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# Review

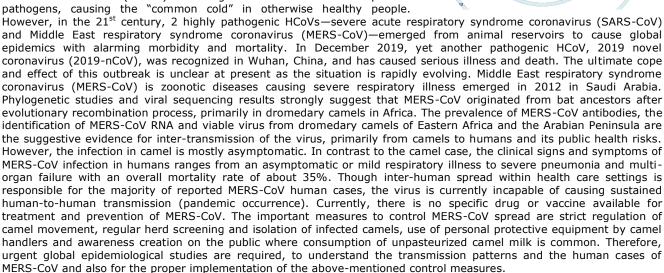
# Review on coronavirus, a middle east respiratory syndrome (MERS-CoV)

Kassahun B, Berhanu T and Damtew B.

Online J. Anim. Feed Res., 10(1): 01-11, 2020; pii: S222877012000001-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr1

#### Abstract

Human coronaviruses (HCoVs) have long been considered in consequential



Keywords: Bats, Dipeptidyl peptidase 4, Dromedary camels, MERS-CoV, SARS-CoV, Transmission

[Full text-PDF]

# Research Paper

# Morpho-histological study of spleen ontogenesis in lambs during antenatal and posnatal period.

Eddine Djallal R, Amine Mohamed F, Manel H, Khaoula M and Marina L.

Online J. Anim. Feed Res., 10(1): 12-16, 2020; pii: S222877012000002-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr2

#### Abstract

In an experiment on the spleen of healthy lambs at two periods (prenatal and postnatal), using anatomo-topographic and histo-cytological research

methods and with subsequent statistical analysis, it was found that the changes in the anatomical and histological structure related to the age of this organ, including during the early stages of the postnatal period of ontogenesis. Note that the surface of the spleen is covered with a capsule of connective tissue, from which leave trabeculae which divide the parenchyma of the organ into lobes. At the time of physiological maturity, the ratio of the structures changes in the direction of an increase in the white pulp and a decrease in the component of the red pulp. During all periods of prenatal fetal development, the percentages of spleen tissue components corresponding to fetal analogs with normal clinical development are determined. The morphological structure of the spleen is described for the first time for this breed in Algeria, age-related changes in the structures of the parenchyma are traced, the formation of follicles began during the terminal phase of gestation, and the development age-related white pulp.

Keywords: Algeria, Follicle, Parenchyma, Postnatal, Spleen, Lambs, White pulp.

[Full text-PDF]

## Research Paper

# Conservation-based breeding program for indigenous sheep breeds in Ethiopia: the way forward.

Molla M.

Online J. Anim. Feed Res., 10(1): 17-24, 2020; pii: S222877012000003-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr3

#### **Abstract**

This paper review briefly Ethiopian sheep genetic resources, description of farming systems, breeding objectives of the community, and to quantify

threats with previous sheep genetic improvement and conservation strategies in Ethiopia. Thus, the aims of this paper focus on the role of characterizing indigenous sheep breed, including their threat status in genetic improvement programs. Indigenous sheep genetic resources have contributed considerable portion to the integrated crop-livestock farming systems in Ethiopia. Ethiopia endowed with a diverse sheep genetic resource maintained under different production systems. Sheep research and development in Ethiopia has largely focused on characterization of sheep genetic resources, description of farming systems and genetic improvements using crossbreeding and selection within breed. In Ethiopia, there are a total of 14 traditional sheep populations in Ethiopia fall into six breed groups based on DNA and morphological data. The main cause of threatening the sheep genetic resource in Ethiopia is indiscriminate crossbreeding with exotic germplasm. Few research institutes and agricultural universities are involved in the conservation and management of sheep breeds in Ethiopia. Currently the emphasis is on village based rather than centre-based genetic improvement program. It is possible to conserve sheep genetic resources in Ethiopia through quantifying past sheep characterization works and developing breeding programs suitable to the conditions of smallholder farmers that increase the economic values of indigenous sheep breeds.

Keywords: Conservation, Indigenous, Sheep, Smallholder farmer, Village breeding practice

[Full text-PDF]

### Research Paper

# Phenotypic characterization of donkeys in Benishangul Gumuz National Regional State.

Mustefa A, Assefa A, Misganaw M, Getachew F, Abegaz S, Hailu A and Emshaw Y.

Online J. Anim. Feed Res., 10(1): 25-35, 2020; pii: S222877012000004-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr4

#### **Abstract**

Fifteen morphometric measurements and eighteen qualitative traits were recorded on 323 randomly sampled adult donkeys (123 jacks and 200 jennets) to meet an objective of characterizing a heterogeneous donkey population of three phenotypic types (Sinnar, the locals and their crosses) found in Benishangul Gumuz region of Ethiopia. The General Linear Model and non-parametric test (chi-square) procedures of SAS software were used for the analysis of the morphometric data and qualitative traits, respectively. Means were separated using the Tukey-Kramer test. The studied morphometric measurements were significantly affected by the phenotypic type and partially affected by sex and sample location /district. Sinnar donkeys were significantly bigger and heavier than the local and the crosses. However, for some of the morphometric measurements no significance difference was observed between Sinnar and crosses implying the presence of heterosis. Heart girth measurements for Sinnar, local and crosses were  $110.61\pm0.436$ ,  $106.18\pm0.448$  and  $108.87\pm1.251$ , respectively. Body weight estimates of  $127.26\pm1.277$ ,  $113.40\pm1.312$ and 121.13± 3.665 kg were obtained for Sinnar, the local and the crosses. There was sexual size dimorphism and depending on the type of morphometric trait either jacks or jennets show significantly (P < 0.05) higher values as compared to the opposite sex. Jacks had wider chest (22.61 vs 22.09 cm.), thicker (24.40 vs 23.24 cm.), and longer cannon bone (31.00 vs 31.32 cm) than jennets, while the jennets possess wider hip (33.00 vs 31.69 cm.) and longer body (90.49 vs 88.52 cm.), back (67.37 vs 66.17), ear (24.42 vs 23.90 cm) and heavier estimated weight (122.47 vs 118.71 kg) than the jacks. Limited location effect was recorded showing donkeys from Guba district were comparatively the largest. Majority of the studied donkeys possess white abdominal color, unpigmented hoof and muzzle, short and medium hair size, plain body color pattern with long dorsal stripe without leg stripe, straight face and sloppy rump profile, medium tail length and thickness at the base of the tail. Qualitative differences (P < 0.05) were also observed among the class categories. Further molecular level studies could supplement the current study and provide more refined classification of the various genotypes in the studied area. Similarly, characterization of the local donkeys found in other parts of the region and the Abyssinian donkeys in the adjoining areas is required.

**Keywords:** Heart girth, Morphometric, Qualitative, Phenotypic types, Sinnar

[Full text-PDF]



Local Marie

Senior Male

Cost Male

Cost Male

Cost France

Cost France

Cost France

Mustefa A, Assefa A, Misganaw M, Getachew F, Abegaz S, Hailu A and Emshaw Y (2020). Phenotypic characterization of don in Registrated Guerra National Registrate Coding C Aging Seed Res 10 (1): 25-35 was a circle.

## Research Paper

# Effect of phase feeding on broiler performance.

Algam TA, Osman RH, Zomrawi WB and Abdalhag MA.

Online J. Anim. Feed Res., 10(1): 36-40, 2020; pii: S222877012000005-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr5

#### **Abstract**

This study was conducted to assess the effects of phase feeding on growth performance of broiler chicks. The experimental work consisted of a 7-week trial feeding, in which three different feeding programme. Three

experimental diets formulated to contain different levels of metabolizable energy (ME) and crude protein (CP); namely a broiler starter diet containing approximately 3072 Kcal ME/kg and 23.9% crude protein, a grower diet containing 3118 Kcal ME/Kg and 21.8% crude protein, and a finisher diet containing 3200 Kcal ME/kg and 18% crude protein. The results diets not reveal any significant differences in productive parameters, but indicated marked trends in differences among the experimental treatment. These differences showed that the highest feed consumption and live weight gain were attained by the group of birds fed the starter diet throughout the experimental period. The starter diet followed by the finisher diet for four weeks resulted in the lowest total feed consumption, reasonably high body weight gain, lowest feed conversion ratio and highest dressing percentage. It can, however, be considered under the conditions of the present experiment that the three experimental feeding programmes supported similar productive performance of broiler, indicating a slight economic advantage of feeding the starter diet followed by the finisher diet.

Keywords: Broiler chicken, Growth performance, Phase feeding

[Full text-PDF]



# Assessment of dairy value chain sustainability, constraints and opportunities in Aksum, Central Tigray, Ethiopia.

Misganaw G.

Online J. Anim. Feed Res., 10(1): 41-52, 2020; pii: S222877012000006-10 DOI: https://dx.doi.org/10.36380/scil.2020.ojafr6

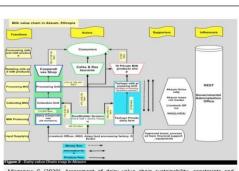
#### Abstract

Describing the sustainability of dairy value chain and showing the clear

gaps of the sector is important for bringing continual improvement that can support the livelihoods of dairy farmers and the wellbeing of environment. To facilitate a balanced practice on the three pillars of sustainability (people, planet and profit), a regular updating of the existing situation of dairy production is crucial. Therefore, the objective of this report was describing the sustainability of dairy value chain in Aksum district, and shows clearly the gaps of the sector that needs urgent action for continual improvement. Desk study for gathering secondary information was employed. Different analytical tools were used to analyze and present the result. In Aksum, milk is produced by mainly smallholder farmers, dairy cooperatives and few commercial medium scale farms. The production system is operated by gender inclusive system which is in line with sustainable dairy. The production potential of milking cows is very poor that is mainly due to poor genetic makeup and management system. The common feed is roughage that causes to the low production performance (poor economic viability) and high greenhouse gas emissions from enteric fermentation (risky for environment). The highest value addition, risks and costs are belonged to producers but the high share of margin is for processors and retailers. Therefore, this unfair share of profits, costs and risks is not a good sign of sustainable dairy development. Hence, an attempt to improve the dairy sector should consider the three pillars of sustainability.

