnel for each treatment and allowed to pass through the hole of plastic funnel and measured the time elapsed. Figure 1 shows dimensions and height of posthumous funnel and plastic funnel used for this experiment.



(A) (B)

Figure 1. Posthumous funnel (A) and plastic funnel (B)

Note: D_0 = the lower hole diameter (**4.5mm**), H= height (**108mm)** and **D2**= the upper hole diameter (**60mm**). Cow milk yogurt was measured using plastic funnel (the lower hole diameter =**10mm** and the upper hole diameter=**100mm**).

Syneresis Analysis

The syneresis of yogurt samples were monitored after 24h of storage at 4°C by measuring the quantity of whey separated. Ten gram of yogurt sample was poured into a graduated cylinder and put in to a centrifuge (350xg, 20°C for 10 minutes) (Centurion scientific Ltd, West Sussex BN16b1AW, UK). The amount of the watery part, which was separated on the top in graduated cylinder was measured and calculated according to the following formula (Farnsworth *et al*., 2006 and Jacek Domagała, 2012) with slight modification.

$$\text{Syneresis (\%)} = \frac{\text{Whey expelled (g)}}{\text{Initial yogurt (g)}} 100$$

Texture profile Analysis of Yogurt

Texture analysis was performed by using a TA XT2 texture analyzer (Stable Micro Systems Ltd, court, surrey GU7 1 YL, UK). A cylindrical probe of 35mm diameter was used with a pre-test, compression and post-test of a sample, where the speed of the probe used in the procedure was 1.0mm/s, 2.0mm/s and 10.0mm/s, respectively. Compression distance was 20 mm in to the sample. All samples height was 80ml. Four parameters were evaluated for texture; (1) Firmness (hardness) defined as the maximum peak force during the first compression cycle (first bite), (2) Elasticity (springiness) height that the food recovers during the time elapses between the end of the first bite and the start of the second bite, (3) Adhesiveness the negative area for the first bite, representing the work

necessary to pull compressing probe away from sample, (4) Cohesiveness defined as the ratio of the positive force area during the second compression to that during the first compression. Figure 2 shows the four parameters used in this experiment.

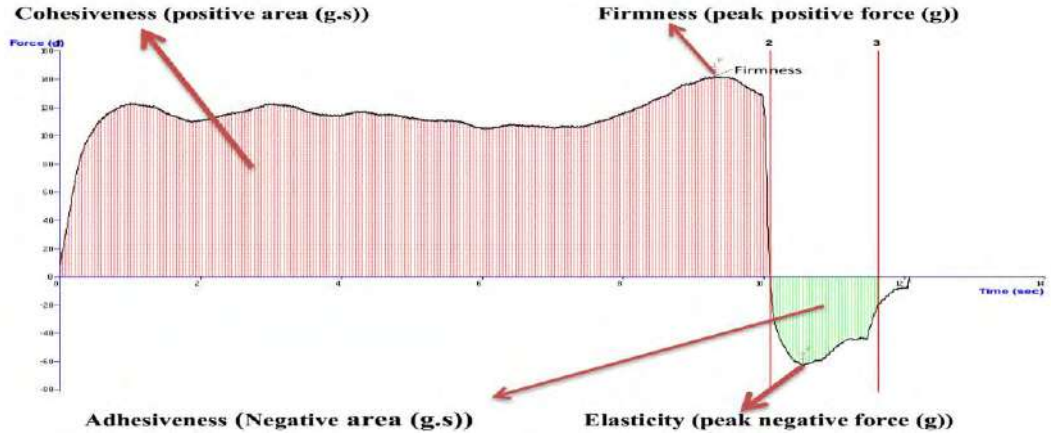


Figure 2. Example of Texture Profile Analysis curve

Acceptability Test

Sensory quality and acceptability of yogurt was performed by ten voluntary panellists selected based on the criteria suggested by Hashim (2002): age between 18 and 34 years, and usual consumers of camel milk or fermented camel milk and yogurt from milk of other species to rate the acceptability of the yogurt based on colour, taste, aroma, texture and overall acceptability using a 7-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately and 7 = like extremely). The sample was coded with three digits before given to the panellists. The sample was taken out from the refrigerator and thawed to the room temperature before presented to the panellists. Pure water was given to the panellists to drink and rinse their mouth before testing and evaluating the next yogurt sample.

Statistical Analysis

A Complete Randomized Design (CRD) with one-way analysis of variance (ANOVA) was performed to analyse data on physicochemical analysis, textural properties and sensory evaluation. Means were separated by Least Significant Difference (LSD) procedure using SAS statistical software version 9.1 (SAS institute. Cary, NC, USA). All values were reported as mean ± standard error mean (SEM) and significances were determined at $P<0.05$. All samples were conducted in triplicates.

RESULTS

Physicochemical Properties of Camel and Cow Milk

Results of the physicochemical properties of raw camel milk used for the yogurt making experiment observed in the current study (Table 1) are within the range reported by different authors.

Table 1. Physicochemical composition of raw camel and cow milk samples used for yogurt making

Variables	Values	
	Camel Milk	**Cow Milk**
Fat (%)	3.67±0.84	3.55±0.19
Protein (%)	2.50±0.09	3.16±0.15
Lactose (%)	4.93±0.06	4.40±0.18
SNF (%)	7.88±0.19	8.33±0.33
TS (%)	11.81±0.86	11.95±0.48
pH	6.54±0.01	6.60±0.01

Values in the table are mean±SE of three replications; Total Solid (TS), Solid Not Fat (SNF)

Yogurt Physicochemical Composition

Differences in physicochemical compositions were observed between the yogurt made without additives (T1 and T2) and with additives (T3, T4 and T5) (Table 2). Accordingly, treatment 4 (YEPS+CMP) and treatment 5 (YEPS+S+CMP) were significantly higher (*P<0.05*) protein, SNF, and TS compared to the other treatment samples. This was due to the added CMP that could increase the chemical compositions compared to yogurt produced without CMP additions. Treatment 3 (YEPS+S) and treatment 5 (YEPS+S+CMP) were also showed significantly (*P<0.05*) higher lactose content due to the addition of starch (Table 2). The presence of exopolysaccharides produced by LAB did not bring any change on physicochemical composition. However, it contributed to the specific rheology and texture of the products. Cow milk yogurt produced with starch (YEPS+S) also had significantly (*P<0.05*) higher lactose (7.72±0.75) compared with the other treatment groups of cow milk yogurt sample.

Table 2. Physicochemical properties of camel and cow milk yogurt (%)

Parameters	Treatments (Mean±SE)				
	T1 (YNEPS)	T2 (YEPS)	T3 (YEPS+S)	T4 (YEPS+CMP)	T5 (YEPS+S+CMP)
Camel milk yogurt					
Fat	3.42±0.58	3.27±0.45	3.21±0.62	4.37±0.46	3.75±0.49
Protein	2.54±0.08^{c}	2.50±0.10^{c}	2.32±0.06^{c}	3.36±0.12^{a}	2.93±0.07^{b}
Lactose	3.73±0.14^{b}	3.84±0.10^{b}	6.97±0.94^{a}	5.29±0.50^{ab}	5.63±0.53^{a}
TS	11.23±0.60^{b}	11.07±0.50^{b}	12.65±0.81^{ab}	14.48±0.49^{a}	13.80±0.46^{a}
SNF	7.76±0.23^{b}	7.76±0.35^{b}	9.65±0.71^{a}	10.11±0.29^{a}	9.94±0.56^{a}
Cow milk yogurt					
Fat	3.31±0.18	3.29±0.09	3.49±0.12	3.90±0.68	3.86±0.25
Protein	3.10±0.15	3.02±0.12	2.84±0.13	3.59±0.44	3.21±0.17
Lactose	4.65±0.10^{b}	4.52±0.06^{b}	7.72±0.75^{a}	4.67±0.49^{b}	5.67±0.43^{b}
TS	11.39±0.41	11.08±0.28	13.54±0.38	12.63±1.85	13.66±0.17
SNF	8.11±0.27^{ab}	7.89±0.15^{b}	9.93±0.24^{a}	9.27±1.36^{ab}	9.82±0.15^{ab}

Note: T1(Y_{NEPS}) =yogurt produced with non-EPS LAB, T2(Y_{EPS}) = yogurt produced with EPS LAB, T3(Y_{EPS+S}) = yogurt produced with EPS producing LAB and starch, T4($Y_{EPS+CMP}$)= yogurt produced with EPS producing LAB and camel milk powder and T5($Y_{EPS+S+CMP}$)= yogurt produced with EPS producing LAB, starch and camel milk powder. Means with the different letter within the same row are significantly different at (P< 0.05) with LSD. Each value is the mean of three replication (n=3). Total Solid (TS), Solid Not Fat (SNF). The same procedure was followed for cow milk yogurt.

Yogurt Rheological Properties

Viscosity and syneresis

The yogurt produced from camel milk with exopolysaccharides producing lactic acid bacteria and starch had a very good viscosity and less amount of whey separation compared to the yogurt produced with non-exopolysaccharides producing LAB (Figure 3). The syneresis value (71%) for camel milk yoghurt made with non-EPS was higher that yoghurts with EPS (67%)). Less viscosity was observed in camel milk yogurt produced with non-EPS (19.23 sec.) due to the absence of EPS when compared to T2 (21.6 sec.) (Figure 3). Therefore, the level of syneresis of T3 was significantly ($P<0.05$) lower than the other yoghurt samples.

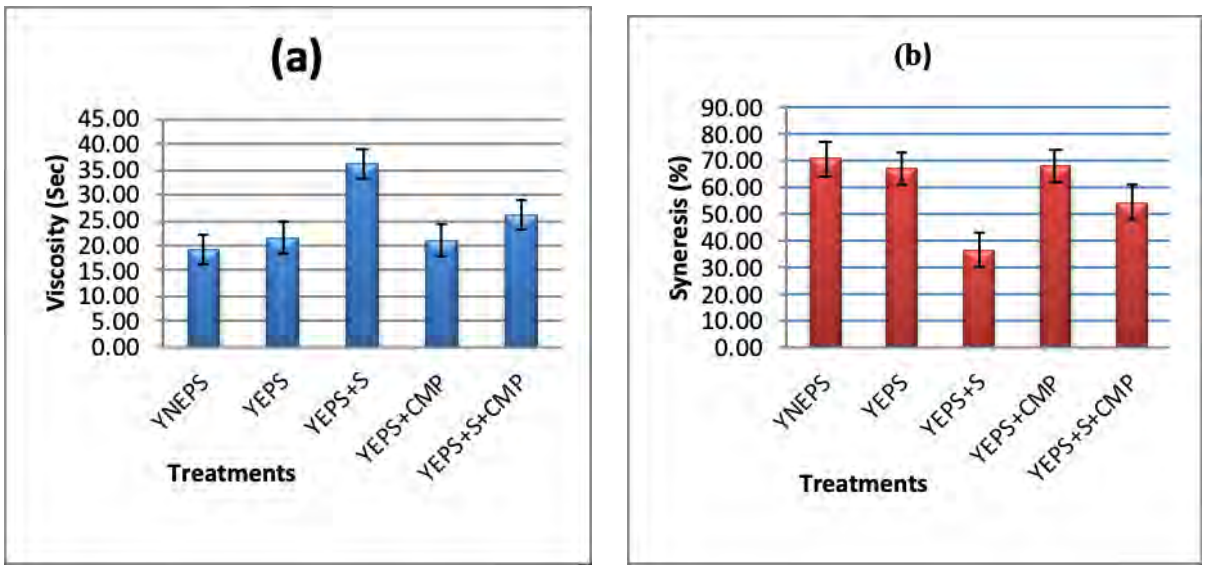


Figure 3. Camel milk yogurt viscosity and syneresis:

Yogurt produced with non-EPS producing Lactic Acid Bacteria (YNEPS), Yogurt produced with EPS-producing Lactic Acid Bacteria (YEPS), Yogurt produced with EPS-producing Lactic Acid Bacteria and Starch (YEPS+S), Yogurt produced with EPS-producing Lactic Acid Bacteria and Camel Milk Powder (YEPS+CMP) and Yogurt produced with EPS-producing Lactic Acid Bacteria, starch and camel milk powder (YEPS+S+CMP). Vertical bars indicate standard errors (SE) of least square means (n = 3).