Keywords: Dairy value chain, gender inclusive, smallholder farmers, Sustainability

[Full text-PDF]



Misganaw G (2020). Assessment of dairy value chain sustainability, constraints and opportunities in Aksum, Central Tigray, Ethiopia. Online J. Anim. Feed Res., 10(1): 41-52.

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# REVIEW ON CORONAVIRUS, A MIDDLE EAST RESPIRATORY SYNDROME (MERS-CoV)

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ABSTRACT: Human coronaviruses (HCoVs) have long been considered in consequential pathogens, causing the "common cold" in otherwise healthy people. However, in the 21st century, 2 highly pathogenic HCoVs—severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)-emerged from animal reservoirs to cause global epidemics with alarming morbidity and mortality. In December 2019, yet another pathogenic HCoV, 2019 novel coronavirus (2019-nCoV), was recognized in Wuhan, China, and has caused serious illness and death. The ultimate cope and effect of this outbreak is unclear at present as the situation is rapidly evolving. Middle East respiratory syndrome coronavirus (MERS-CoV) is zoonotic diseases causing severe respiratory illness emerged in 2012 in Saudi Arabia. Phylogenetic studies and viral sequencing results strongly suggest that MERS-CoV originated from bat ancestors after evolutionary recombination process, primarily in dromedary camels in Africa. The prevalence of MERS-CoV antibodies, the identification of MERS-CoV RNA and viable virus from dromedary camels of Eastern Africa and the Arabian Peninsula are the suggestive evidence for inter-transmission of the virus, primarily from camels to humans and its public health risks. However, the infection in camel is mostly asymptomatic. In contrast to the camel case, the clinical signs and symptoms of MERS-CoV infection in humans ranges from an asymptomatic or mild respiratory illness to severe pneumonia and multi-organ failure with an overall mortality rate of about 35%. Though inter-human spread within health care settings is responsible for the majority of reported MERS-CoV human cases, the virus is currently incapable of causing sustained human-to-human transmission (pandemic occurrence). Currently, there is no specific drug or vaccine available for treatment and prevention of MERS-CoV. The important measures to control MERS-CoV spread are strict regulation of camel movement, regular herd screening and isolation of infected camels, use of personal protective equipment by camel handlers and awareness creation on the public where consumption of unpasteurized camel milk is common. Therefore, urgent global epidemiological studies are required, to understand the transmission patterns and the human cases of MERS-CoV and also for the proper implementation of the above-mentioned control measures.

Keywords: Bats, Dipeptidyl peptidase 4, Dromedary camels, MERS-CoV, SARS-CoV, Transmission

#### **Abbreviations**

COV Coronavirus

MERS-CoV Middle East respiratory syndrome coronavirus SARS-CoV Severe respiratory syndrome corona virus

DPP4 Dipeptyl peptidase 4
WHO World Health Organization

ICTV International Committee on Taxonomy of Virus

PCR Polymerase Chain Reaction

Rt-PCR Real Time Polymerase Chain Reaction

Hcov Human Coronaviruses
ORF Open Reading Frame

CDC Centers for Disease Control and Prevention
ECDC European Centre for Disease Prevention and Control

ELISA Enzyme-linked immune sorbent assay
UpE Upstream of the envelope gene

BtCOV Bat coronaviruses

#### INTRODUCTION

Coronaviruses (CoV) are enveloped, positive-sense RNA viruses with large genomes (29–32 kb) packaged in particles with corona-like morphology (Lai et al., 2007). They can infect humans, as well as a variety of animals, such as bats, mice, birds, dogs, pigs, and cattle, causing mainly respiratory and enteric diseases (Perlman and Net land, 2009).

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Supporting Information

Before the 21st century, it was believed that human corona viruses, represented by the virus's hCoV-OC43 and hCoV-229E, can only cause mild respiratory symptoms (Saif, 2004). This notion changed after the outbreak of the severe acute respiratory syndrome (SARS) in 2002-2003, when a previously unknown human corona virus, named severe acute respiratory syndrome corona virus (SARS-CoV), caused the first corona virus-associated human epidemic, infecting approximately 8000 and killing 774 people (Peiris et al., 2004). In the years that followed, two additional human corona viruses were discovered, namely hCoV-NL63 and hCoV-HKU1 (Woo et al., 2005). All known human corona viruses are believed to have a zoonotic origin, with bats playing a major role in the interspecies transmission (To et al., 013).

MERS-CoV is the second newly discovered Beta corona virus lineage 2C and initially recognized in the Kingdom of Saudi Arabia in June 2012, when an elderly Saudi Arabian man was admitted to a local hospital with acute pneumonia and later died of progressive severe respiratory illness and renal failure (Zaki et al., 2012). The World Health Organization (WHO) global case count for MERS was 1952 laboratory-confirmed cases, including at least 693 deaths (case fatality rate 36%) from September 2012 to 3 April 2017 (WHO, 2017).

MER-CoV previously called human corona virus-Erasmus Medical Center was discovered by Zaki et al. (2012) in Saudi Arabia in 2012. In May 2013, the Corona Virus Study Group of the International Committee on Taxonomy of Viruses renamed the virus "Middle East respiratory syndrome corona virus (Murphy et al., 2012; Degroot, 2013). MERS-CoV marks the second known zoonotic introduction of a highly pathogenic corona virus, probably originating from bats (Sharif and Kanj, 2014). Three lines of evidence currently support this theory: Firstly, the very close phylogenetic similarity with the bat Beta corona viruses: BtCoV-HKU4 and BtCoV-HKU5 (Van bohemeen et al., 2012). Secondly closely related corona virus sequences have been recovered from bats in Africa, Asia, the Americas, and Eurasia; and thirdly MERS-CoV uses the evolutionary conserved dipeptidylpeptidase 4 (DPP4) protein in *Pipistrellus pipistrellus* bats for cell entry (Raj et al., 2013).

Since human-bat contact is limited, camels have been implicated as probable intermediate hosts. MERS-CoV appears to have been circulating in dromedary camels for over 20 years (Corman et al., 2014). Many studies have now identified dromedary camels (*Camelus dromedarius*) as a natural host for MERS-CoV, and there appears to be ample evidence of wide spread infection in dromedaries in the Middle East and in many parts of Africa (Reusken et al., 2014a, Hamid et al., 2015). MERS-CoV strains isolated from dromedaries are genetically and phenotypically very similar or identical to those infecting humans (Farag et al., 2015).

While corona viruses affect wide animal species (Woo et al., 2012a), MERS-CoV have affects limited host ranges. In the last few years, a large spectrum of domestic species has been negative after MERS-CoV serology tests, including horses, cattle, water buffalo, chickens, goats, and Bactrian camels (hemida et al., 2013). An exception was published recently when antibodies were detected in Alpaca (*Vicugna pacos*) in Qatar (Reusken et al., 2016) and susceptibility of pigs and Ilamas to MERS-CoV infection (Vergera et al., 2017).

In the case of MERS-CoV transmission, there is a large uncertainty about the various exposure pathways associated with new dromedary camel or human cases, and, although published research on MERS-CoV is actively increasing (Zyoud et al., 2016), few transmission risks have yet been quantified.

Therefore, the objective of this paper was to review the public health risk and transmission of Middle East Respiratory Syndrome.

#### **ETIOLOGY AND TAXONOMY**

Middle East respiratory syndrome (MERS), an emerging infectious disease, is caused by the MERS-corona virus (MERS-CoV) (Zaki et al., 2012). CoVs taxonomically belong to the subfamily Coronavirine, family Coronaviridae, in the order Nidoviales, and can be further classified into four genera: Alpha corona virus, Beta-corona virus, Gamma corona virus and Delta corona virus (De Groot, 2011).

The genus Beta corona virus contains four different lineages, A, B, C and D. The human corona virus's hCoV-229 and hCoV-NL63 belong to the genus Alpha corona virus, while hCoV-OC43 and hCoV-HKU1 belong to the lineage A of the genus Beta corona virus. SARS-CoV belongs to the genus Beta corona virus of lineage B while MERS-CoV grouped under lineage c beta corona virus. The genera Gamma and Delta corona virus contain only viruses that infect animals (ICTV, 2012).

Phylogenetic analysis performed by Zaki et al. (2012) after the isolation of MERS-CoV from the Saudi patient suggested that the virus belongs to the lineage C of the genus Beta coronavirus, together with the bat coronaviruses BtCoV-HKU4 and BtCoV-HKU5, which have been isolated from the species *Tylony cterispachypus* and *Pipistrellus abramus*, respectively (Woo et al., 2012a).

As stated by the ICTV, viruses that present greater than 90% sequence identity in their replicase domains belong to the same species. To investigate whether the newly identified virus is the prototype of a novel virus species, the amino acid sequence of the replicase gene obtained by sequencing of the PCR fragments that the pan-coronavirus PCR yielded was aligned with the respective sequences of its closest relatives, BtCoV-HKU4 and BtCoV-HKU5 (ICTV, 2012).

The comparison showed that the identity the viruses shared was greater than 80%, suggesting that the discovered virus represents a novel Beta corona virus species, the first human coronavirus described in lineage C of this genus. These results were repeated by the study of Van Boheemen et al. (2012), after they obtained the complete genome sequence of the the virus.

#### **EPIDEMIOLOGY OF THE DISEASE**

#### **Geographic distribution**

According to WHO report, MERS cases reported from 27 countries including Arabian peninsula (Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, the United Arab Emirates, Iran, Yemen), Europe (Austria, France, Germany, Greece, Italy, Netherland Turkey and UK), Asia (China, Philippines, Malaysia, Thailand, Republic of Korea), Africa (Algeria, Egypt, Tunisia) and USA. In the European and Asian countries as well as in Algeria, Egypt, Tunisia, and the United States, patients developed illness after returning from the Arabian Peninsula (WHO, 2019). In the United Kingdom, France, Italy, and Tunisia, limited human-to-human transmission occurred among close contacts of the index cases (WHO, 2015). MERS outbreak is ongoing in South Korea since May 2015; the index case was a man who had traveled to Bahrain, the United Arab Emirates, Saudi Arabia, and Qatar. All of the cases outside of the Middle East have had a direct or indirect connection to the Middle East.

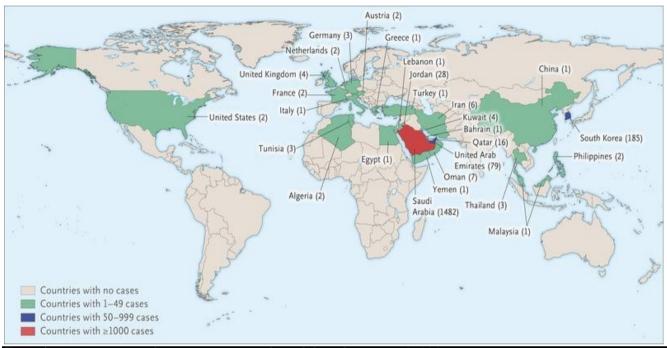


Figure 1 - Map showing confirmed global cases of MERS-CoV. Source: WHO (2017)

#### Virus replication on cyclophilin A

CypA is one of the most abundant cytosolic proteins constituting 0.1–0.4% of the total cellular protein content (Harding et al., 1986). Data from different labs suggest that relatively low levels of CypA may suffice to support efficient coronavirus replication. Cyclophilins were initially implicated as host factors in coronavirus replication during studies with general Cyp inhibitors such as cyclosporine A (CsA). In cell culture, the replication of a variety of coronaviruses was found to be strongly inhibited by low micromolar concentrations of cyclosporine A and the non-immunosuppressive Cs A analogs Alisporivir. The cyclosporine A dependence of the replication corona viruses in the same cell line (Huh7), in which CypA expression was knocked-out using CRISPR/Cas9 gene editing technology (de wilde et al , 2017).

### Reservoirs and host susceptibility

Dipeptidyl peptidase 4 (DPP4; also, known as CD26) has been identified as the receptor for the MERS-CoV spike protein and is required for viral binding and entry into host cells (Raj et al., 2013). DPP4 is a type II Transmembrane glycoprotein that is expressed on epithelial and endothelial cells throughout the body (Lambeir et al., 2003). Although DPP4 is evolutionarily conserved, differences in the amino acids present in its extracellular domain, which interacts with MERS-CoV spike protein, have been noted among various animal species and humans. Specifically, 14 amino acids in DPP4 appear to be critical in determining whether the MERS-CoV spike protein can bind to DPP4 (Wang et al., 2013).

#### **Bats**

Bats are known natural reservoirs for several emerging viral infections in humans including rabies, Nipah virus, Hendra virus and Ebola virus (Han et al., 2015). Several features enable bats to be efficient sources of emerging human viral infections. As an extremely diverse species with a long evolutionary history, bats have co-evolved with a variety of viruses. Their lack of B-cell-mediated immune responses allows them to carry viruses without showing overt signs of illness (Brook and Dobson, 2015). Low metabolic rate and suppressed immune response during bats' hibernation result in delayed viral clearance (George et al., 2011). Bats live closely together in extremely large numbers facilitating stable circulation of viruses amongst them (Calisher et al., 2008).

Furthermore, bats are capable of flying and hence carrying potentially infectious pathogens over considerable distances (Drexler et al., 2014). A pertinent feature of bats is that they chew fruits to absorb their sugars and spit out the remains. The discarded fruits can be contaminated with viruses from the oral cavity, urine and feces providing a ready source for transmission to other potential hosts such animals and humans (Brook and Dobson, 2015).

Bats have been implicated as the main reservoir of members of the genera Alpha corona virus and Beta corona virus (Woo et al., 2012b) and play pivotal roles in interspecies transmission of CoVs. This is best exemplified by SARS-CoV, which was shown to originate from Chinese horseshoe bats (Lau et al., 2005), and was probably transmitted directly to humans (Ge et al., 2013) or through an intermediate host, such as the palm civet (Guan et al., 2004). MERS-CoV, together with bat CoV-HKU4 and HKU5, phylogenetically belongs to lineage C in the genus Beta corona virus (Van Boheemen et al., 2012). Thus, it is suspected that the emerging MERS-CoV might also originate from bats.

A large screening study for beta corona viruses was conducted on fecal specimens taken from 4758 bats of ten different species in Ghana, and 272 *Pipistrellus* bats from four European countries showed that a bat derived corona virus that has a very close phylogenetic relationship to MERS-CoV (Annan et al., 2013).

A report from South Africa and Saudi Arabia identified a bat derived corona virus that has a very close phylogenetic relationship to MERS-CoV (Ithete et al., 2013; Memish et al., 2013c). An experimental study conducted on Jamaican fruit bat (*Artibeusja maicensis*) showed evidence of infection as they shed the virus from their respiratory and intestinal tract (Munster et al., 2014). Therefore MERS-CoV, like many other coronaviruses, originated in bats. This is based on the isolation of other lineage C beta-corona viruses that are very closely related to MERS-CoV on phylogenetic analysis (De Benedicts et al., 2014).

#### **Dromedary camels**

The close phylogenetic relationship of human MERS-CoV isolates with those obtained from bats initially suggested that MERS-CoV might have originated from bats. However, bats were unlikely to be the direct source of the MERS outbreak, since MERS cases were rarely found to have a history of contact with bats. Therefore, other animals were searched as direct sources of zoonotic transmission of MERS-CoV (Han et al., 2016). Multiple lines of evidence implicate dromedary camels in the emergence and transmission of MERS CoV.

MERS-CoV antibodies are highly prevalent in dromedary camels from across the Arabian Peninsula, North Africa and Eastern Africa (Nowotny and Kolodziejek, 2014). The high prevalence of MERS-CoV seropositivity in Africa and the Middle East suggests that animal movement has facilitated the transmission and circulation of MERS-CoV amongst dromedary camels in these regions. MERS-CoV antibodies have neither been found in Mongolian or Dutch Bactrian camels nor in South American camelids such as ilamas, alpacas and guanacos (Reusken et al., 2013a).

MERS-CoV reported by RT-PCR in oro-nasal and faecal samples from dromedary camels in multiple locations in the Arabian Peninsula (Hemida et al., 2013). The four of 110 dromedary camels in which MERS-CoV RNA was detected in Egypt were all imported from Sudan or Ethiopia for slaughter (Chu et al., 2014). MERS-CoV was also detected by RT-PCR in symptomatic camels. Dromedaries with active MERS-CoV infection exhibited symptoms such muco-purulent nasal and lachrymal discharge, cough, sneezing, fever and loss of appetite (Memish et al., 2013b).

The potential infectiousness of MERS-CoV recovered from dromedary camels was evident its capability to cause exvivo infection in human respiratory cells and human hepatoma cells. Successful MERS-CoV cultures usually coincide with corresponding high viral loads in the same specimens (Chan et al., 2014). Experimental MERS-CoV infection of dromedary camels with result mild clinical infection manifesting as fever and rhinorhea also implicate dromedary camels in the emergence and transmission of MERS CoV (Adney et al., 2014a,b).

## Other animal species

Differences in virus susceptibility and pathogenicity between animals of different species could be explained by a distinct tissue distribution of DPP4, the MERS-CoV receptor. Species with few or no differences in the 14 amino acids seem to be susceptible to MERS-CoV, including rhesus macaques, common marmosets and result cytopathic cellular changes and mild to severe respiratory illness (Munster et al., 2013; Falzarano et al., 2014). Macaques and marmosets have already proved useful animal models for the investigation of MERS-CoV (Eckerle et al., 2014).

Dromedary camels seems to be the only domestic animal reservoir for MERS-CoV until a recent study by Reusken et al. (2016) and Vergera et al. (2017). Reusken et al. (2016) investigated the MERS-CoV infection status of 15 healthy alpacas (*Vicugnapacos*) in a herd of 20 that shared a barn complex with dromedaries. All tested alpacas were seropositive to MERS-CoV.

Recent study on livestock susceptibility conducted in 2017 indicate that pigs and llamas are susceptible to MERS-CoV infection (Vergera et al., 2017), but the level of MERS-CoV excreted in the nose of dromedaries seems to be much higher than that of other animal species described so far (Adney et al., 2014a,b). On the other hands the study showed that sheep did not show clinical sign. These results are in concordance with those reported by Adney et al. (2016) that MERS-CoV experimentally inoculated sheep showed no clinical disease and that only small amounts of virus were detected in nasal swab samples. Even though the receptor binding domain, and in particular key amino acids on the docking site are identical in horses and human (Van Doremalen et al., 2014), horses were not susceptible to MERS-CoV (Vergera et al., 2017). Screening study on equids including horses, donkeys and mules from UAE and Spain result in sero-

negative for MERS-CoV (Meyer et al., 2015). These results highlight that other mechanisms, such as epithelial cell permissibility or strong innate immune responses, may influence the establishment of infection. Differences in the number of goblet cells in the lining epithelium and mucus covering epithelial surfaces, which may have impeded the binding of the virus to the respiratory epithelium of horses (Vergera et al., 2017). Serological study, conducted in Saudi Arabia and Europe from sheep, goats, cattle, and chickens representing different geographical areas within the country were resulted sero negative for MERS-CoV (Hemida et al., 2013; Reusken et al., 2013a). In addition ferrets, hamsters, and mice are resistant to infection (Van et al., 2014).