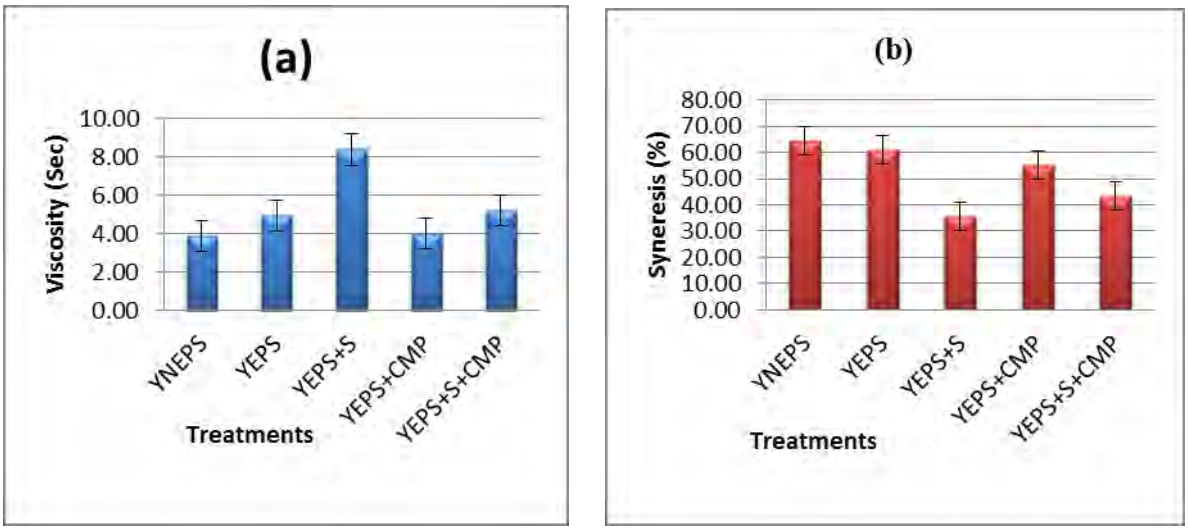


Figure 4. Cow milk yogurt viscosity and syneresis:

Yogurt produced with non-EPS producing Lactic Acid Bacteria (YNEPS), Yogurt produced with EPS-producing Lactic Acid Bacteria (YEPS), Yogurt produced with EPS-producing Lactic Acid Bacteria and Starch (YEPS+S), Yogurt produced with EPS-producing Lactic Acid Bacteria and Camel Milk Powder (YEPS+CMP) and Yogurt produced with EPS-producing Lactic Acid Bacteria, starch and camel milk powder (YEPS+S+CMP). Vertical bars indicate standard errors (SE) of least square means (n = 3).

The highest level of syneresis was observed with the yogurt treated with CMP (T4, 68.33%). On the other hand, the amount of starch added to the yogurt showed significant effects ($P<0.05$) in reducing syneresis and increasing viscosity (Figures 3 and 4).

Yogurt texture

T1 yogurt found to be lower in firmness in both camel and cow milk yogurt compared with T2 (Figures 5a and 6a). T3 Yogurt was significantly different ($P<0.05$) in firmness of camel and cow milk yogurt as compared to T4 and T5 (Figures 5a and 6a). In general, firmness, cohesiveness, elasticity and adhesiveness of the yogurt texture were positively affected in T3 yogurt samples made from both cow and camel milk (Figures 5 and 6). T3 yogurt was also more gel stable due to the mixture with tapioca starch.

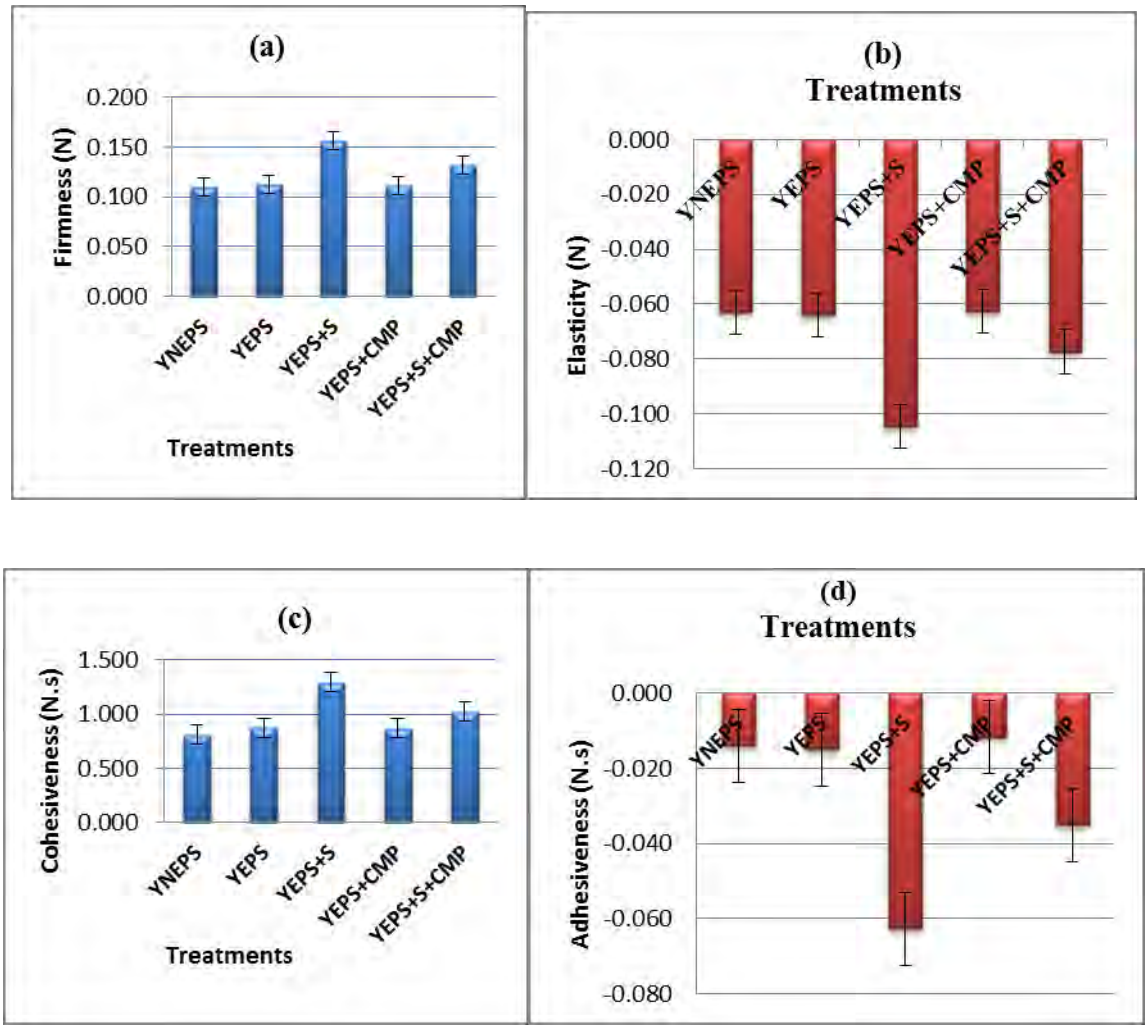


Figure 5. Texture properties of camel milk yogurt

Yogurt produced with non-EPS producing LAB (YNEPS), yogurt produced with EPS-producing LAB (YEPS), yogurt produced with EPS-producing LAB and Starch(YEPS+S), yogurt produced with EPS-producing LAB and camel milk powder (YEPS+CMP) and yogurt produced with EPS-producing LAB, starch and camel milk powder(YEPS+S+CMP. Vertical bars indicate standard errors (SE) of least square means (n = 3). Firmness (a), Elasticity (b), Cohesiveness (c) and Adhesiveness (d)

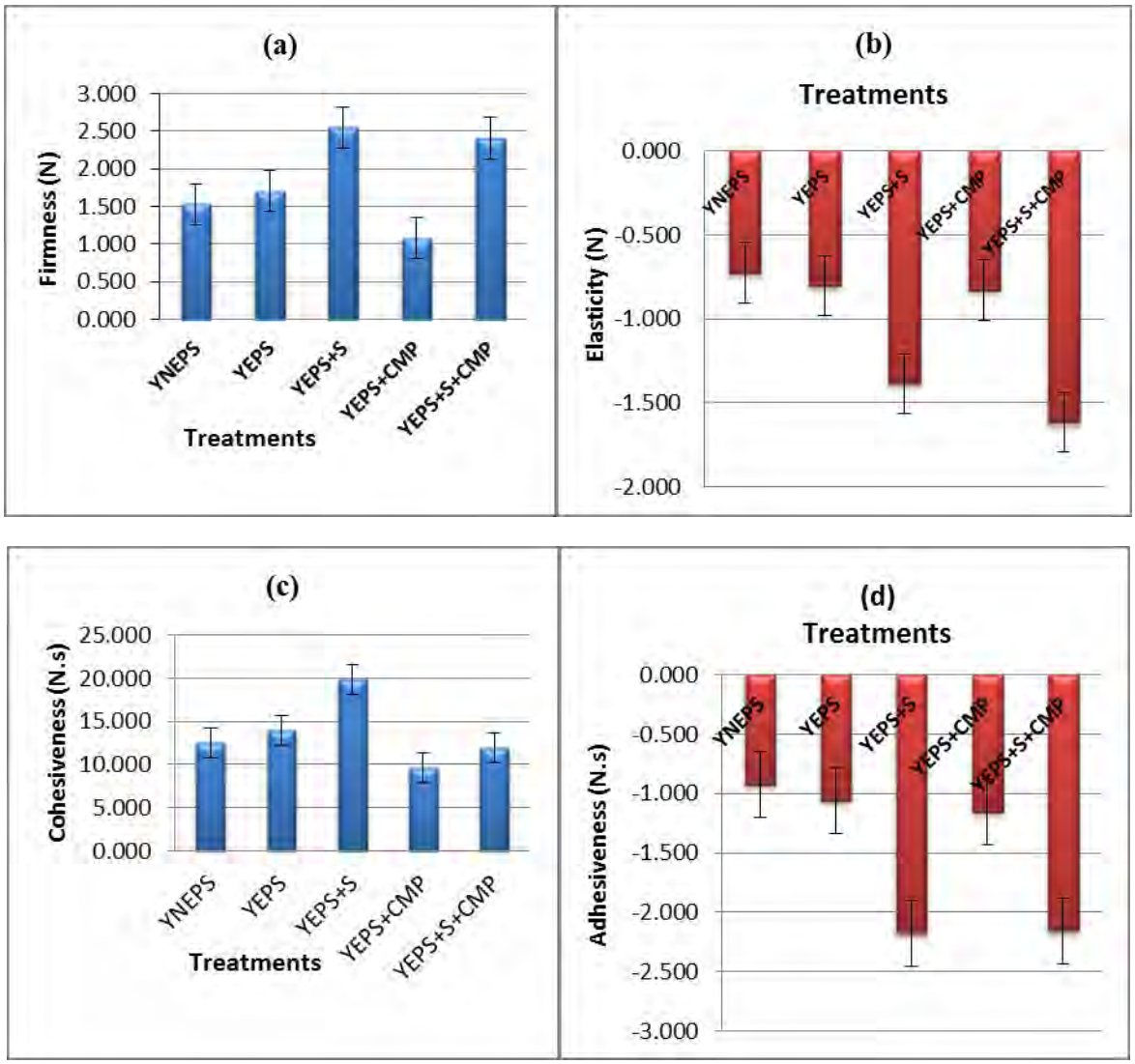


Figure 6. Texture properties of cow milk yogurt

Yogurt produced with non-EPS producing LAB (YNEPS), yogurt produced with EPS-producing LAB (YEPS), yogurt produced with EPS-producing LAB and Starch (YEPS+S), yogurt produced with EPS-producing LAB and camel milk powder (YEPS+CMP) and yogurt produced with EPS-producing LAB, starch and camel milk powder (YEPS+S+CMP. Vertical bars indicate standard errors (SE) of least square means (n = 3). Firmness (a), Elasticity (b), Cohesiveness (c) and Adhesiveness (d)

Acceptability of Camel and Cow Milk Yogurt

There was no significant difference ($P>0.05$) in the average mean scores of all treatments in camel milk yogurt (Table 3). Most of the panellists described that some of the camel milk yogurt taste was slightly salty. However, cow milk yoghurt produced with non-EPS producing LAB (YNEPS) was rated the most preferred in color and was significantly different ($P<0.05$); On the other hand, the color of T4 yoghurt (YEPS+CMP) from cow milk yogurt was rated less preferred and significantly different ($P<0.05$) from the other all cow yogurt samples. This was most probably because of an addition of CMP to cow milk. The color of T1 (YNEPS) of cow milk yogurt was accepted due to the absence of any addition. However, there was no significantly different ($P>0.05$) in the average mean scores of aroma, texture and overall acceptability (Table 3).