#### Source of infection and transmission

There is growing evidences that the dromedary camel is host species for MERS-CoV and plays an important role in the transmission of the viruses to human (Azhar et al., 2014). In August 2013, for the first time, dromedary camels were implicated as a possible source of virus causing human infection because of the presences of MERS-CoV specific neutralizing antibodies in dromedary camels from Oman and other countries in the Arabian Peninsula and North Africa.

An analysis of an outbreak of MERS-CoV infection in humans in Qatar in October 2013 found that dromedary camels and humans were infected with a nearly identical strain of MERS-CoV (Hemida et al., 2013). Widespread circulation of different genetic variants of MERS-CoV has been found in camels and the presence of MERS-CoV specific antibodies in samples taken from camels, years earlier. Although dromedary camels are suspected to be the primary source of MERS-CoV leading to human infections, the true routes of zoonotic transmission remain to be determined (Azhar et al., 2014).

Chu et al. (2014) reported that Middle East respiratory syndrome corona virus from Egypt has been fully genetically sequenced and biologically characterized. While this virus was genetically diverse from viruses causing zoonotic infections in the Arabian Peninsula, the receptor binding domain of the Egyptian viruses is conserved, indicating that these viruses would be able to infect the human respiratory tract. This contention is supported by the finding that tropism and virus replication competence of MERS-CoV from Egypt in ex vivo cultures of the human bronchial and lung is comparable to that of camel of human virus isolates in the Arabian Peninsula (Chu et al., 2014).

#### **Camel to human transmission**

Whole MERS-CoV genome sequences obtained from viral cultures of the human and camel isolates were 100% identical. Importantly, 4-fold rise in MERS-CoV antibody titres was documented in the camels, indicating that active MERS-CoV infection was probably circulating in the dromedary herd. Later, rising MERS-CoV antibodies were documented in the patient, suggesting that MERS-CoV infection was transmitted from the camels to the human and not vice versa (Azhar et al., 2014). MERS-CoV was detected in eight asymptomatic dromedary camels at entry into UAE from Oman. Two asymptomatic men, aged 29 and 33 years, who were in contact with the camels, were found to be positive for MERS-CoV RNA in their respiratory samples. Partial sequences of MERS-CoV spike and nucleocapsid regions from the human and linked camels were identical. Within 4–8 days from diagnosis, both patients had undetectable MERS-CoV RNA (Al Hammadi et al., 2015).

MERS-CoV sequences have been detected more commonly in nasal swabs than in rectal specimens of camels (Hemida et al., 2014). Infection of camels in the laboratory also confirmed susceptibility, with a large quantity of virus shedding from the upper respiratory tract (Adney et al., 2014a,b). Therefore, droplet transmission or direct contact with infected camels may be the most likely mode of camel-to-human transmission of MERS-CoV. Direct contact with camels can only explain some of the primary cases, since some MERS cases did not report any direct contact with camels (Han et al., 2016).

Other possible routes for camel-to-human transmission include food-borne transmission through consumption of unpasteurized camel milk, raw meat and the camel urine. Camels are an important source of milk in some Middle East countries and parts of Africa, and more than half of the camel milk is sold as unpasteurized fresh or fermented milk to local and urban consumers in Saudi Arabia (Faye et al., 2014). A survey found the presence of MERS-CoV RNA in the milk of camels actively shedding the virus (Reusken et al., 2014b).

An experimental study of the stability of MERS-CoV in milk showed that viable viruses could still be recovered after 48 h regardless of reduction in virus titre, indicating that infection could happen by consumption of unpasteurized fresh raw milk (Van Doremalen et al., 2014). Consumption of undercooked meat from infected camels and handling of infected raw camel meat without proper protective equipment may also pose risks for getting MERS CoV from camel. An oral-faecal transmission mode was also suspected. Using protein intrinsic disorder prediction, MERS-CoV was placed into disorder group C and was likely to persist in the environment for a rather long period of time, and showed high oral-faecal transmission chances (Goh et al., 2013).

The single study, by Van Doremalen et al. (2013), on Plastic or steel surfaces inoculated with MERS-CoV at different temperature and relative humidity (RH) shows that the virus remained viable for 24 h. The study reported that MERS-CoV was more stable under low-temperature/low-humidity conditions, suggesting the potential for MERS-CoV to be transmitted via contact or fomite transmission due to prolonged environmental presence. By comparison, a well-known and efficiently transmitted respiratory virus, influenza A virus, could not be recovered in culture beyond four hours under any conditions. Aerosol experiments found MERS-CoV viability only decreased 7% at low relative humidity at 20 °C. In comparison, influenza A virus decreased by 95 % (Van Doremalen et al., 2013).

#### **Enter-human transmission**

While the introduction of MERS-CoV to the human species from an animal reservoir seems to be the reason for the initial infections, the occurrence of clusters suggests that the virus hasadapted to human-to-human transmission. Person-to-person transmission of MERS-CoV has been documented in several human clusters associated with healthcare facilities, households and workplace (Assiri et al., 2013a; Memish et al., 2013a).

Nosocomial outbreak is a distinct hallmark in MERS-CoV transmission involving hospitalized patients, healthcare workers and close family contacts in healthcare facilities in affected countries in the Middle East and in some countries where the disease had been exported to, the most recent being the Republic of Korea (RoK). Droplet spread between humans is considered the mechanism of human-to-human transmission and the need for droplet precautions was emphasized after the Al-Ahsa hospital, the KSA (Khalafalla et al., 2015) and the South Korean outbreaks (Assiri et al., 2013a).

MERS-CoV's ability to remain viable over long time periods gives it the capacity to thoroughly contaminate a room's surfaces when occupied by an infected and symptomatic patient (Van Doremalen et al., 2013). Whether MERS-CoV can remain a drift and infectious for extended periods (truly airborne) remains unknown. Such findings expand our understanding of the possibilities for droplets to transmit respiratory viruses in many settings, including hospital waiting rooms, emergency departments, treatment rooms, open intensive care facilities and private patient rooms. The nature and quality of air exchange, circulation and filtration are important variables in risk measurement and reduction as is the use of negative pressure rooms to contain known cases (Assiri et al., 2013a).

Furthermore, given the high concentration of the virus in the lower respiratory tract of infected patients, airway suctions or use of bronchoscopes could also serve as a source of transmission (Guberina, 2014). The infectiousness of urine and stool is currently under investigation, since the virus has been detected in urine and stool samples of patients and based on the fact that cluster patients had been sharing toilet rooms during hospitalization. Transmission via blood should also be considered a possible route, since scientists claim that the virus might be present in blood (Guery, 2013). This could be correlated to the reported person-to-person transmission in hem dialysis units of a hospital in Saudi Arabia (Assiri et al., 2013b).

Generally, corona viruses are transmitted among humans via aerosol droplets and/or through direct contact with other secretions (stool, urine etc.) (Danielsson, 2012). Currently, the pathways used by MERS-CoV for inter human transmission remain unknown. Several case investigations have suggested that airborne transmission seems to be the most likely route (Memish et al., 2013a).

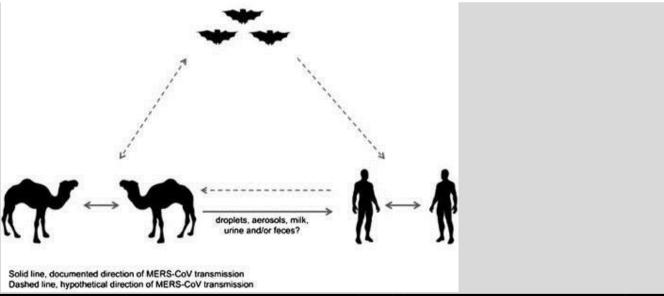


Figure 2 - Reported Infection sources and transmission routes of MERS-CoV between bats, camels and human (Source: Omrani et al., 2015).

#### **Public health importance**

This novel virus can cause severe acute respiratory disease, mainly in patient with immunosuppressant condition and underlying disease including diabetes, heart disease, renal failure, hypertension, chronic lung disease, including asthma and cystic fibrosis. Moreover, history of travel in at risk countries and smoking might have considered as a risk factors of severe disease (Al Barrak et al., 2012). Washing hands, face and hair in camel urine is a traditional custom among Bedouins and camel-herding peoples in the Arabian Peninsula and East Africa. There are currently no published data about MERS-CoV in the urine of infected camels, but the virus has been found in low concentration in human urine samples (Drosten et al., 2013), and therefore, consumption of camel urine may represent a risk factor for infection.

Dromedary camel meat represents 0.45% of the red meat produced worldwide (Faye, 2013). While there is no evidence of MERS-CoV in camel meat, by analogy with what is known about other viruses like Rift Valley fever virus, we can assume that the fall in pH of meat with maturation could inactivate the virus (Food and Agriculture Organization) and that proper cooking would kill the virus. However, handling of raw meat and slaughtering of animals should not be excluded as a risk factor. The list of at-risk countries, as defined in European Centre for Disease Prevention and Control (ECDC) rapid risk assessment included Iraq, Israel, Jordan, Qatar, Saudi Arabia, Syria, Kuwait, Lebanon, Palestine, Oman, UAE, Yemen, Bahrain and Iran (ECDC, 2013). People working closely with camels (e.g. farm workers, slaughterhouse workers and veterinarians) may be at higher risk of MERS-CoV infection than people who do not have regular close contacts with camels and also health care workers (WHO, 2014).

#### **PATHOGENESIS**

MERS-CoV pathogenicity is based on the extent pathogen-host interaction. It elicits maximum pathogenic potential especially in humans. This is due to the fact that MERS-CoV shows a strong tropism for bronchial non-ciliated epithelia. Furthermore, the virus arrests host bronchial interferon synthesis. It should be noted that most of other viruses causing respiratory diseases attack and damage epithelial cilia, including Influenza type A.

Molecular studies revealed that cellular receptors for MERS-CoV are exopeptidase (angiotensin converting enzyme 2) (Coleman et al., 2014). Moreover, it was found that neutralization of angiotensin converting enzyme 2 by specific antibodies did not arrest the spread of infection into bronchus and lung alveolus. Extensive investigations showed that another functional cellular receptor called DDP4 was also involved in the severity of MERS-CoV disease spread into the lungs (de Wit et al., 2013). Of note, receptors for DDP4 are also located in nephrons of kidneys and heart.

During the acute stage of the MERS-CoV infection, there is a severe viremia, leading o spread of MERS-CoV viral particles in the bloodstream. Hence, MERS-CoV leads not only to the damage of lungs but also kidneys and heart, thereby resulting in respiratory, renal and cardiac failure, ultimately ending to coma and death (Van Boheemen et al., 2012). The severity is worsened by concurrent secondary bacterial infections. Recent research showed that bacterial infections due to Staphylococcus aureus, Group A Streptococcus, Streptococcus pneumonia and Haemophilus influenzae type b augment the pathogenic potential of MERS-CoV, particularly in humans. These bacteria particularly dwell in the oral cavities, tonsils and pharynx of humans (Lau et al., 2013).

#### **CLINICAL FEATURES**

The median incubation period of a MERS-CoV infection is 5 days. The clinical manifestations in patients of MERS-CoV range from subclinical infection to severe respiratory disease. Symptomatic patients often present with fever, myalgia, and sore throat, shortness of breath, cough, and occasionally hemoptysis. Gastrointestinal symptoms such as diarrhea and vomiting are also common. Hematological abnormalities reported for clinical cases include thrombocytopenia, lymphopenia, lymphocytosis, and neutrophilia (Assiriet al., 2013a; Gueryet al., 2013). Radiographs with a spectrum of lower pulmonary infiltrates and consolidation consistent with viral pneumonia (Zakiet al., 2012; Assiriet al., 2013b).

In contrast to the human cases, camel showed minor clinical signs of the disease, including of rhinorrhea and a mild increase in body temperature but no other clinical signs were observed (Khalafalla et al., 2013) and the nasal discharge drained from both nostrils varied in character from serous to purulent (Daniella et al., 2014). In humans, after the entry of MERS-CoV viral particles in to lung alveoles, alveolar macrophages fail to contain the spread of infection. The strong host cellular immune response and cytokine release leads to inflammation and fluid accumulation in lungs.

#### **DIAGNOSIS**

Sputum from lower respiratory tract, nasopharyngeal swab, whole blood, tissue from biopsy or autopsy including from lung and serum for serology are important for virus detection. Lower respiratory tract specimens (such as tracheal aspirates and Broncho alveolar lavage) appear to have the highest virus titre. Upper respiratory tract specimens are also recommended, especially when lower respiratory tract specimens cannot be collected (Guery et al., 2013).

Rapid verification of cases of novel corona virus infection will be based on detection of unique sequences of viral RNA by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and immune fluorescence. However, antibodies against beta corona viruses are identified to cross react within the genus. Therefore, immunofluorescence effectively limits their use to confirmatory applications (Corman et al., 2012b). However, for detection of MERS-CoV in particular, alternative RT-PCR assays are required, detecting certain targets that have been described to be specific for the virus (Van Boheemen et al., 2012).

Corman (2012a) proposed two RT-PCR assays for the detection of the virus, each one targeting different parts of the viral genome. The first assay targets a region upstream of the envelope (E) gene (upE assay), while the second assay targets part of ORF1b (ORF1b assay), which does not overlap with the target of pan-coronavirus assay. The upE assay was found to be more sensitive in comparison to the ORF1b assay. Thus, the use of the upE assay is recommended for screening, while the ORF1b assay can be used for confirmation (Corman et al., 2012b). The specificity of both assays was

confirmed by excluding cross-reactivity with the other known human corona viruses. A third assay, optimized for sensitivity, was described by the same group, this time targeting ORF1a. Overall, a combination of the upE and ORF1a assay seems to be the optimal approach for MERS-CoV detection (Corman et al., 2012a).

Several serology assays have been developed for the detection of MERS-CoV antibodies, including immuno-fluorescence assays and a protein microarray assay (Reusken et al., 2013b). The Center of Disease Control and Prevention (CDC) has developed a two-stage approach, which uses an enzyme-linked immune sorbent assay (ELISA) for screening followed by an indirect immunofluorescence test or micro neutralization test for confirmation.

#### **PREVENTION AND CONTROL**

Since there are currently no effective drug therapies to treat or prevent the infection, clinical management of patients with severe disease largely relies on meticulous intensive care support and prevention of complications. This includes hydration, antipyretic, analgesics, respiratory support, and antibiotics, if needed, for bacterial super infection. Current treatment is based on previous experience with the Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV), in vitro studies, and case series. Various agents have been tried, including those that block virus entry, inhibit viral replication, or interfere with host immune response (Al-Tawfiq and Memish, 2014).

Monoclonal antibodies that efficiently block the interaction between the MERS-CoV envelope spike glycoprotein and a human protein DDP4 have been developed using a humanized mouse (transgenic mouse). These researchers are now working to move the antibodies into human trials. Based on experience with SARS-CoV, the use of convalescent plasma, hyper-immune globulin, or human monoclonal antibodies that contain neutralizing antibodies may be efficacious and is recommended as first-line treatment when available (Jiang et al., 2014).

Understanding the zoonotic sources of MERS-CoV might guide control and prevention of the disease. The WHO advises people at risk of MERS-CoV infection to avoid contact with camels, to practice good hand hygiene, and to avoid drinking raw milk or eating contaminated food unless it is properly washed, peeled or cooked (WHO, 2014). Since most of the cases occur in the health care setting, it is thoughtful that all health care workers practice appropriate infection control measures when taking care of patients with suspected or confirmed MERS-CoV (WHO, 2015). Currently, there is no specific drug or vaccine available for treatment of infection caused by MERS-CoV. Even though, a number of antiviral medicines are currently under study (Zumla et al., 2015), there is no licensed vaccine to prevent MERS-CoV infection. However, one company has developed an experimental candidate MERS-CoV vaccine (Novavax, 2013). O also developed other candidate vaccines which are being studied as full-length infectious DNA clone of the MERS-CoV genome in a bacterial artificial chromosome.

#### **CONCLUSION**

Middle East respiratory syndrome is zoonotic diseases causing severe lower respiratory illness and now considered a threat to global public health. The current knowledge about virus is limited, since many important epidemiological and clinical aspects remain unknown. Transmission of MERS-CoV from camel to human is well documented and studied by different researchers but is generally not very efficient because transmission route of the virus back from human to camel is still hypothetical.

The exact mechanism of transmission is not clear, including whether other intermediate hosts are involved, which will be a risk for new incidence of the disease, especially for those countries in which infection cases were not reported. Although MERS-CoV displays lower transmissibility among humans than SARS-CoV, the possibility that future mutations will render the virus highly transmittable, with a devastating outcome, cannot be discarded.

In the light of aforementioned conclusions the following general and specific points are recommended.

- Urgent epidemiologic investigations through surveillance on environmental, animal and testing around sporadic unexplained cases are needed to find other animal reservoirs.
  - Extensive efforts are required to speed up the development of an effective therapy and vaccine.
- Camels may play important role in transmission of the virus, and the common practices in pastoral areas of consuming unpasteurized camels' milk and raw meat should be avoided.
- Health care workers caring for patients under investigation for MERS-CoV or confirmed cases should exercise standard precautions including hand hygiene, as well as contact or air borne precautions.

## **DECLARATION**

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#### **Authors' contribution**

Dr. Birhanu and Dr. Kassahun participate in design the topic of the review. Dr. Damtew performed by gathering the data. In addition, Dr. kassahun carefully revised and write the manuscript. Finally, all authors read and approved the final manuscript.

#### **Availability of data**

The data can be availed to the journal upon request.

#### **Conflict of interest**

The authors declare they have no competing of interests.

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# MORPHO-HISTOLOGICAL STUDY OF SPLEEN ONTOGENESIS IN LAMBS DURING ANTENATAL AND POSNATAL PERIOD

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ABSTRACT: In an experiment on the spleen of healthy lambs at two periods (prenatal and postnatal), using anatomo-topographic and histo-cytological research methods and with subsequent statistical analysis, it was found that the changes in the anatomical and histological structure related to the age of this organ, including during the early stages of the postnatal period of ontogenesis. Note that the surface of the spleen is covered with a capsule of connective tissue, from which leave trabeculae which divide the parenchyma of the organ into lobes. At the time of physiological maturity, the ratio of the structures changes in the direction of an increase in the white pulp and a decrease in the component of the red pulp. During all periods of prenatal fetal development, the percentages of spleen tissue components corresponding to fetal analogs with normal clinical development are determined. The morphological structure of the spleen is described for the first time for this breed in Algeria, age-related changes in the structures of the parenchyma are traced, the formation of follicles began during the terminal phase of gestation, and the development age-related white pulp.