Table 3. Sensory properties of camel and cow milk yogurt

Treatments	Sensory Parameters (Mean±SE)				
	Colour	Taste	Aroma	Texture	Overall Acceptability
Camel milk yogurt					
T1(YNEPS)	6.33±0.20	5.33±0.20	6.00±0.17	5.10±0.10	5.23±0.53
T2 (YEPS)	6.10±0.20	5.23±0.29	5.66±0.52	5.33±0.37	5.53±0.23
T3 (YEPS+S)	6.43±0.29	5.80±0.10	5.86±0.29	5.66±0.20	5.93±0.34
T4 (YEPS+CMP)	6.66±0.20	5.56±0.29	6.10±0.72	5.56±0.29	6.00±0.17
T5 (YEPS+S+CMP)	6.66±0.20	5.66±0.20	6.10±0.20	5.70±0.00	6.00±0.17
Cow milk yogurt					
T1(YNEPS)	6.55±0.11^{a}	5.89±0.22^{ab}	5.89±0.48	6.00±0.19	5.88±0.29
T2 (YEPS)	6.44±0.29^{ab}	6.00±0.19^{ab}	5.89±0.22	6.11±0.11	6.11±0.22
T3 (YEPS+S)	6.44±0.11^{ab}	6.66±0.19^{a}	6.11±0.11	6.00±0.57	6.44±0.22
T4 (YEPS+CMP)	5.77±0.39^{b}	5.67±0.57^{b}	5.55±0.22	5.89±0.61	5.55±0.58
T5 (YEPS+S+CMP)	6.22±0.11^{ab}	6.00±0.19^{ab}	6.11±0.11	6.00±0.19	6.00±0.19

^{a,b,c}: Means with different superscript in the same column are significantly different at $p<0.05$. Each value was the mean of three replications (n=3)

DISCUSSION

Physicochemical Properties of Milk and Yogurt

The results observed for milk physicochemical properties in the current study are found within the range reported by Al-Zoreky and Al-Otaibi (2015); and Mortada and Omar (2013). The average mean value of fat, protein and lactose of the current findings were also found in the range reported by Knoess *et al*. (1986). The presence of exopolysaccharides which was produced by LAB did not bring any change on physicochemical composition. However, it contributed a lot to the specific rheology and texture of the yogurt samples.

Yogurt Rheological Properties

In the present study, yogurt produced from camel milk with EPS producing LAB and starch had a very good viscosity and less amount of whey separation. The result agrees with the findings of Early (1998) who reported that the viscosity of yoghurt is usually enhanced by the addition of stabilizers and thickeners such as modified or natural starches, alginates, carrageenan, edible gums, pectin and celluloses. Corn starch was also reported to be better in reducing syneresis and increasing viscosity compared with the other stabilizers used in the experiments (Athar *et al*., 2000). Moreover, Vedamuthu (1991) and Hess *et al.* (1997) found that ropy strain of *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* used to produce smooth and viscous yogurt. These bacteria, often called slime-producing bacteria, produce exopolysaccharides, which helps to increase the viscosity.

Bouzar *et al*. (1996) and Folkenberg *et al. (*2006) also reported that some EPS-producing LAB showed a higher viscosity and a lower degree of syneresis compared with non-EPS-producing LAB. Using ropy-exopolysaccharide (ropy-EPS) producing starter cultures, syneresis could be overcome since non-EPS starter cultures had the highest level of syneresis (Amatayakul *et al*., 2006). Therefore, the level of syneresis of T3 (yoghurt with EPS and starch) was significantly ($P<0.05$) lower than that of the other yoghurt samples.

According to the report of Sodini *et al* (2004), as cow milk powder increases the protein content of yogurts, the viscosity, gel strength, and whey retaining ability of the yogurt made from cow milk also increase. However, addition of camel milk powder to both cow and camel milk did not

contribute for reducing syneresis and increasing viscosity and also did not improve the texture in general (Figures 3 and 4). In contrast, viscosity and syneresis were positively affected by the addition of starch; this might have arisen from the high-water binding capacity of starch both in camel and cow milk (Figures 3 and 4).

The addition of CMP weakens the gel matrix and weak gel that could lead to spontaneous whey separation, having poor texture. Improved yogurt viscosity is observed when the total solids content of milk is increased (Guirguis *et al*., 1984; Becker and Puhan, 1989; Wacher-Rodarte *et al*., 1993). However, T4 yogurt (YEPS+CMP) of the current result observed did not agree with the above findings since the addition of CMP to both camel and cow milk yogurt did not reduce the syneresis of the yogurt and also did not increase the viscosity of the final product. This report clearly shows the difficulty of producing yogurts with acceptable rheological properties with the addition of CMP. Nevertheless, the report of Mortada and Omer (2013) indicated that camel milk yoghurt treated with 5 and 7% skim milk powder improved the viscosity value ($P \leq 0.01$) during storage period. The report of Todoric and Bajic (1979) also demonstrated that addition of skim milk powder to yoghurt improved viscosity and prevented whey separation. According to Tamime and Robinson (1985), viscosity of the product is directly proportional to the level of protein present. Added CMP, which comprised of extra protein, however, could not improve the viscosity of the current yogurts. This means that the current finding did not agree with the report of Tamime and Robinson (1985). The possible reason for poor interaction between proteins needs to be investigated.

The highest firmness values observed in T3 (YEPS+S) were due to the combined effects of high solid content of starch and the presence of exopolysaccharides (EPS). This result agrees with the finding of Kessler (1981) who reported the importance of starch for the yogurt firmness. Ropiness and the protein matrix that are more responsible for hardness (Tunick, 2000). Other researchers (De Vuyst and Degeest, 1999; Hassan *et al*., 2002) also reported that EPS could improve the texture of bovine yogurt, because exopolysaccharide produced by LAB interacts with the free water in the gel-like structure. Thus, the yogurts made from EPS producing starters showed better textural characteristics.

The added starch with the presence of EPS entered in to the protein matrix and strengthens the internal bonds thereby improved the cohesive properties of the product. This might be due to the protein matrix in yogurt are more responsible for cohesiveness (Tunick, 2000). Rawson and Marschall (1997) also revealed that adhesiveness and cohesiveness could be linked to EPS produced by specific strains of yoghurt *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

In general, firmness, cohesiveness, elasticity and adhesiveness of the yogurt texture were positively affected in T3 (YEPS+S) yogurt samples made from both cow and camel milk. The T3 samples were more gel stable due to the mixture with tapioca starch. According to the report of Sandoval-Castilla *et al*. (2004) the solubilized molecules of modified tapioca starch might be integrated to the casein micelle network and be responsible for structure openness. Yogurt produced with EPS-producing LAB (YEPS) also had a positive contribution for textural properties as compared with non-EPS producing LAB. Therefore, desirable texture properties of yoghurts with low syneresis, especially camel milk yogurt, were achieved using EPS producing LAB together with Starch. However, yogurt produced with CMP, showed no positive effect on viscosity, syneresis and textural properties. For camel milk yogurt, this might be attributed to the highest antimicrobial factors present in camel milk that may cause the difficulty of producing fermented camel milk products with good consistency (El-Agamy *et al*., 1992). This might be related to the added camel milk powder comprised of extra antimicrobial factors. The second reason for both camel and cow milk yogurt could be due to the larger casein micelle present in camel milk and camel milk powder. Eksterend *et al*. (1980) found out that the content of k-casein decreases with increasing casein micelle size.

There was no significant difference in the average mean scores of acceptability of all treatments in camel milk yogurt and the majority of the panellists described that some of the camel milk yogurt taste was slightly salty. This was in line with the report of El-Agamy (1994) and Indra and Erdenebaatar (1994) who reported that the taste of camel milk is salty due to camels' feeding system.

CONCLUSION

We found that the use of exopolysaccharides producing LAB for developing yogurt from camel or cow milk can improve sensory and textural qualities of the products. The viscosity of yogurt was increased and similarly the level of whey separation (syneresis) was decreased in product made with EPS producing LAB. Yogurt produced with EPS producing LAB and starch had a better texture compared to that of yogurt produced with CMP. Viscosity and syneresis was significantly improved by the addition of tapioca starch which may arise from high water binding capacity of starch. Generally, tested EPS producing LAB and addition of tapioca starch positively affect textural properties (such as firmness, elasticity, cohesiveness and adhesiveness), of both camel and cow milk yogurt. However, addition of CMP to both camel and cow milk could not improve the texture and viscosity of both camel and cow milk yogurt.

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Effect of Fertilizer Level and Harvesting Date on Yield and Nutritive Value of Desho Grass (*Pennisetum pedicellatum)* in Hula and Bule districts of the Southern Region of Ethiopia

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ABSTRACT

A study was conducted to investigate the effect of fertilizer level and harvesting date on agronomic traits, biomass yield and chemical composition of Desho grass (Pennisetum pedicellatum) at Hula and Bule districts of Sidama and Gedeo zones, respectively, Southern Nations, Nationalities and Peoples` Region of Ethiopia. A factorial arrangement was employed using a randomized complete block design with 3 level of nitrogen fertilizer (0, 41 and 73 kg N/ha) and 3 harvesting dates (112, 133 and 154 days). Plant height, number of tillers per plant, leaf length and leaf to stem ratio were determined. The ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) contents were analyzed. In vitro organic matter digestibility (IVOMD), dry matter yield (DMY) and CP yield were also quantified. Data were analyzed using the general linear model procedures of the Statistical Analysis System. Plant height was greater (P<0.05) at 154 days of harvesting than at the other two harvesting dates and when 73 kg N/ha was applied compared to application of 41 kg N/ha or no fertilizer. The tiller number was lower (P<0.05) when harvested at 112 days after planting compared to those harvested at 133 and 154 days with no significant differences between the latter two harvesting dates. Tiller number was higher (P<0.05) at the application of 73 kg N/ha than no fertilization application (0 kg N/ha). The leaf length was greater (P<0.05) when 73 kg N/ha was applied compared to the other two N fertilization levels. The leaf: stem ratio decreased (P<0.05) with increasing harvesting date. The CP content was higher (P<0.05) when 41 and 73 kg N/ha was applied compared to the unfertilized treatment (0 kg N/ha). Ash and CP contents were higher (P<0.05) at 154 days than at 112 days of harvesting. The NDF, ADF and ADL contents increased (P<0.05) with increasing harvesting date but showed a decreasing trend with increasing N fertilization level. The IVOMD was higher (P<0.05) at early (112 days) harvesting date than at the intermediate (133 days) and late (154 days) harvesting date. The DMY and CP yield increased (P<0.05) with increasing harvesting date and N fertilizer level. In conclusion, higher yield could be obtained at later harvesting date (154 days) with application of 73 kg N/ha, without affecting quality attributes. However, further study is needed to verify the cost benefit and environmental impact of increased fertilizer level.

Keywords: *Desho grass, fertilizer level, harvesting date, biomass yield, nutritional value*

INTRODUCTION

Livestock keeping is an integral part of the farming system in Ethiopia. However, the productivity of the Ethiopian livestock is low. The major setback is shortage in terms of both quantity and quality of feed resource (Shapiro *et al.,* 2015; Bimrew *et al*., 2017). Provision of adequate feed supply is essential to ensure economically viable and environmentally friendly livestock production. This requires supplementing the traditionally available feed resource with improved and suitable forage species.

Desho grass *(Pennisetum pedicellatum)* is a grass indigenous to Ethiopia, which is highly popular and widely cultivated in southern Ethiopia as source of livestock feed. The grass is also widely used in

soil and water conservation activities to combat land degradation and to improve productivity of land. Farmers in many parts of Ethiopian highlands showed spontaneous adoption of Desho grass production because of its merits as animal feed and in soil and water conservation and management (Getahun *et al.*, 2015; Tekalegn *et al*., 2017). It has the potential to meet the challenges of feed scarcity as it gives high biomass yield per unit area and ensures year round forage supply due to its rapid growth and drought tolerance (Abebe *et al.,* 2011). So far, there is little information on Desho grass production and agronomic managements. Fertilization determines soil and plant nutrient contents, which influence yield and chemical composition of grass pasture. Fertilization is also a major factor that increases pasture yield and nutritive value, including the crude protein content and digestibility, leading to improvement in livestock production (Peyraud and Astigarraga, 1998).

The correct use of relatively inexpensive and simple management practices such as appropriate harvesting date and fertilizer level can help increase the level of fodder production (Yasin *et al.*, 2003). Effect of altitude and harvesting dates, and effect of harvesting dates and spacing on morphological characteristics, yield and nutritive value of Desho grass have been investigated in Ethiopia in earlier studies (Bimrew *et al*., 2017). However, the optimum nitrogen fertilizer level and harvesting date are not well known for Desho grass. Thus, the present study was designed to determine the effect of nitrogen fertilizer level and harvesting date on agronomic traits, biomass yield and nutritive value of Desho grass under highland rain fed conditions of southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted for two years from May 2016 to December 2017 at Hula and Bule districts of Sidama and Gedeo zones, respectively, Southern Nations, Nationalities and Peoples` Region of Ethiopia. Hula and Bule districts lie in the highland agro-ecology, where mixed crop livestock production is the predominant farming system. Rainfall pattern is characterized by two rainy seasons (main and short rainy seasons). The main rainy season extends from June to October and the short rainy season is from March to April. During the experimental period, the mean annual rainfall was about 1187mm and 1149 mm for Hula and Bule districts, respectively. The maximum annual temperatures were reported to be $20.5C^0$ and $19.4C^0$ at Hula and Bule, respectively, with minimum annual temperatures of $4.3C^0$ and $9.9C^0$ at Hula and Bule, respectively. Map of the study areas is given in Figure1.

Hula district is located between 6o 29`Nourth latitude and 38o 31`East longitude with an elevation of 2759 meters above sea level; Bule is located between 6^o18`Nourth latitude and $38^o$ 24` East longitude and an elevation of 2793 meters above sea level. The soils of Hula site are characterized by sandy loam, low in soil p^H (4.25), total organic matter (2.02%), total available nitrogen (0.21%) and medium available phosphorus (16.3%). The soil type of Bule is loam, low in soil p^H (4.74), total organic matter (3.53%), total available nitrogen (0.17%) and medium available phosphorus (22.6%).

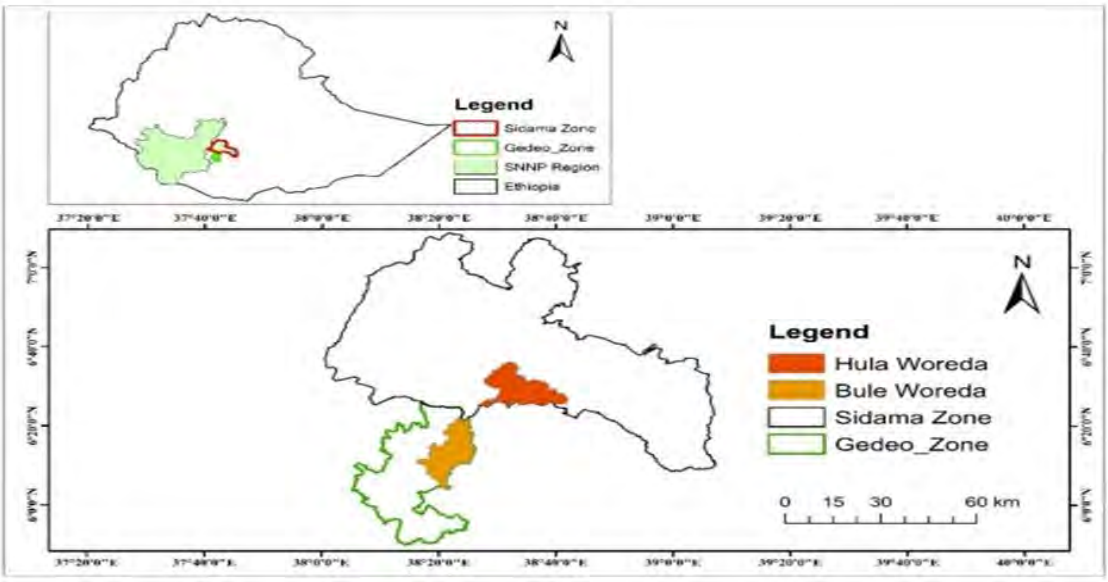

Figure 1: Map of the study districts (woredas) in Sidama and Gedeo Zones, Ethiopia

Treatments and experimental design

A factorial arrangement of treatments was employed using a randomized complete block design with 2 factors (fertilizer level and harvesting stage); 3 nitrogen fertilizer levels (0, 41 and 73 kg N/ha) and 3 harvesting stages (112,133 and 154 days after planting) with 3 replications, which makes nine treatments combinations. The land was ploughed by a tractor and leveled at the start of the main rainy season. The experimental field was divided into three blocks, each containing 9 plots resulting in a total of 27 plots, with each plot measuring 2x5 meter ($10m^2$). The net harvestable plot size was 1×5 meter ($5m^2$). The total number of rows per plot was 4. Samples were taken from the two middle rows. Distance between plots and blocks were 1 and 1.5 meter, respectively. Plots in each block were randomly assigned to one of the 9 treatments. The cuttings were planted into a well-prepared seedbed with one root split per hill.

Data collection

The plant height, tillers per plant, leaf length, leaf to stem ratio and forage dry matter yield data were recorded. The number of tillers was counted from the five culms after harvesting. Plant height was based on five culms taken randomly in each plot, measured using a steel tape from the ground level to the highest leaf. Leaf length per plant was taken from five randomly selected plants per plot. Leaves were separated from stems and the leaf to stem ratio was estimated based on the dry weight of each component. The total herbage on each plot at the fixed dates was harvested leaving out border rows. From each plot, an area of 5m2 was used to calculate dry matter yield (DMY). Harvesting was done by hand using a sickle, leaving a stubble height of 5 cm, and the harvested herbage was weighed fresh in the field using a field balance. Samples of 500g of fresh forage were taken from each plot and oven-dried at 60 °C for 72 hours to determine DM content, which was used in the calculation of DMY.

Chemical Analysis

The pre-dried grass samples were ground to pass through a 1 mm sieve (Wiley mill) and stored in airtight plastic bags until required for chemical analysis. Total ash content was determined by combusting the samples in a muffle furnace at 550^{o}C for 6 hours. Nitrogen content was determined by the Kjeldahl method (AOAC, 1995) and crude protein (CP) content was calculated as N% × 6.25. Standard procedures were used to determine the neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) contents (Van Soest and Robertson, 1985) and *in vitro* organic matter digestibility (IVOMD) determination (Tilley and Terry, 1963).

Data analysis

Analysis of variance (ANOVA) was employed and differences among treatments were tested using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 2002). Least significance difference (LSD) at 5% significance level was used for comparison of means. The data were analyzed using the following model: $Y_{ijk} = \mu + A_i + B_k + N_j + A_i * N_j + e_{ijk}$. Where: Y_{ijk} = individual observation, μ = overall mean, A_i = effect of fertilizer, B_k = k^{th} block effect, N_j = effect of harvesting stage, $A_i * N_j$ = interaction effect of fertilizer and cutting and eijk = the random error.

RESULTS

Agronomic traits of Desho grass as affected by fertilizer level and harvesting date

The effects of fertilizer level, harvesting date and interaction between fertilizer level and harvesting date on agronomic traits of Desho grass are shown in Table 1. Plant height was higher ($P<0.05$) at 154 days of harvesting than at the other two harvesting dates and it was higher ($P<0.05$) when 73 kg N/ha was applied compared to application of 41 kg N/ha or no fertilizer. The tiller number was lower ($P<0.05$) when harvested at 112 days after planting compared to those harvested at 133 and 154 days after planting with no significant differences ($P>0.05$) between the latter two harvesting dates. Fertilizer level also had significant ($P<0.05$) effects on tiller number.

The tiller number increased with increasing levels of fertilizer application, the number being higher ($P<0.05$) at the application of 73 kg N/ha than without fertilizer application (0 kg N/ha). The leaf length showed a significant increase ($P<0.05$) with increasing level of N fertilizer application and it was higher ($P<0.05$) when 73 kg N/ha was applied compared to application of 41 kg N/ha or no fertilizer. However, the harvesting stage did not have a significant effect ($P>0.05$) on leaf length. There was interaction ($P < 0.05$) between harvesting stage and level of fertilizer application on leaf length. The leaf: stem ratio was not affected by the level of fertilizer application ($P>0.05$) but it showed a significant decrease ($P<0.05$) with increasing stage of harvesting.

Table 1 Agronomic traits of Desho grass as affected by fertilizer level and harvesting dates

Variable	Harvesting date(HD)	nitrogen fertilizer Level (FL) (kg N/ha)			Mean	SEM	Significance level		
		0	41	73			HD	FL	HD x FL
Plant height(cm)	112	21.25	22.08	23.92	22.42^{B}	0.79	*	***	NS
	133	20.75	24.08	27.08	23.97^{B}	1.83			
	154	27.25	28.08	29.33	28.22^{A}	0.60			
	Mean	23.08^{B}	24.75^{B}	26.78^{A}					
	SEM	2.09	1.76	1.57					
Tiller number	112	43.03	44.33	48	45.12^{B}	1.49	***	*	NS
	133	48.58	55.67	55.92	53.39^{A}	2.41			
	154	50.42	52.75	63.25	55.47^{A}	3.95			
	Mean	47.34^{B}	50.92^{AB}	55.72^{A}					
	SEM	2.22	3.40	4.40					
Leaf length(cm)	112	14.83	17.33	17.67	16.61	0.90	NS	***	*
	133	13.33	16.33	19.50	16.39	1.78			
	154	16.00	16.33	18.33	16.89	0.73			
	Mean	14.72^{C}	16.66^{B}	18.50^{A}					
		0.77	0.33	0.54					
Leaf: Stem	112	0.93	0.8	0.85	0.86^{A}	0.04	***	NS	NS
	133	0.78	0.72	0.72	0.74^{B}	0.02			
	154	0.38	0.43	0.37	0.39^{C}	0.02			
	Mean	0.70	0.65	0.65					
	SEM	0.16	0.11	0.14					

HD x FL=interaction between fertilizer level and harvesting dates; SEM = Standard error of means, Not significant (NS) = P>0.05, significant (*) = P<0.05, *(*P<0.05*); ** (*P<0.01*); *** (*P<0.001*), A, B, C mean values with different superscripts differ significantly for harvesting date within a column and fertilizer level, within a row

Chemical composition of Desho grass as affected by fertilizer level and harvesting dates

The chemical composition of Desho grass as affected by nitrogen fertilizer level and harvesting date is shown in Table2. Ash content was lower (P<0.05) at the intermediate (41 kg N/ha) fertilizer level than at low (0 kg N/ha) and high (73 kg N/ha) fertilizer level. However, the CP content was higher (P<0.05) when 41 and 73 kg N/ha was applied compared to the unfertilized treatment (0 kg N/ha).The DM, ash and CP contents increased with increasing harvesting date, the differences being higher (P<0.05) at 154 days than at 112 days of harvesting after planting.

The fiber (NDF, ADF and ADL) contents showed a significant increase (P<0.05) with increasing harvesting date but showed a decreasing trend with increasing N fertilizer level. The NDF content was lower (P<0.05) when 73 kg N/ha was applied whereas the ADF content was lower (P<0.05) in both fertilized treatments (i.e. 41 and 73 kg N/ha) than in the unfertilized one. The ADL content was not affected (P>0.05) by N fertilization. The IVOMD was higher (P<0.05) at early (112 days) of harvesting date than at the intermediate (133 days) and late (154 days) harvesting date. The application of N fertilizer did not show a clear pattern on IVOMD.

Table 2 Chemical composition and *In vitro* organic matter digestibility of Desho grass as affected by level of fertilizer and harvesting stages

Composition (% DM)	Harvesting date (HD)	nitrogen fertilizer level (FL) (kg N/ha)			Mean	SEM	Significance level		
		0	41	73			HD	FL	HD*FL
Ash	112	15.58	16.32	15.99	15.96^{B}	0.21	*	**	***
	133	18.05	14.01	16.65	16.24^{AB}	1.18			
	154	16.41	16.66	16.68	16.58^{A}	0.09			
	Mean	16.68^{A}	15.66^{B}	16.44^{A}					
	SEM	0.73	0.83	0.23					
CP	112	11.44	11.99	10.65	11.36^{B}	0.39	*	***	***
	133	9.97	12.67	12.26	11.63^{AB}	0.84			
	154	10.18	12.27	13.68	12.04^{A}	1.02			
	Mean	10.53^{B}	12.31^{A}	12.20^{A}					
	SEM	0.46	0.20	0.88					
NDF	112	62.84	61.65	62.12	62.20^{C}	0.35	***	**	***
	133	62.22	63.50	63.34	63.02^{B}	0.40			
	154	67.61	67.46	65.16	66.74^{A}	0.79			
	Mean	64.22^{A}	64.20^{A}	63.54^{B}					
	SEM	1.70	1.71	0.88					
ADF	112	31.74	31.2	30.84	31.26^{C}	0.26	***	**	***
	133	36.12	33.11	36.24	35.16^{B}	1.02			
	154	39.99	40.56	37.68	39.41^{A}	0.88			
	Mean	35.95^{A}	34.96^{B}	34.92^{B}	35.28				
	SEM	2.38	2.86	2.08					
ADL	112	2.19	2.28	1.90	2.12^{C}	0.11	***	NS	***
	133	3.38	2.75	3.26	3.13^{B}	0.19			
	154	3.46	3.94	3.58	3.66^{A}	0.14			
	Mean	3.01	2.99	2.91					
	SEM	0.41	0.49	0.52					
IVOMD (%)	112	68.25	67.13	65.09	66.82^{A}	0.93	***	**	***
	133	57.86	65.02	60.68	61.19^{B}	2.08			
	154	57.41	60.19	61.37	59.66^{B}	1.17			
	Mean	61.17^{B}	64.11^{A}	62.38^{B}					
	SEM	3.54	2.05	1.37					

CP=Crude Protein; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; ADL= Acid Detergent Lignin; IVOMD= In-vitro Organic Matter Digestibility; FL x HD=interaction between fertilizer level and harvesting date; SEM = Standard error of means; NS=Not significant (P>0.05), significant (*) = P<0.05, *(*P<0.05*); ** (*P<0.01*); *** (*P<0.001*); A, B, C mean values with different superscripts differ significantly for harvesting date within a column and fertilizer level, within a row

Dry matter yield and crude protein yield of Desho grass as affected by fertilizer level and harvesting dates

The effects of fertilizer level and harvesting date on dry matter yield (DMY) and crude protein yield (CPY) of Desho grass are shown in Table 3. The DMY showed a significant increase (P<0.05) with increasing harvesting date and it was higher (P<0.05) at the highest level (73 kg N/ha) of fertilizer application than at 41 and 0 kg N/ha with no significant differences between the latter two levels. The CPY was lower (P<0.05) in the unfertilized treatments (0 kg N/ha) than in the fertilized ones (41 and 73 kg N/ha). On the other hand, the CPY was higher (P<0.05) when the grass was harvested at 154 days after planting than at the other two harvesting stages.