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RESEARCH ARTICLE

Keywords: Algeria, Follicle, Parenchyma, Postnatal, Spleen, Lambs, White pulp.

#### INTRODUCTION

The spleen is a secondary lymphoid organ with dual function; Immune and hematopoietic at the same time (Short et al., 2016). Spleen studies in sheep have been done by Gnanadevi et al. (2019), in addition, extensive studies on the human spleen have been performed by Almenar et al. (2019). Also the white pulp of spleen is mainly populated by lymphocytes. The areas of white pulp include arterial vessels, surrounded by lymphoid tissue, with clear boundaries. In addition, in white pulp of rabbit's spleen there are lymphoid follicles grouped by lymphoid cells. According to Rahman et al. (2016), they are surrounded by a well-defined connective tissue. These lymphoid follicles belong to B-dependent lymphoid tissue that the researchers found by Mollejo et al. (2002).

With regard to other animals, the white and red pulps are distinguished in the spleen of rabbits previously studied by Rahmoun et al. (2019), also white pulp of spleen is mainly populated by lymphocytes. The areas of white pulp include arterial vessels, surrounded by lymphoid tissue, with clear boundaries (Quesada et al., 1990). In addition, in white pulp in rabbit's spleen there were lymphoid follicles grouped by lymphoid cells, surrounded by well-defined connective tissue. These lymphoid follicles belong to the B-dependent lymphoid tissue that found by the researchers (Rebelatto et al., 2018). Papenfuss et al. (2017) described that at the level of the white pulp, the lymphoid sleeve is presented by a mass of T lymphocytes around the central arteries lymphoid follicles, also attached to the sleeves formed by B lymphocytes are composed of a clear germ center corresponding to the transformation zone of the lymphocytes into plasma cells and a darker crown where lymphocytes proliferate.

Our research based on the study of growth and development of this organ in antenatal and postnatal period according to the norms of descriptive immunology of lymphoid organs theories and highlighting the activation of follicles after the contact with antigen.

#### **MATERIALS AND METHODS**

Spleens from fetuses, as well as lambs healthy and unvaccinated from the slaughterhouse city of souk ahras, Algeria, were the subject of our study, the collected spleens were performed immediately after slaughter dissection of animals for the antenatal period, and from the pregnant female during the last month of gestation. After collecting organs, the spleens are weighed with an electronic High precision balance KERN KB 0.001 g and the length and width are taken with electronic Ruler Measuring Stainless Steel LCD, the data is entered in the note-book.

Supporting Information

Organ fixation was performed with 5.0% and 10.0% formalin. The research was performed in the histology and histopathology laboratory of Taoura Veterinary Institute, University of Souk Ahras, Algeria. Organ fixation was performed with 5.0% and 10.0% formalin. The organs have been sectioned and placed in a coating cassette, placed in the initial pot of automat impregnation SLEE MEDICAL, which includes 12 stations, ten glass beakers for xylene and alcohol with digressive degrees of treatment and two pots of paraffin in anodized aluminum. For dehydration and preparation of tissue for microtome cutting Programmable thanks to the LCD screen, for control of temperature and elapsed time. The step of coating the tissue samples with the MPS / P1 system. Thin sections of 5.00 7.00 microns were made with a CUT 4062 microtome with manual rotation, put on slide for different staining, Hematoxylin-eosin, impregnation with silver nitrate, and immuno-histochemistry with markers identification of T lymphocytes.

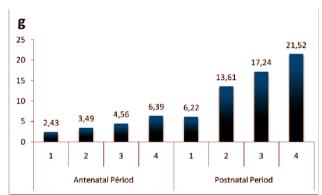
Examination with an ocular micrometer, distances between divisions of 5 µm. The histological structure of the parenchyma has been detected. The measurements of each ocular field were carried out in five fields of vision of five histological sections of each histological slide.

Other sections were subjected to an impregnation with silver nitrate by serial passage in silver nitrate and potassium permanganate tanks at a decreasing concentration and this in order to visualize the reticular structure of this organ. For localization of lymphocytes in the functional areas of the spleen, immunohistochemistry is used for the determination and localization of lymphocytes and macrophages in the parenchyma of organs, also the determination of the cytological characteristics was carried out using an ocular microscope, antibodies were used to elucidate the T lymphocytes in the functional areas of the spleen.

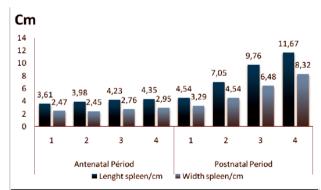
The examinations and the taking of the data were carried out on personal computer. The determination of the histological and cytological characteristics was carried out using an ocular microscope and a stereo MBS-10 microscope. The quantitative study for the tissue compartments was carried out using the point S system; the data obtained were analyzed with R program.

#### **RESULTS AND DISCUSSION**

The study of the mass of spleens during the antenatal period revealed that the minimum value was 2.43 g found in the first group, while the maximum value was 6.39 g found in the fourth group (Graph 1), concerning lambs. From the postnatal period, the minimum value was 6.22 g found in the first group, while the maximum value was 21.52 g found in the fourth group (Graph 1). The results of morphometric study and mass of this organ on other mammals were conducted by Vashishtha et al. (2018). Finding significant variations according to age, also, discussed a progressive increase of mass and revealed that sex has no influence on its development, results found by Jaji et al. (2019). Measurement of fetal spleens, minimum value of length was 3.61 cm found in first group while maximum value was 4.35 cm found in fourth group. About width, minimum value was 2.47 cm found in second group, whereas maximum value was 2.95 cm for the fourth group (Graph 2). Regarding lambs spleen, the minimum length value of 4.54 cm found in first group and maximum value of 11.67 cm found in fourth group. While minimum width value was 3.29 cm found in first group, and maximum width was 8.32 cm found in fourth group (Graph 2).



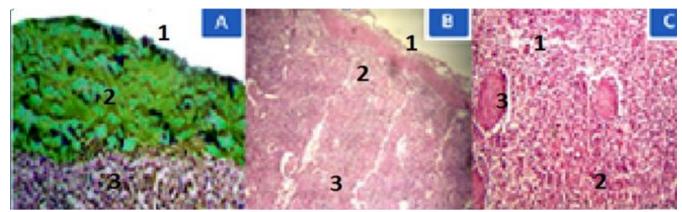
**Graph 1** - Mass index of spleens (g) according to antenatal and postnatal period (days).



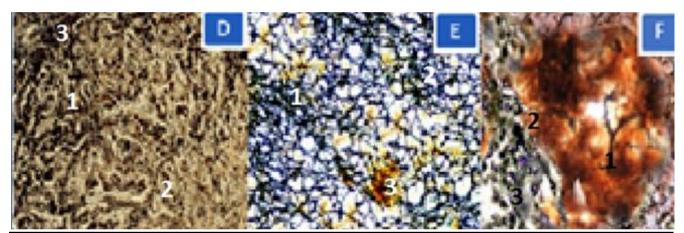
**Graph 2** - Morphometric index of some spleens, (cm) according to the age (days).

Optical Microscopic exams revealed that lambs' spleen consisted of stroma and parenchyma (Graph 3). Stroma mainly formed by capsule and trabeculae that enter into parenchyma. The capsule of this organ consists of two layers, an external connective tissue (elastic) and internal (muscle), according to Weigert's Hematoxylin and picrofushin (Van Gieson) staining allowing the organ to change its size and maintain an increase significant of its volume (Figure 1-A). It has been found that red pulp is composed of splenic sinusoidal capillaries and cellular cords. Splenic sinusoidal capillaries are limited by single discontinuous epithelium, resting on basement membrane which surrounds sinusoids in discontinuous way, species situated between sinusoidal capillaries occupied by cellular cords, in the researches of Silva-O'Hare et al. (2017) have achieved almost the same results, although follicles appeared in the antenatal phase according to our data, which is not in theory as immunological norm, this result would open new research later.

The results obtained after silver nitrate staining revealed white pulp is composed of lymphatic follicles, integrated in different places of red pulp in the form of small rounded growths. Arteries that cross the periphery were observed. Vascularization of red pulp takes its origin from penicillate arteries that give rise to arterioles (Figure 2-F), whereas endothelial cells and smooth muscle cells are clearly visible in the center (Figure 2-E). Gavrilin et al. (2017) noted that fibers were arranged vertically in trabeculae and gradually became shallow in terminal branches. Elastic and muscular fibers oriented in a parallel way to trabeculae direction, their function is changing volume, whereas smooth muscle function is discharging blood from the organ. White pulp rich in reticular tissue found to have silver nitrate impregnation of histological sections also was observed (Ikpegbu et al., 2019).



**Figure 1**: (a) Histological section of newborn lamb spleen, Hematoxylin & eosin, × 40: 1-capsule; 2-muscle fiber; 3-Cortical zone. (b) Histological section of lamb fetus spleen, Hematoxylin & eosin, × 40: 1-capsule; 2-cortical area; 3-Medullar area; 4-Trabeculae artery. (c) Hematoxylin & eosin, × 40: 1- Pulp artery; 2-Trabeculae artery; 3-red pulp.



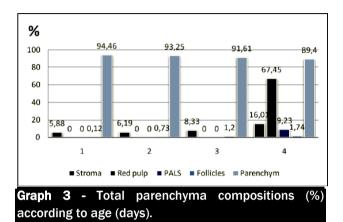
**Figure 2:** (D) Histological section of lamb fetus spleen, silver nitrate impregnation, × 40: 1-sinus; 2-early formation of reticular tissue; 3-medullary area. (E) Histological section of the lamb spleen, silver nitrate impregnation, × 40: 1-zone of reticular fiber formation; 2-pulp artery. 3-cortical area. (F) Histological section of the newly born lamb spleen: 1-pulp artery, 2-PALS, 3-lymphocyte cell accumulation.