Table 3 Dry matter and crude protein yield of Desho grass as affected by fertilizer level and harvesting dates

Yield (t/ha)	Harvesting date (HD)	nitrogen fertilizer level (FL) (kg N/ha)			Mean	SEM	Significance level		
		0	41	73			HD	FL	HD x FL
Dry matter yield	112	0.86	1.04	1.12	1.01^{C}	0.08	***	*	*
	133	1.03	1.19	1.38	1.20^{B}	0.10			
	154	1.69	1.71	2.08	1.83^{A}	0.13			
	Mean	1.19	1.31	1.53					
	SEM	0.25	0.20	0.29					
Crude protein yield	112	0.10	0.12	0.12	0.11^{B}	0.01	***	**	**
	133	0.10	0.15	0.17	0.14^{B}	0.02			
	154	0.17	0.21	0.29	0.22^{A}	0.03			
	Mean	0.12^{B}	0.16^{A}	0.19^{A}					
	SEM	0.02	0.03	0.05					

HD x FL=interaction between fertilizer level and harvesting dates; +SE = Standard error of means, Not significant (NS) = P>0.05, significant (*) = P<0.05, *(*P<0.05*); ** (*P<0.01*); *** (*P<0.001*), A, B, C mean values with different superscripts differ significantly for harvesting date within a column and fertilizer level, within a row.

DISCUSSION

Agronomic traits as affected by fertilizer level and harvesting dates

The increase in plant height at higher fertilizer level is consistent with the findings of previous studies of (Chaparro *et al.,* 1996; Yasin *et al.,* 2003), who reported similar result for Napier grass. The findings also indicated that the changes in plant heights with the changes in the harvesting date are indicative of increased fodder production with application of appropriate management practices for Napier grass. The increased plant height found in this study with increasing harvested date is contrary to previous report of Worku *et al.* (2017), who reported that the plant height of similar grass is not increased significantly as fertilizer level increased. Similarly, this is in agreement with previous study of Abdi Hassan (2014), who reported that no difference were observed at harvesting height among the different nitrogen fertilizer rates for *Cenchrus ciliaris* and *Panicum maximum.*

The number of tillers per plant increased with increasing fertilizer level, which is consistent with findings of Tessema *et al.* (2003) who reported similar results for Napier grass. The increase in number of tillers per plant with increasing harvesting date is also in line with the findings of Tessema and Alemayehu (2010) for Napier grass who reported that Napier grass produced many tillers and dense vegetative growth as the pasture consolidates due to perennial nature of the grass. High tiller productions do not only indicate stable productivity (Mukhtar, 2006) but also is linked to better persistence after periods of unfavorable environmental conditions (Assuero and Tognetti, 2010). Moreover, the increase in tiller number with increasing fertilizer level in the current study agrees with findings of Worku *et al.* (2017). This is in agreement with the finding by Kizima *et al*. (2014) who reported that application of optimal nitrogen fertilization level significantly affects the appearance of new tillers and increases the dynamics of tiller population of *Cenchrus ciliaris*. Generally, tiller production is a key factor in the resistance of grasslands to deterioration by ageing.

The increase in leaf length at later harvesting date is consistent with the findings of Molla *et al.* (2018) who reported that the difference in leaf length between early and late harvesting might be due to the differences between physiological changes of plants observed during the growing periods. The present results are also supported by the findings Asmare *et al.* (2017) who noted increased leaf length in C*hloris gayana* as N fertilizer levels and harvesting stages increased. The decrease in leaf: stem ratio as harvesting date increased agrees with the findings of Yasin *et al.* (2003) that showed reduction in leaf proportion and an increase in the stem fraction of the grass at the advanced harvesting date. Similar observations were reported for tropical forage grasses (Seyoum *et al.,* 1997).

Chemical composition of Desho grass as affected by fertilizer level and harvesting dates

The increased ash content found in this study with increasing harvesting date is contrary to previous report (Kitabe and Tamir, 2005), who found that the ash concentration of grasses declined significantly as cutting interval increased for Napier grass. In a similar manner, previous studies in Kenya (Kariuki *et al.,* 1999; Mukhtar, 2006) showed a decline in macro-mineral content of Napier grass with advancing date of growth. The divergence of the results in this study may be due to the fact that grasses vary in their genetic capacity to take up minerals from the soil and in their mineral requirements for growth. Similarly, Asmare *et al.* (2019) reported that the uptake of soil nutrients is determined by soil characteristics and climatic condition.

In the current study, application of N fertilizer improved CP content of Desho grass as compared to unfertilized plots. The result is in agreement with the previous finding (Ram and Trivedi, 2014) for Guinea grass. Nitrogen fertilization may have resulted in increased leaf N, tissue protein, and digestible carbohydrates as reported earlier for Bermuda grass (Kering *et al.,* 2011).

The fiber (NDF, ADF and ADL) contents increased with increasing harvest date, which is consistent with the previous findings of Bimrew *et al.* (2017) for the same grass species. The NDF of grass was lower than the value (62.20 to 66.74%) that obtained before by Bimrew *et al*., 2017), where NDF content ranged from 73.58 to 76.03%. Similarly, the ADF content was lower than the value (31.26 to 35.95%) reported by the same authors where ADF concentration increased from 41.28% at 90 days to 43.69% at 150 days of age. And also ADL content was lower than the value (5.24 to 5.48%) reported by Bimrew *et al*. (2017). On the other hand, as the level of fertilizer increases there is a reduction in fiber components in conformity with a previous report by Taye *et al.* (2004) for Napier grass, which showed a decreasing trend with increasing N fertilizer level. Moreover, the decrease in the fiber content with increasing fertilizer level agrees with other study (Ram and Trivedi, 2014) for Guinea grass. The difference in fiber contents among the study might be due to difference in variety or species, soil and climate and treatment factors combination (i.e. fertilizer date and harvesting date).

The lower IVOMD observed at higher harvesting date could be due to increased fiber content with increasing harvesting date. This is consistent with the findings of Nelson (1995), who reported highest IVOMD at early harvesting date for Bermuda, Bahia and star grasses. However, the application of N fertilizer did not show a clear pattern on IVOMD in the current study as the IVOMD value was higher when 41 kg N/ha was applied as compared to the non-fertilized treatment and higher rate of fertilization (73 kg N/ha). Thus, under the conditions of the current study, 41 kg N/ha appears to be an optimal level of fertilizer application although there is no clear explanation for it.

Dry matter yield and crude protein yield of Desho grass as affected by fertilizer level and harvesting dates

The increased DMY with increasing harvesting date might be due to additional tillers development with the increase in leaf formation and leaf elongation and development. This is consistent with other findings (Mukhtar, 2006; Leta *et al.*2013) who reported increased herbage yield with increased stage of maturity for elephant grass. Similarly, significant increases in DMY with advancing age of plants were reported by Ramamurthy and Shankar (1998), Choubey *et al.* (1999) and Ram and Trivedi (2014) for *Pennisetum transpacific* hybrid, *Brachiaria mutica* and Guinea grass, respectively. Amongst the major agronomic practices required, harvesting of grass at appropriate harvesting date and fertilizer level are very important to improve DM yield of Desho grass. The highest CPY observed in the late harvest date was due to higher DMY. The higher CPY in the fertilized Desho grass could be attributed to the fact that nitrogen is the main constituent of protein and is involved in the synthesis of amino acids and accumulation of protein in plants.

CONCLUSION

Fertilizer level and harvesting date have the combined effect on dry matter yield and quality of Desho grass. Higher dry matter yield could be harvested at later harvesting date (154 days) with application of 73 kg N/ha, without affecting quality attributes. However; further study is needed to verify the cost benefit and environmental impact of increased N fertilizer level.

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On-Farm Phenotypic Characterization of Begaria Cattle Population and Their Production System in Guba District, North Western Ethiopia

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ABSTRACT

Production system and phenotypic characterization study on Begaria cattle was carried out in Guba district of Metekel Zone, Benishangul Gumz Region of Ethiopia. Based on a result of a reconnaissance survey three different localities, namely, Mankush, Fanguso, and Almahal were purposively selected for the study. Questionnaires were administered to 40 cattle owners in the area to assess the production system and document the performance of Begaria cattle. Quantitative and qualitative measurements were made on a total of 134 (124 female and 10 male) adult cattle identified as Begaria by producers. Quantitative data were analyzed using PROC GLM procedure of SAS by fitting the three locations as a fixed factor and different body measurements as a dependent variable. Multivariate analysis of morphometric measurements was also done to quantify the distance between the sub-populations from the three sites. Sedentary and transhumant ways of life were practiced in the study area. Among livestock, cattle were ranked first in terms of importance with an index of 0.45 followed by goat and chicken with an index of 0.32 and 0.11, respectively. Chi-square goodness of fit test for coat colour pattern in Begaria cattle was significant (Chi-square=164.1, df=3, P<0.01). The majority (71.6%) of the cattle had plain or uniform coat colour pattern followed by shaded and pied 18.7% and 9%, respectively. There was also a highly significant association (Chi-square = 23.32, P<0.01) between study locations and coat colour pattern. White or cream coat colours were dominant among Begaria cattle and together they accounted for 58.2% of the total variation. Multivariate discriminant analysis has shown a high misclassification error of individuals belonging to the three different sites implying similarity among the populations and high within site variation. Begaria cattle had multipurpose roles and males were mainly kept for income generation, draught power, and breeding while females were mainly used for milk production, breeding and income generation. Lower milk yield was reported with high variation between villages. This calls for identification of the sources of variation and subsequent improvement of milk production. Mixing of Begaria cattle (large Zebu) with the short sized Zebu from adjacent highland districts in the region and Felata breed from the adjoining areas of the Republic of Sudan is considered as a potential threat for dilution of Begaria cattle population. Thus, setting up and implementing in-situ conservation and genetic improvement program is of high priority to conserve the diversity and sustainably utilize Begaria cattle.

***Keywords**: Begaria cattle, Ethiopia, Multivariate analysis, Phenotypic characterization, Production System,*

INTRODUCTION

Smallholder livestock production is an integral part of agriculture in Ethiopia and contributes tremendously to the livelihood and the national economy. When contribution to ploughing service is taken into consideration about 45% of the Agricultural GDP is estimated to be generated from livestock (Behnke, 2010). A total population of 59.5 million cattle (CSA, 2015) and 28 distinct populations (EBI, 2016) have been documented in Ethiopia. Due to the diverse agroecology and proximity to domestication route, Ethiopia is endowed with diverse animal genetic resources.

It is generally believed that most domestic animals were first domesticated in southwest Asia. The origin and development of African livestock have been a subject of studies in the past and

additional African origin has been forwarded for cattle by some of the studies (Grigson, 1991; Stock and Fifford-Gonzalez, 2013, Decker *et al.*, 2014; Okeyo *et al.*, 2015; Kim *et al.*, 2017). The presence of diverse breeds of livestock in the continent has called for characterizing the breeds for the purposes of utilization and conservation and, to date, numerous studies have been undertaken. However, there are still a population of livestock in some remote areas which have not been characterized. In Ethiopia, one such population is Begaria cattle in the northwestern part of the country. The breed is believed to have unique adaptive and productive traits suitable for survival and performance under the low-input and hot climatic condition of the area. In addition to that, the area is among parts of Ethiopia where Trypanosomiasis is prevalent (Solomon and Fitta, 2011; Samson *et al*., 2016) and the breed is possibly trypanotolerant.

Characterization of a population of livestock is necessary for proper identification of breeds and to command appropriate breeding program for sustainable improvement, conservation and sustainable utilization of animal genetic resources (FAO, (2015). The present study was undertaken to characterize Begaria cattle of Ethiopia with the objectives of assessing the morphological and quantitative characteristics of Begaria cattle; evaluating its productive and reproductive performances; and understanding the breed's status, breeding objectives, origin, distribution and production system.

MATERIALS AND METHODS

Study areas

The study was conducted in Guba district, Metekel zone of the Beneshangul Gumuz Regional State in northwestern Ethiopia. Three villages (Mankush, Fanguso and Almahal) which had higher and more homogenous population of Begaria cattle in terms of size and color were purposively selected. Study areas bordered with Sudan to the West, Amhara Regional State to the north and east and other districts of Beneshangul Gumuz Region in the South. The sampling frame was defined after a review of available secondary information on the presence of unique cattle populations in Metekel zone and a subsequent exploratory field visit. A rapid exploratory survey of farm animal genetic resources was conducted in Benishangul Gumuz Regional State from January to February 2012. The survey involved on-farm observations of cattle populations and key informant discussions (with livestock researchers, agricultural experts, and knowledgeable farmers) revealed the presence of an apparently distinct population of cattle in Guba district.

The climate of the study areas can be classified as hot and humid. Agro-ecologically the study area is found in the lowlands within an altitude ranging from 531 to 860 m a.s.l. The district receives mean annual precipitation of 965 mm during June to September with uni-modal distribution (Addisu, 2010).

Data collection

Survey data

A formal survey was conducted in March, 2012 during which farmers were interviewed with the help of semi-structured questionnaires. Questionnaires were administered to 40 cattle owners (12 in Mankush, 12 in Fanguso and 16 in Almahal). The criteria used to select respondents were ownership of cattle, experience in cattle husbandry in the area, and willingness to participate in the study. The main issues addressed in the questions were herd structure; importance of cattle in the livelihoods of the community; management system; milk production, growth and reproductive performance; and resilience of the cattle population.

Qualitative and quantitative measurements

Measurements on qualitative and quantitative (linear) characters were performed on a total of 134 (124 female and 10 male) randomly selected adult cattle (Table 1) from herds encountered in a transect walk in each of the sites (villages). A limited number of animals were sampled due to their low population and difficulty in restraining animals with little contact with humans in the wilderness except for their keepers. The physical maturity of the cattle subject to measurements was confirmed from observation of their dentition and verification of age by their keepers. Data were collected according to the FAO guidelines (FAO, 2012).

Table 1. Number of animals sampled and questionnaires administered by study site

Site	Quantitative and qualitative traits		Questionnaires administered
	Males	Females	
Mankush	-	33	12
Fanguso	3	47	12
Almahal	7	44	16
Total	10	124	40

A total of 25 qualitative traits (including some size traits categorized qualitatively) and 9 quantitative measurements were recorded. Qualitative traits included in the study were: coat colour pattern, coat colour type, muzzle colour, eyelid colour, hoof colour, horn presence, horn condition, horn spacing, horn shape, horn orientation, ear shape, ear orientation, hump shape, hump size, hump position, udder size, teat size, facial profile, back profile, rump profile, testes size, tail length, naval flap width, preputial sheath, and dewlap width. Quantitative measurements were made on body length, heart girth, height at wither, pelvic width, mouth circumference, ear length, horn length, cannon bone length and hock circumference. Measurements were taken using a textile measuring tape to the nearest unit centimeter.

Data analysis

All data were entered, cleaned and managed on MS Excel© worksheet. Multiple range test was performed on all area means of body measurement traits of females. Cross-tabulation was performed with SPSS v. 22.0 (IBM Corp, 2013) on categorical data to describe the proportion and to test association among fixed factors (sites) and dependent (categorical) variables. Chi-square test was used to test the significance of the association. Fisher's exact test was used when the number of samples in each cell was less than the expected value (when 75 % have less than 5 counts).

Indices were calculated to provide ranking on the importance of domestic livestock species in the area. The formula used in the calculation of the indices is Index= sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) given for each species divided by sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) for all species ranking.

PROC FREQ in SAS was used to know the relative frequency of qualitative characters per sampling site. Quantitative data were analysed separately for the two sexes using the PROC GLM in SAS. Taking site and sex as fixed main effects, the following model was used in the analyses:

$$Y_{ijk}=\mu + S_i + D_j + e_{ijk}$$

where Y_{ijk} is the observed value of the linear body measurements, S_i is the fixed effect of site i (i=1, 2,3), D_j is the effect of the jth sex (j=1,2), and e_{ijk} is the residual error. Interaction effect of the ith site with the jth sex was not statistically significant and was dropped out from the final model.

Computation of Pearson correlations among body measurements was done with PROC CORR. Stepwise discriminant procedure was applied on female sample populations using PROC STEPDISC to determine which morphological traits had more discriminant power than the others. The CANDISC procedure was used to perform canonical analysis to derive canonical functions, linear combinations of the quantitative variables that summarize variation between areas and compute Mahalanobis distance matrix. The percent assignment of cattle populations into their sampling area was made by using DISCRIM procedure.

RESULTS AND DISCUSSION

Production system

All the cattle owners involved in this study practice mixed crop-livestock production. However, there was a significant association between the study site and priority in farming activity (Chi-square 8.865, $P<0.05$). For most of the farmers (91.7%) in Mankush, livestock production was their main activity. Contrary to this, crop production was considered as the main activity in the other two locations, 71.4% in Fanguso and 54.5% in Almahal. Cropland holding was highest in Almahal site with mean size of 9.5 ± 1.39 ha per household. This was significantly higher than the land holdings in Fanguso (3.2 ± 1.65 ha). Landholding in Mankush was intermediate between the two sites (6.0 ± 1.51 ha) and not significantly different from the other two sites. There was a significant association (Chi-square=19.336, P<0.01) between livestock mobility and study sites. Most of the cattle owners interviewed at Almahal were leading a sedentary way of life while all the cattle owners from Mankush were practicing transhumance. Both sedentary farming and transhumance were identified among cattle owners at Fanguso site (Table 2). Respondents in all sites reported the practice of communal grazing, which is a good opportunity to facilitate sharing of best bulls among herds if the communities are willing to cull unselected males.

Table 2. Agricultural production system and livestock management practices in Guba district

Variable	Overall	Site			Chi-square	*P* values
		Mankush	Fanguso	Almahal		
Production system						
Crop livestock	40(100.0)*	12(100.0)	12(100.0)	16(100.0)		
Main farming activity					8.865	0.007
Livestock	18(60)	11(91.7)	2(28.6)	5(45.5)		
Crop	12(40)	1(8.3)	5(71.4)	6(54.5)		
Livestock management					4.542	0.152
Extensive	34(85.0)	11(91.7)	8(66.7)	15(93.8)		
Semi-intensive	6(15.0)	1(8.3)	4(33.3)	1(6.2)		
Mobility					19.336	0.000
Sedentary	19(57.6)	0(0.0)	4(40.0)	15(93.8)		
Transhumance	14(42.4)	7(100.0)	6(60.0)	1(6.2)		

*Number within parenthesis in the table indicate percent out of total respondents

Livestock species herd size and ranking

About 80%, 67.5%, 60% and 25% of cattle owners reported keeping goats, donkeys, chicken and sheep, respectively. Ownership of horse or mule was not reported except by one farmer. Based on the perception of the farmers, the population of Begaria cattle in the study area was reported to be increasing (75%), 11% reported that the population was stable whereas rh remaining14% said that it

was decreasing. The increasing trend was attributed to growing interest of farmers to maintain the breed due to its fast growth and good market price.

There was no significant difference (P>0.05) on cattle, equine and sheep holding per household among different sites (Table 3). However, goat and chicken holdings were affected by location where farmers in Fanguso had a larger number of goats and chickens. Overall mean livestock holding per household were 19.3, 11.2, 3.8, 8.6 and 1.3 for cattle, goat, sheep, chicken, and donkey, respectively. The number of cattle per household is higher than 2 to 4 and 10 to 15 animals reported by Asfaw *et al*. (2013) for the highland and pastoral lowland areas, respectively. Comparison of livestock holding within site revealed that cattle number was higher in Mankush and Almahal followed by goat and chicken. However, in Fanguso goat was largest in number followed by chicken and cattle. The larger possession of cattle per household indicates the availability of ample feed and grazing area, larger land holding (Table 3) as compared to other areas of the country and also the importance of cattle in the livelihood of the framers in the area.

Table 3. Mean domestic animal and land holding ± standard errors per household in Guba district

Species/land	Overall	Site			*P* value
		Mankush	Fanguso	Almahal	
Domestic animal					
Cattle	19.3 ± 3.08	19.25 ± 5.58	13.3 ± 5.58	25.3 ± 4.83	0.278
Goat	11.2 ± 1.43	7.5 ± 2.49^{a}	16.5 ± 2.73^{b}	9.6 ± 2.16^{a}	0.049
Sheep	3.8 ± 1.12	3.14 ± 1.97	6.2 ± 2.06	1.7 ± 1.76	0.263
Chicken	8.6 ± 1.20	5.1 ± 2.18^{a}	14.1 ± 2.18^{b}	6.1 ± 1.89^{a}	0.005
Equines	1.3 ± 0.21	0.8 ± 0.37	1.8 ± 0.37	1.3 ± 0.32	0.229
Land holding	6.2 ± 0.88	9.5 ± 1.39^{a}	3.2 ± 1.65^{b}	6.0 ± 1.51^{b,a}	0.020

Values with different superscript letter within a row are significantly different at P = 0.05.

Among livestock, cattle were ranked first in terms of importance with an index of 0.45 followed by goat and chicken with an index of 0.32 and 0.11, respectively (Table 4). Extensive livestock management system was predominant (85%) in all of the sites. However, 15% reported that they practiced semi-intensive management where supplementary feeding was given apart from grazing and shelters were provided.

Table 4. Ranking of livestock species based on perceived importance by households in Guba district

Species	Rank 1st	Rank 2nd	Rank 3rd	Index
Cattle	13	3	3	0.449
Sheep	1	0	5	0.075
Goat	5	9	1	0.318
Chicken	0	4	4	0.112
Equines	0	2	1	0.047
Sum	19	18	14	1.00

Index= sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) given for each species divided by sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) for all species ranking.

Cattle herd structure

Cattle holding per household observed in this area was higher than the average cattle holding of 9.54 in northwestern lowlands (Ftiwi and Tamir, 2015). With larger land holding per person in the area there is a possibility of establishing ranching type of production system. Herd composition of Begaria cattle are presented in Table 5. About 30% of the total cattle population were cows with above 3 years old.

Table 5. Cattle herd structure by age and sex reported by households in Guba district

Sex and age category	Mean	Percent
Male		
Less than 1 year	2.57	13.32
1 to 3 year	3.01	15.60
Above 3 years	2.35	12.20
Female		
Less than 1 year	2.51	12.98
1 to 3 year	3.01	15.61
Above 3 years	5.85	30.29
Total	19.3	100

Coat color pattern, type and other morphological characters

Chi-square test for coat colour pattern was significant (Chi-square=164.1, *df*=3, *P*<0.01). Majority (71.6%) of the cattle had plain or uniform coat colour pattern followed by shaded and pied 18.7% and 9%, respectively (Table 6). Spotty coat colour pattern was found rarely (0.7%). Coat colour pattern of Begaria cattle observed in this study area varied remarkably from the findings of Getachew (2006) who found predominantly uniform coat colour pattern (56.7%) among the adjacent West Gojjam Highland Zebu cattle populations. There was also highly significant association (Chi-square = 23.32, P<0.01) between study locations and coat colour pattern. Thus the odds of being uniform in colour were calculated as the ratio of frequency of uniform colour to frequency of non-uniform colour. The odds of being uniform colour in Begaria cattle was 2.52, 1.06, 2.57 and 5.38 for the overall location, Mankush, Fanguso and Almahal, respectively. This result showed cattle in Almahal were more likely to be uniform in colour as compared to cattle from other locations.

Table 6. Colour pattern and type of Begaria cattle in different districts

Coat colour pattern and type	Overall	Site			Chi-square	P value
		Mankush	Fanguso	Almahal		
Pattern						
Plain	96(71.6)	17(51.5)	36(72.0)	43(84.3)	23.316	<0.0003
Pied	12(9.0)	1(3.0)	6(12.0)	5(9.8)		
Spotty	1(0.7)	-	-	1(2.0)		
Shaded	25(18.7)	15(45.0)	8(16.0)	2(3.9)		
Odds of being uniform	2.5	1.06	2.57	5.38		
Muzzle colour						
Non-pigmented	14(10.7)	7(14.0)	2(4.0)	5(16.1)	3.879	0.136
Pigmeneted	117(89.3)	43(86.0)	48(96.0)	26(83.9)		
Eyelid colour						
Non-pigmented	10(7.5)	4(7.8)	1(2.0)	5(15.6)	5.222	0.063
Pigmeneted	123(92.5)	47(92.2)	49(98.0)	27(84.4)		
Hoof colour						
Non-pigmented	2(1.5)	1(2.0)	0(0.0)	1(3.0)	1.364	0.714
Pigmeneted	132(98.5)	50(98.0)	50(100.0)	32(97.0)		

White or cream colour (Figure 1) were the dominant coat colour in Begaria cattle and accounted for 58.2% of the coat colour variation (Table 7). Association between coat colour type and location was at the margin of significance level (*P*=0.05). White or creamy colour was most frequent in Fanguso

and Almahal with a percentage value of 64.7 and 60.0 %, respectively compared to in Mankush (45.5 %). Based on a study on two Sub-types of Baggara cattle in Sudan Alsiddig *et al*. (2010) reported about 45% of the cattle to be of white colour while 34% were light to dark red brown. Albeit in different proportions, all the colour types indicated in the study of Alsiddig *et al*. (2010) were also found in the current study. In addition to coat colour, cervico-thoracic hump position and white and medium to small horn size with curved shape observed in this study was in agreement with the description of Baggara breed reported in Sudan (Alsiddig *et al.*, 2010).