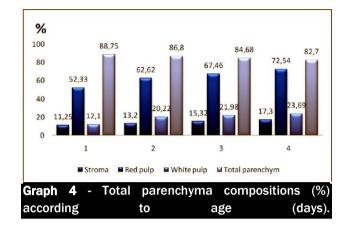
Hassal corpuscles and a peri-arterial lymphatic sheath also were noted (Figure 1-F). Results are similar to those reported by Shringi et al. (2018). Splenic follicles were well visualized, of different sizes, as an ovoid mass, composed of aggregates of lymphatic tissue and small pulpal arteries (Figure 1-C), as described by Dubey et al. (2018), space between white pulp and trabeculae occupied by red pulp, similar results were observed in other studies. It has also been noted that red pulp is composed of pulpal arterioles, splenic follicles, and splenic cords (Nicander et al., 1993). Quantitative results in antenatal period revealed after microscopic examination that spleen is composed of capsule which envelops the whole organ (Figure 1-B), its index increases according to groups,  $02.34 \pm 0.74$ % for first group,  $03.48 \pm 0.83$ % for second group, third group of fetal spleen present value of  $04.84 \pm 0.67$ % finally a sum of  $06.66 \pm 0.24$ % assigned for the fourth group. For trabeculae, minimum percentages value of  $02.71 \pm 0.87$ % found in second group, while maximum value is  $09.35 \pm 0.24$ % for fourth group.

Red pulp and arteriolar peripheral lymphoid sheaths (PALS) begin to form only in fourth group antenatal spleens, while they are absent entirely in other groups of. Follicles in these groups have variable values, minimal value of 0.12% found if the first group, and maximal value of 1.74% in the fourth group. Whereas total parenchyma has a variable percentage occupies discrete invisible neo-formations, the Maximum value is  $94.46\pm0.72\%$  for the first group of fetal spleens, while it is at least  $89.40\pm3.45\%$  in fourth group. For lamb's spleens, it was noted that all parenchyma components are present, a noticeable increase in percentages of components tissue, capsule exhibited minimal value in second group  $06.10\pm0.17\%$ , while maximum value was in fourth group  $11.65\pm0.82\%$ . For trabeculae, the minimum

value of  $04.53 \pm 0.35\%$  found in the first group and reaches  $07.10 \pm 0.17\%$  in the second group. For the red pulp, the minimal surface of  $52.33 \pm 1.65\%$  found in the first group, and a maximum of  $72.54 \pm 1.62\%$  in the fourth group. These results are similar to those found by (Dunaevskaya, 2019).

Peri-arteriolar lymphoid sheaths (PALS), revealed minimum surface of  $07.70 \pm 0.78\%$  lambs first group and maximal value of  $13.34 \pm 0.76\%$  in fourth group. Regarding follicles, inactive follicles percentage shows minimum value of  $1.74 \pm 1.26\%$  in first group and maximum value of  $5.79 \pm 1.35\%$  in fourth group. For active follicles, minimum percentage of  $2.66 \pm 1.21\%$  found in first group; whereas maximum value was in second group  $8.29 \pm 2.54\%$ . Looking at total parenchyma percentage a maximum value of  $88.75 \pm 1.87\%$  in first group, and  $82.7 \pm 1.85\%$  as lowest value found in fourth group.





#### CONCLUSION

Spleen in ovine species in Algeria appears as an important multifunctional organ of peripheral element of immune system and hematopoiesis process. Results of this study obtained at macroscopic and microscopic level indicate that development index begins already in antenatal period then in post-natal period, stroma, red pulp and white pulp have development index appears normal whereas follicles appear already in antenatal period about gestation's end. The objective is to establish basic anatomical characters of spleen in Algerian breed Ouled Djellal, in order to obtain safe animal production. For this morphometricall and histologic spleens research gave an idea about the development index.

#### **DECLARATIONS**

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#### **Authors' Contribution**

All authors contributed equally to this work.

#### **Conflict of interests**

The authors declare that they have no competing interests.

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# CONSERVATION-BASED BREEDING PROGRAM FOR INDIGENOUS SHEEP BREEDS IN ETHIOPIA: THE WAY FORWARD

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Supporting Information

ABSTRACT: This paper review briefly Ethiopian sheep genetic resources, description of farming systems, breeding objectives of the community, and to quantify threats with previous sheep genetic improvement and conservation strategies in Ethiopia. Thus, the aims of this paper focus on the role of characterizing indigenous sheep breed, including their threat status in genetic improvement programs. Indigenous sheep genetic resources have contributed considerable portion to the integrated crop-livestock farming systems in Ethiopia. Ethiopia endowed with a diverse sheep genetic resource maintained under different production systems. Sheep research and development in Ethiopia has largely focused on characterization of sheep genetic resources, description of farming systems and genetic improvements using crossbreeding and selection within breed. In Ethiopia, there are a total of 14 traditional sheep populations in Ethiopia fall into six breed groups based on DNA and morphological data. The main cause of threatening the sheep genetic resource in Ethiopia is indiscriminate crossbreeding with exotic germplasm. Few research institutes and agricultural universities are involved in the conservation and management of sheep breeds in Ethiopia. Currently the emphasis is on village based rather than centre-based genetic improvement program. It is possible to conserve sheep genetic resources in Ethiopia through quantifying past sheep characterization works and developing breeding programs suitable to the conditions of smallholder farmers that increase the economic values of indigenous sheep breeds.

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**REVIEW ARTICLE** 

Keywords: Conservation, Indigenous, Sheep, Smallholder farmer, Village breeding practice

# INTRODUCTION

The issue of loss of diversity and conservation of farm animal genetic resources has gained momentum in the last few decades. Correspondingly, there have been tremendous efforts globally to study genetic diversity in livestock species addressing the needs of both development and conservation of animal genetic resources in the different parts of the world. Livestock are essential components of Ethiopian agriculture and the country is reported to have the largest livestock resource than any other African country. Ethiopia is recognized to possess one of the most diversified livestock populations in Africa. The population of small ruminants owned by the country is estimated to be 56.6 million heads of small ruminants (CSA, 2015). Of this number, the sheep population accounts for about 28 million heads. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to play significant role in the economic development of the country. It is eminent that sheep products in the form of meat could play a pivotal role as a good source of animal protein since they have short generation interval and affordable by resource poor farmers (Markos, 2006; Tsegaye et al., 2013).

The sheep in the country is produced under an extensive low input subsistence system. Similar to other tropical countries, studies noted that productivity of indigenous sheep in terms of meat has been limited by poor genetic potential (Markos, 2006; ILRI, 201; Getahun, 2008). Among the various factors, absence of planned genetic improvement programs for local breeds is one of the causes for losing their competitive advantage, especially where production systems or external conditions are subject to change (Hiemstra et al., 2007).

Moreover, lack of adequate information on indigenous livestock genetic resources has been recognized to be a serious constraint to effective prioritization and planning of sustainable breeding strategies and breed conservation measures. As a result, a number of activities aimed at the characterization of the major livestock genetic resources have been carried out by different governmental and non-governmental organizations (IBC, 2004).

This review paper provides an overview of the current state of knowledge on characterization of sheep genetic resources, production system, existing breed management, traditional breeding practice and identification of important traits of indigenous sheep breeds in Ethiopia. Finally, gaps in current knowledge are identified, and priorities for future research are proposed.

#### LITERATURE REVIEW

#### Sheep genetic resources of Ethiopia

Ethiopia is one of the countries in the world, which has contributed richly to the global livestock gene pool. Estimates indicated that about 99.9% of the total sheep population in Ethiopia is indigenous breeds (CSA, 2015). According to (Solomon et al., 2007b; Solomon et al., 2008a), Ethiopian sheep are classified into 15 traditional sheep populations, which fall into four breed groups and nine breeds based on diversity analysis using microsatellite markers and morphological diversity.

Exotic breeds represent a very insignificant proportion of the national flock and some of the exotic sheep breeds that have been introduced into the country include: Dorper, Merino, Romney, Corriedale, Hampshire, Awassi, and Blue de Main. Only very few of these or their crosses are seen either in rural or urban areas. Some were limited to research stations while others have been and are still being distributed to farmers (Sisay, 2009; Solomon and Tesfaye, 2009). Sisay (2009) phenotypic ally characterized local sheep population of Amhara National Regional State of Ethiopia into five distinct major groups.

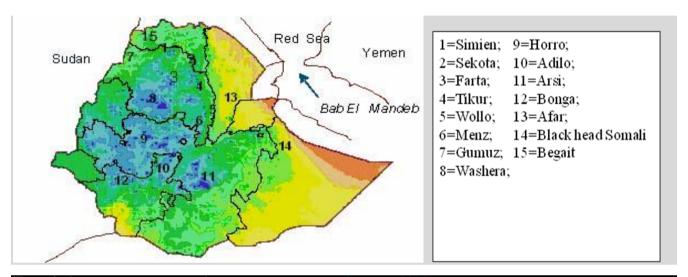
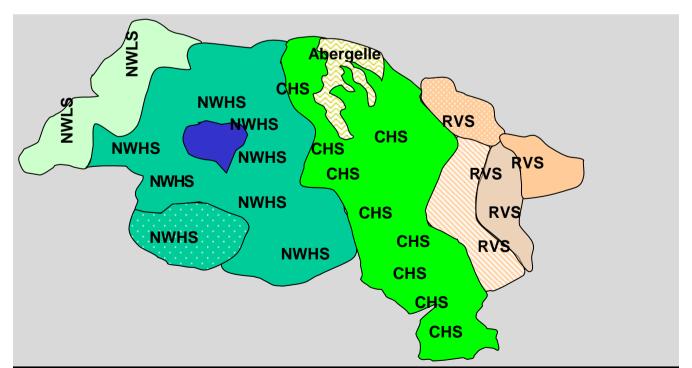


Figure 1 - Geographic distribution of Ethiopian sheep breeds (Solomon, 2008)



**Figure 2 -** Agro-ecological distribution of the five major sheep types in the Amhara region. CHS: Central Highland sheep; NWHS: North-western Highland sheep; RVS: Rift Valley (Afar) sheep; Abergelle: Abergelle sheep; NWLS: North-western Lowland (Gumez) sheep.