The cattle breeds of Ethiopia in areas adjacent to the study area include Horro and Begait cattle. Horro cattle which is distributed along the eastern and south-eastern borders of Benishangul-Gumuz region are dominated by uniform brown and reddish colour (Hassen *et al.*, 2007) while the Begait breed found in the far North Ethiopia along the Sudan border and managed in similar production environment, are of predominantly non- uniform coat colour pattern with black and white combination (Ftiwi, 2015). This is an indication of the uniqueness of the breed to the other breeds in adjacent areas. However, the dominant coat color of Begaria cattle has similarity with the white and grey colour of Ogaden and Boran cattle breed found in south eastern part of Ethiopia and inhabiting similar lowland agro-ecology (Sisay, 1996; Getnet *et al*., 2009; Getachew *et al*., 2014, Mekuriaw and Kebede, 2015). Begaria breed had shown some similarity in coat colour with the Mahebere Selassie composite cattle population (Zewdu *et al.,* 2008) indicating the later could belong a sub-population of the Begaria breed. Further molecular study considering the similar transboundary or inland populations would give clue on the ancestral similarity among populations.

Table 7. Coat colour type of Begaria cattle in different sites in Guba district

Coat colour type	Overall	Site			*P* value
		Mankush	Almahal	Fanguso	
White or creamy	78 (58.2)	15 (45.5)	33 (64.7)	30 (60.0)	0.05
Black	2 (1.5)	0 (0.0)	0 (0.0)	2 (4.0)	
Red or brown	15 (11.2)	2 (6.06)	9 (17.7)	4 (8.0)	
White and black or grey	23 (17.2)	10 (30.3)	4 (7.8)	9 (18.0)	
White and red or brown	16 (11.2)	6 (18.2)	5 (9.8)	5 (10.0)	

Light colour of the breed might be associated with thermoregulation. Light-colored hair coats and hair coats that are sleek and shiny reflect a greater proportion of incident solar radiation than hair coats that are dark in color or more dense and wooly (Hansen, 2004). Finch and Western (1977) also found that the proportion of light colour increased with heat stress telling us cattle evolved in high-temperature area become progressively lighter in colour. Pigmentation on the muzzle, eyelid, and hoof had no association with study sites (P>0.05). Pigmentations were common on the muzzle (89.3%), eyelid (92.5%) and hoof (98.5%) of the Begaria cattle population in the study area.

All of the Begaria cattle sampled during the study had horns, flat facial profile, lateral ear orientation with straight-edged shape and erect cervico-thoracically positioned hump (Table 8). Majority (89.23%) had narrow (<30 cm) horn spacing. Most (79.89%) of the cattle were characterized by possessing curved horns. Predominantly the breed had small humps (84.96 percent). A large number of the cows had small udder (59.68%) with medium teat size (37.90%). Most of the cattle (96.27%) had straight back profile with sloppy ramp (98.51%). A considerable number (57.14%) of mature Begaria bulls had large testes and large preputial sheath (40.00%). On the other hand, a large proportion (48.39%) of the cows were observed to have medium naval flap width. Long tail length (well below the hocks) was common (86.47%) among the cattle population while almost half of them (50.75%) exhibited medium dewlap width. The horns of Begaria cattle is short 18.7 and

20.8 cm for male and female, respectively. Males included in the sample were small in number and relatively younger than the females. Horn growth continues throughout the animals life and that might explain the larger horn length in females than males. Loose horns/lateral orientation of horns is commonly found with 21.6% of the total population. Despite the extensive variation, morphological features observed here are similar with Baggara and Kenana cattle breed found in western and southern Sudan (Yousif and Moula, 2006; Alsiddig *et al.*, 2010) and Borena and Ogaden breeds in Ethiopia (Mekuriaw and Kebede, 2015). Baggara cattle of Sudan which the population of cattle under investigation is believed to have originated from have been reported not to be a uniform type of cattle (Elkhalifa *et al*.,1985 Cit. Bashir and El Zubeir, 2013a).

Table 8. Frequency of discrete variables in Begaria cattle of sampled populations

Discrete variable		Number	Per cent
Horn presence	Present	134	100.00
Horn condition	Horned	115	85.82
	Scurs	14	14.18
Horn spacing	Narrow (<30cm)	116	89.23
	Wide (>30cm)	14	10.77
Horn shape	Curved	107	79.89
	Straight	27	20.15
Horn orientation	Upright	63	47.01
	Lateral	29	21.64
	Forward	27	20.15
Ear shape	Straight edged	134	100.00
Ear orientation	Lateral	134	100.00
Hump shape	Erect	134	100.00
Hump size	Small	113	84.96
	Medium	16	12.03
	Absent	3	2.26
Hump position	Cervico-thoracic	134	100.00
Udder size	Small	74	59.68
	Medium	47	37.90
	Large	3	2.42
Teat size	Small	41	33.06
	Medium	47	37.90
	Large	36	29.03
Face profile	Flat	134	100.00
Back profile	Straight	129	96.27
	Curved	5	3.73
Rump profile	Sloppy	132	98.51
Testes size	Medium	3	42.86
	Large	4	57.14
Tail length	Nearly below hocks (medium)	11	8.27
	Well below hocks (long)	115	86.47
Naval flap width	Small	38	30.65
	Medium	60	48.39
	Large	21	16.94
Preputial sheath	Small	1	20.00
	Medium	2	40.00
	Large	2	40.00
Dewlap width	Small	8	5.97
	Medium	68	50.75
	Large	57	42.54

Figure 1. Begaria cow (left) and Begaria bull (right) from Almahal site.

Linear body measurements

The analysis was done for male and female separately. Descriptive statistics for linear body measurement and least square means with a standard error by district for both and female Begaria cattle are presented in Table 9, and 10, respectively. As expected males were larger (P<0.05) in size (body length, heart girth, and height at wither) than females (results not presented here). Among linear body measurements, body length, heart girth, height at wither and pelvic width were not affected by the study districts (P>0.05). On the other hand, among the variables, ear length (EL), horn length (HL), hock cirumference (HC) and cannon bone length (CBL) were significantly affected by district (P < 0.01) for females.

Table 9. Least square means (± SE) of quantitative body measurements (cm) for all sites by sex

Dependent variable	Sex		
	Male (N=10)	Female (N=124)	Probability of difference
	119.00 ± 2.28	111.44± 0.61	P<0.0017**
EL	21.67 ± 0.67	22.02 ± 0.18	P<0.6169
HL	18.67 ± 2.70	20.70 ± 0.72	P<0.4676
HC	37.89 ± 0.73	34.45 ± 0.20	P<0.0001**
HG	172.89 ± 2.75	156.17 ± 0.74	P<0.0001**
HW	131.56 ± 1.93	125.31 ± 0.52	P<0.0022**
PW	40.11 ± 0.84	39.56 ± 0.22	P<0.5263
CL	25.11 ± 0.58	24.10 ± 0.15	P<0.0938
MC	42.67 ± 1.04	40.02 ± 0.28	P<0.0153*

*P<0.05, **P<0.01; BL=Body length, HG=Heart girth, HW=Height at withers, PW=Pelvic width, MC=Mouth circumference, EL=Ear length, HL=Horn length, CL=Cannon bone length, HC=Hock circumference

Table 10. Least square means ± standard errors and coefficient of variation (CV) of different linear body measurements of female Begaria cattle by study site.

	Overall	CV (%)	Site			Signifcance level (P)
			Mankush	Funguso	Almahal	
BL	111.5±0.64	6.3	112.3 ± 1.23	111.1 ± 1.03	111.2 ± 1.06	0.7148
HG	155.6±0.76	5.3	153.4 ± 1.45	156.7 ± 1.20	157.7 ± 1.26	0.0765
HW	125.1±0.50	4.4	124.4 ± 0.97	124.9 ± 0.79	125.9 ± 0.83	0.4855
PW	39.6±0.20	5.6	39.1 ± 0.39	40.2 ± 0.32	39.5 ± 0.33	0.0891
MC	40.2±0.19	5.3	39.8 ± 0.37	40.1 ± 0.32	40.6 ± 0.32	0.265
EL	22.2±0.14	6.8	22.8 ± 0.26^{a}	22.4 ± 0.22^{a}	21.3 ± 0.23^{b}	0.0001
HL	20.8±0.69	37.7	21.1 ± 1.32^{a}	17.3 ± 1.11^{b}	24.0 ± 1.14^{a}	0.0003
HC	34.4±0.19	6.1	34.0 ± 0.37^{a}	35.4 ± 0.31^{b}	33.8 ± 0.32^{a}	0.0007
CBL	24.0±0.15	6.8	23.4 ± 0.29^{a}	23.9 ± 0.24^{a}	24.8 ± 0.25^{b}	0.0011

BL= body length, HG= heart girth, HW= height at wither, PW= pelvic width, MC= mouth circumference, EL= ear length, HL= horn length, HC= hock circumference, CBL= cannon bone length

Body length, height at wither and heart girth of female Begaria cattle were lower than 128.1 cm, 131.5 and 159.6 cm, respectively, reported for the Begayit breed (Ftiwi and Tamir, 2015). However, Begaria was found to be larger than Gojjam Highland Zebu and Horro cattle in the adjoining areas and Ogaden cattle in eastern Ethiopia (Getachew and Ayalew, 2014; Dereje, 2015; Getachew *et al*., 2014). Begaria cattle was shorter in body length compared to the composite Mahibere selassie (Zewdu *et al.*, 2008), and Mursi breeds (Terefe *et al.* 2015). However, Begaria was larger in height at wither and heart girth than these breeds.

Correlation coefficients among morphological traits of Begaria cattle combined from the three site are shown in Table 11. High and significant correlations were found between BL and HG; and HW and HG (0.42). The lowest and significant correlation coefficient was recorded between MC and EL (0.17). Nakachew (2009) reported higher correlation (0.49) between HG and BL in Abigar cattle than the correlation obtained in the current study. Edouard *et al*. (2018) also reported higher (0.59) correlation between HG and BL.

Table 11. Phenotypic correlation coefficient values (r) among body measurements in Begaria cattle populations of both sexes (n=132)

Trait	**BL**	**HG**	**HW**	**PW**	**MC**	**EL**	**HL**	**CL**
HG	0.41**							
HW	0.33**	0.42**						
PW	0.25**	0.33**	0.38**					
MC	0.28**	0.47**	0.31**	0.18*				
EL	0.15	0.18*	0.10	0.15	0.17*			
HL	0.21*	0.15	0.19*	-0.03	0.26**	0.14		
CL	0.27**	0.38**	0.49**	0.30**	0.22*	-0.02	0.15	
HC	0.33**	0.49**	0.47**	0.36**	0.24**	0.24**	0.02	0.34**

*P<0.05, **P<0.01; BL=Body length, HG=Heart girth, HW=Height at withers, PW=Pelvic width, MC=Mouth circumference, EL=Ear length, HL=Horn length, CL=Cannon bone length, HC=Hock circumference

Multivariate analyses

Stepwise discriminant analysis

Out of the nine quantitative variables separately subjected to the STEPDISC procedure of SAS (2003) from females (Table 12), five were found significant ($P < 0.01$) and more important in differentiating cattle between sites. However, CL followed by HC had more discriminant power as shown by their higher R^2 and F-values. The variable in the model that contributed the least to the discriminatory power of the model as measured by Wilks' lambda failed to meet the criterion to stay, then that variable was removed. Similarly, the importance of ear length as a discriminant variable has been observed in the study of three different indigenous cattle populations from North East states of India (Pundir *et al*. 2015).

Table 12. Stepwise selection summary table for female sample population

Step	Entered	Partial R^2	*F* value	*Pr >F*	Wilk's Lambda	Pr<lambda	Average squared canonical correlation	ASCC
1	HL	0.12	8.31	*P*=0.0004	0.88	*P*=0.0004	0.06	*P*=0.0004
2	HC	0.13	8.83	*P*=0.0003	0.76	*P*<0.0001	0.12	*P*<0.0001
3	CL	0.15	10.26	*P*<0.0001	0.65	*P*<0.0001	0.18	*P*<0.0001
4	EL	0.09	5.63	0.0046	0.59	*P*<0.0001	0.22	*P*<0.0001
5	HG	0.04	2.22	0.1135	0.57	*P*<0.0001	0.24	*P*<0.0001

Note: HL=horn length; HC=hock circumference; CL=cannon bone length; EL=ear length; HG=heart girth

Discriminant analysis

The variables with low discriminatory power from STEPDISC procedure were observed to be useful in improving the correct classification percentage and Mahalanobis distances and hence were not removed from the model used in DISCRIM and CANDISC procedures. The discriminant analysis showed a high misclassification error of individuals (Table 13) belonging to Mankush (36.4 percent), Fanguso (30.4 percent), and Almahal (20.4 percent) indicating similarity among the three sample populations on morphological basis and the existence sizeable within site variation. Animals sampled from Almahal were relatively more homogenous than animals from the other two sites. For two population of N'Dama cattle, Edouard *et al*. (2018) have reported a very high percentage (96.3) of correct classification of animals in their respective populations. Elkhalifa *et al*. (1985 Cit. Bashir and El Zubeir, 2013a) indicated that Baggara cattle of Sudan (which is assumed to be the main origin of Begraia cattle) not to be a uniform type of cattle with sizeable variation within the breed.

Table 13. Number of observations and percent classified (below) in different sites for the female sample population using discriminant analysis

From site	Mankush	Fanguso	Almahal	Total
Mankush	21 (63.6)	5 (15.5)	7 (21.2)	33 (100.00)
Fanguso	7 (15.2)	32 (69.6)	7 (15.22)	46 (100.00)
Almahal	3 (6.8)	6 (13.6)	35 (79.6)	44 (100.00)

Canonical discriminant analysis

The Mahalanobis distance between sites for female sample populations was generally low. The pair-wise squared Mahalanobis distance between sites for female sample populations were significant ($P<0.01$) at Almahal when measured from samples at Mankush (2.4) and Fanguso (2.7) indicating that populations from this site were slightly different from the other two. The distance ($P<0.01$) between Mankush and Fanguso was even shorter (1.45). Unlike in the current study, Edouard *et al*. (2018) have reported higher distance (3.69) between two populations of N'Dama cattle in Côte d'Ivoire.

Purpose of keeping Begaria cattle

Results of this survey revealed that Begaria cattle had multi-purpose roles. Males were mainly kept for income generation, as sources of draught power and for breeding purpose, in their order, with ranking index (I) of 0.299, 0.266 and 0.179, respectively (Table, 14). Draught power was more important in areas where crop production was a main agricultural activity. However, during the group discussion, respondents reported that Begaria cattle, despite its large size, is not as good as cattle from adjacent areas in terms of ploughing performance. The reason could be Begaria cattle has its origin from the Baggara and other cattle of Sudan which are mainly developed under pastoral livestock production system (FAO, 1957) and selection (natural and/or artificial) for draught purpose is unlikely to take place. The main purpose of keeping female Begaria cattle was for milk production ($I = 0.396$), followed by use as breeding animal ($I = 0.358$) and source of income ($I = 0.139$). Multipurpose role of cattle observed in this study is also well documented in many tropical countries (Mekonnen *et al.*, 2012). However, Begaria cattle are mainly preferred for income generation from the sale of male animals and for milk production as opposed to major use for draught power of Horro (Dereje, 2015) and Gojjam Highland Zebu (Getachew, 2006) cattle found in the adjoining areas. Use of cattle mainly for sale and consumption than traction power implies that livestock production is predominant in the area as compared to crop production. This is in agreement with the use of Begayit cattle in the northwestern lowland part of Ethiopia which are also kept for breeding and milk production (Ftiwi and Tamir, 2015) and Butana and Kenana cattle breed in Sudan primarily kept for income and milk rather than traction power (Musa *et al.*, 2006). Fast growth of males and milk yield traits can be identified as the two most important traits of Begaria cattle. These traits and associated traits like adaptation and reproductive performances need to be incorporated in designing breeding program for the improvement of Begaria cattle which has lived for many generations at extremely low altitude characterized by high temperature and shortage of water.

Table 14. Ranking of purpose of keeping male and female Begaria cattle

Purpose	Males				Females			
	Rank 1st	Rank 2nd	Rank 3rd	Index	Rank 1st	Rank 2nd	Rank 3rd	Index
Meat	1	5	6	0.103	0	1	3	0.027
Milk	0	0	0	0.000	15	13	3	0.396
Draught power	13	4	2	0.266	0	1	0	0.011
Breeding	4	8	5	0.179	15	11	0	0.358
Saving	3	5	4	0.125	0	3	6	0.064
Wealth status	0	0	1	0.005	0	0	0	
Ceremony	0	2		0.022	0	0	1	0.005
Income source	10	7	11	0.299	2	3	14	0.139
Sum	31	31	29	1.000	32	32	27	1.000

Index= sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) given for each purpose divided by sum of (3 x number of household ranked first + 2 x number of household ranked second + 1 x number of household ranked third) for all purpose ranking.

Breed origin, distribution and mating practices

The source of Begaria breed in the area is reported to be within the same district, (47.1%), from the neighboring district (20.6%) or from the adjoining areas of the republic of Sudan (29.4%). The majority (65 %) of the respondents believed that the breed was kept in the area for more than 30 years. Begaria cattle producers describe the breed to have short horn size and white or creamy coat colour with dark gray shades to the neck, head, and hump. They perceived that the breed is large as compared to the breeds in the adjoining areas. They also recognized the breed has fast growth and is highly demanded for meat by the domestic market and export market in the adjoining Sudanese areas. Cross of Begaria cattle with the cattle from the adjoining areas (called by producers as Habesha) is given a name Beladi Cattle. Baggara breed in Republic of Sudan have similar physical appearance with Begaria cattle characterized in this study. Furthermore, dissimilarity of this breed to the other breeds in the adjoining areas of Ethiopia confirmed that the breed might have diverged from the Baggara breed of Sudan and later developed unique features.

Natural mating was the only mating system and predominantly uncontrolled due to communal grazing land and scarcity of labor. Majority of the farmers (82.9%) were reported to have Begaria bulls and the remaining proportion of farmers (14.3%) had mixed (Beladi) and 2.9% had Habesha bulls. Almost half (45.9%) of farmers practiced castration. Better draught power, improve fattening for better market price and controlled breeding were mentioned by farmers as reasons for castration.

Currently use of exotic breeds for crossbreeding is limited in the area however mixing with surrounding short sized Zebu and Felata breed introduced from Sudan has been a potential threat for the dilution of Begaria cattle. Farmers in the area have been introducing the highland Zebu for draught purpose because of the relatively cheaper price and better traction performance of the highland zebu as compared to Begaria cattle.

Performance levels

Age at sexual maturity, first calving and market age

Mean (SE) of age at sexual maturity for male and female Begaria cattle was 30.1 (1.48) and 30.1 (1.06) months, respectively (Table 15). Location has significant ($p<0.05$) effect on this trait for both male and female, where the value for Almahal was higher than for the other two locations. Overall

age at first calving (AFC) and calving interval (CI) for Begaria cattle was 40.7 and 17.3 months, respectively. This was better than a value of 59.8 and 22.6 reported for Horro breed in the adjoining highland areas (Dereje, 2015), 48.8 and 23.5 months, for breeds in Gojjam Highland (Getachew, 2006) and 52 months for Baggara cattle in the South Kordofan state of Sudan (Bashir and El Zubeir, 2013b).

AFC and CI were also significantly influenced by location. AFC at Almahal (46.3 ± 1.32) was higher ($p<0.05$) than the AFC for the other two locations (39.3±1.60 at Fanguso and 36.5 ± 1.45 at Mankush). The AFC for the latter two locations were not different from each other. Significantly lower CI, (13 ± 1.9 months) was reported in Fanguso site compared to the other locations (18.2 and 20.6 months in Almahal and Mankush, respectively.) In addition to the smaller average cattle holding in Fanguso as compared to the other sites (Table 3) the semi-intensive type of management being practiced in more households (Table 2) than in the other sites may partly explain for the difference in CI.

Milk production performance

Mean milk yield of Begaria cattle was 1.9 ± 0.24 litter per day (Table 15). There was no significant difference ($P>0.05$) in the mean milk yield among the different sites. However, lactation length and milking frequencies were significantly affected ($P<0.05$) by study site. Lactation length of Begaria cattle in Almahal was lower compared to the Fanguso and Mankush. Milking frequency was higher in Mankush than the other two locations (Almahal and Fanguso). Longer lactation length and more frequent milking in Mankush is in line with the report of higher dependency of farmers in Mankush on livestock than crop as compared to other sites (Table 2). The mean daily milk yield obtained in the current study is similar with reports of Nakachew (2009) for early lactation yield in Abigar Cattle of Gambela region, Ethiopia.

Table 15. Least square means ± standard errors for some productive and reproductive performance of Begaria cattle by study site

Performance	N	Overall	Site			Sig
			Almahal	Fanguso	Mankush	
ASM_M (months)	30	30.05±1.48	34.4±2.41	27.3±2.41	28.5±2.83	0.10
ASM_F (months)	32	30.1±1.06	35.1±1.55[a]	25.7±2.19[b]	29.5±1.74[b]	0.0035
AFC (months)	33	40.7±0.84	46.3±1.33[a]	39.3±1.60[b]	36.5±1.45[b]	<0.0001
CI (months)	37	17.3±1.04	18.2±1.69a	13.0±1.90b	20.6±1.82a	0.02
MA_M (months)	32	32.5±2.79	38.7±4.73	34.0±5.23	24.8±4.53	0.11
MA_F (months)	30	34.5±2.89	37.6±4.66[a]	42.0±5.84[a]	24.0±4.46[b]	0.0374
MY (litter)	38	1.9±0.24	1.8±0.39	1.5±0.43	2.4±0.43	0.28
LL (months)	36	4.7±0.24	3.5±0.41[a]	5.8±0.41[b]	4.8±0.41[b]	0.0017
MF	36	1.5±0.07	1.3±0.12[a]	1.3±0.12[a]	1.9±0.12[b]	0.0008
Weaning age	36	3.8±0.06	3.7±0.11	4.0±0.12	3.8±0.11	0.13

ASM_M=age at sexual maturity for males, ASM_F= age at sexual maturity for females, AFC= age at first calving, CI= calving interval, MA_M= market age for males, MA_F= market age for females, MY= milk yield, LL=lactation length, MF= milking frequency.

Average lactation milk yield varied from 189 litres for cattle sampled in Almahal to 345.6 for those sampled in Mankush. In the former case, milking is limited to the rainy season as animals are taken to other areas during the dry season while in the latter case milking is practiced throughout the year s, and this may account for the difference in milk yield. The estimated mean lactation yield at Mankush

corroborates with the findings of Getachew (2006) and Abdel Rahman (2007) who reported lactation yield ranging from 326-339 litres under on-farm *condition* for Gojjam Highland Zebu in central Ethiopia and 356 liters for Baggara cattle in Souther Kordofan State of Sudan. The mean values, however, were much lower than the value of 538.26 and 598.73 kg reported for Butana and Kenana cattle under farmers management in Sudan (Musa *et al.*, 2006). Identifying the sources of variation in milk yield between districts can serve in designing ways of improving milk production.

CONCLUSION

The coat colour pattern and type, and the performance and size of the Begaria cattle have shown the uniqueness of the breed from other cattle in the adjoining areas. The breed was found to be larger in size mainly in height at withers and heart girth than many other Ethiopian breeds. However, the productivity in terms of milk yield per lactation was lower and variation exists between study sites. The current practice of increased introduction of small-sized Zebu from the surrounding areas for draught purpose associated with new settlement would be a potential threat for dilution of this genetic resource.

Growth and milk production traits appear to be important in the production system and need to be considered along with associated traits of adaptation and reproductive performances. Breeding objectives of the producers need also to be considered in designing a breeding program for the improvement of the Begaria cattle. Design of a breeding program considering the current production system and the levels of inputs and services used (feeding, health, mating system, extension, marketing, and culture) is crucial to achieve sustainable livestock development.

It is noted that due to the high availability of feed from the grassland, the growing demand for beef and milk from increased population as a result of the construction of the renaissance dam, the potential of the breed for beef production and the potential of the area for live animal export to the Republic of Sudan indicate the presence of conducive condition for increased cattle production.

Due to the small number of mature males in the population, the sample size for males was small and results pertaining to the male are likely to be less dependable. Molecular characterization, further performance evaluation, and genetic studies should be carried out to understand the magnitude of the distinctiveness from, and the relationship of this cattle population with already identified cattle populations within the country and the adjoining areas of the Republic of Sudan. Such information would be of importance in making a decision with regard to conservation. The existing difference in performance traits between sites need to be verified and factors accounting for the difference need to be identified for use in improving the performance of the breed through improved genetics and management. Possession of large land holding per person in excess of that can be covered by crop production is very common in the area and there is a possibility of establishing commercial ranching type of production.

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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

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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}
		Milligram per 100 mL	mg dL^{-1}
		Milliequivalents per litre	Mequiv L^{-1}
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 ^oC
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).

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