This diversity is relevant in terms of specific adaptation to the various agro-ecological zones where the breeds exist, for instance attributes like heat tolerance, disease resistance and drought tolerance. These characters help the breeds to survive and produce under prevailing environmental challenges of the different agro-ecological zones of the country. Despite presence of sizeable amount of information to describe the sheep types in Ethiopia, there is still gap to be filled in terms of comprehensive characterization of the sheep population at district level as an input to prioritize for conservation and development of breeding program towards sustainable utilization (Solomon et al., 2011d; Solomon et al., 2013; Tesfaye et al., 2016). The gaps which require comprehensive characterization like in specific areas which have production system and production environment change in areas in which the current climate change affect the adaptation of certain breeds which initiate the community to cross with other adaptable breed in the neighboring areas. This will be used as an input to prioritize for conservation and development of breeding program towards sustainable utilization. The importance of genetic diversity has not been given adequate attention for the sustainable utilization of various breed by different stakeholders including the community in the past and even at present. There is limited location specific information on components of characterization including description of the production system and environment, performance levels of the breeds, and farmers breeding practices and objectives and the threat status of indigenous sheep population in the country (Solomonet al., 2011d).

The loss of genetic variation within and between breeds is detrimental not only from the perspectives of adaptability but also for utility since lost genes may be of future economic interest. The reasons for loss of genetic diversity within and between breed are many, including the low productivity of the local breeds, neglect the potential of good indigenous breeds, lack of well-defined breeding objectives, inappropriate development policies and management strategies, disease outbreaks, various types of disasters and emergencies (FAO, 2007b). Therefore, research and development on conservation of sheep genetic resources in developing countries and particularly in Ethiopia is the most crucial and urgently needed (Solomon et al., 2013). Thus, location specific characterization on the production system, farmers breeding practices, identification of breeding objective traits in participatory manner and performance evaluation of the breeds should be conducted. There is also a need to verify the threat status of endangered livestock breeds and take measures to conserve them. The information generated might be required to design sustainable conservation-based utilization programs.

#### **Characterization of Sheep Production Systems**

Sheep production systems in Ethiopia are predominantly traditional (Markos, 2006; Solomon et al., 2010b). According to the degree of integration with crop production, level of input, agro-ecology, relation to land and type of commodity to be produced and mobility, the traditional sheep production system can be sub divided into five systems (Solomon et al., 2008a). These are subalpine sheep-cereal system, highland cereal-livestock system, highland perennial crop system, lowland crop-livestock system or agro-pastoral system and pastoral production system. A brief account of these systems as indicated in the literatures is discussed below.

#### A) The subalpine sheep-cereal system

This system is found in the highland areas (above 3000 m.a.s.l.) of Ethiopia where the major crops grown are barley and pulses such as faba beans and lentils. Sheep are the dominant livestock species and reared mainly for source of income, meat, manure, skin and coarse wool (Tesfaye et al., 2010; Shigdaf et al., 2013; Mesfin et al., 2014b). In the subalpine system, numbers of sheep in a flock are larger, typically comprising 10-30 animals (Markos, 2006; Solomon et al., 2010b; Tesfaye et al., 2010). The maintenance of pure-bred Menz sheep flocks at Debre Berhan Research Centre and community based breeding programs at Debre Berhan and Menz area are the conservation attempts made in this production systems (Solomon et al., 2013).

#### B) Highland cereal-livestock system

The system covers an area with an altitude between 2000 to 3000 m.a.s.l. and characterized by adequate rainfall and moderate temperature which is suitable for cereal production. In this system, sheep production is found in association with the different agricultural production systems which vary in potentials, intensity of the mixed farming operation and natural resources including grazing and livestock resources (Samuel, 2005). According to Dereje and Tesfaye (2008), sheep are kept in small flocks and provide cash income, meat, manure and skins. On-station improvement of Horro sheep at Bako Agricultural research center is primarily used as the active breeding of Horro sheep populations (Duguma et al., 2010). In addition, in situ conservation of Horro sheep through community based breeding programa at Bako and Shambu area is also use as part of an ongoing livelihood and conservation strategy in the production system (Tadele et al., 2012).

#### C) Highland perennial crop system

This system is common in the Highlands with an altitude range of 1500 to 2000 m.a.s.l. In this farming system, livestock holdings are small, the topography is uneven and inter-cropping is practiced (Zewdu et al., 2012). Under this system livestock production is of minor importance and is characterized by cultivation of a combination of cash and subsistence crops such as inset and fruit, chat, coffee and tea (Belete, 2009). The integration of crop-livestock mixed

farming is lower in the perennial system. Sheep are kept by smallholders, graze and rear together with goat and/or other livestock species such as cattle (Zewdu et al., 2012).

#### D) Lowland crop-livestock system or agro-pastoral system:

This system found in the sub-moist/moist lowland areas (≤ 1000 m.a.s.l.) with higher rainfall to support short season crops (cereals, sesame and cotton) compared to the pastoral system and it is characterized by less integration with crop production (Tesfaye et al., 2010). This production system is associated with the purely livestock based nomadic and transhumance pastoral production systems dependent largely on range, primarily using natural vegetation. In the lowlands of Ethiopia, livestock is comprised of large flocks and herds of sheep, goats, cattle and camels and it is mainly transhumant. Extensive livestock keeping is the backbone of the economies of this system (Fekerte, 2008).

#### E) Pastoral production system:

This system is located in the arid and semi-arid lowland areas (≤ 1000 m.a.s.l.) where livestock rearing is the mainstay of people (Markos, 2006). It is characterized by high mobility in search of grazing and water. In this system, extensive livestock production is mostly the sole source of livelihood with little or no cropping (Solomon et al., 2010b). Livestock, including small ruminant production is associated with the purely livestock based nomadic and transhumance pastoral production systems based largely on range, primarily using natural vegetation. The variability of rainfall is the greatest threat to crop production. Sheep are kept by nearly all pastoralists, often in mixed flocks with goat (Solomonet al., 2008a; Tesfayeet al., 2010).

#### **Definition of Breeding Objectives**

Defining breeding objectives involves identifying breeding-objective traits, deriving their relative importance, and constructing the aggregate genotype that can subsequently be translated into a selection index (Solomon et al., 2008a and Kosgey et al., 2008). Definition of breeding objectives traits is the basis for designing appropriate animals breeding programs (Sölkner et al., 1998; Gemeda et al., 2010 and Wurzinger et al., 2011). In smallholder and pastoral communities, breeding goals are multi-functional incorporating both tangible and intangible benefits of sheep keeping. Therefore, when defining animal breeding objectives in low input systems, the needs and interests of the target group should be incorporate to increase the success of breed improvement programs (Kosgey et al., 2008 and Tesfaye et al., 2010). Lack of participation of farmers in defining the breeding objective was the main reason for failure of many livestock improvement programs in the tropics (Wurzinger et al., 2011).

Participatory approaches of defining breeding objectives have been described by a number of studies (Kahsa, 2009; Solomon et al., 2010b; Tesfaye et al., 2010; Zewdu et al., 2012). Such approaches have been used for mixed crop-livestock and pastoral systems for definition of breeding objectives in goats and in sheep (Grume et al., 2013 and Tadelle et al., 2012). Participatory approaches have also been used in determining objectives of keeping dairy goats and for studies of farming systems for small ruminants in Kenya (Kosgey et al., 2008). Gemeda et al. (2010) recommended five alternative approaches (direct ranking, workshops, choice cards, own and group flock ranking) to identify the breeding objectives and the breeding objective traits in pastoral and smallholder subsistence system. Direct ranking using structured interviews with the sheep owners have often been used when identifying traits of importance for a breeding program (llatsia, 2011).

After listing of preferred traits, there should be a score assigning for each of the traits or trait categories. Ranking of animals from own flock implies grading of own animals based on reproduction and production performances. The sheep owners are asked to rank their own animals (female and males) from the 1st (best), 2nd (second best) and 3rd (poorest), and should indicate reasons for their ranking (Sölkner et al., 1998; Gemeda et al., 2010). After receiving detailed information from the owners, body weight and other linear body measurements are taken on each individual ranked animal. Gemeda et al. (2010) also suggested that a combination of methods can be used to extract breeding objectives of the community. The information obtained through these methods can be used to design village-based selection breeding programs.

The diverse traits (body size, color, milk yield, lamb survival, twinning, walk ability, lambing interval and longevity) as the selection criteria during direct-ranking for breeding animals are well documented in many research reports (Grume et al., 2013; Tesfaye et al., 2010; Zewduet al., 2012; Gemeda et al., 2010; Tadelle et al., 2012). According to Grume et al. (2013) and Tadelle et al. (2012) and Solomon (2014), productive and reproductive traits were frequently mentioned during the own flock-rankings for breeding animals. Moreover, Zewdu (2008) also argued in favor of including reproductive traits not only based on economics of production, but also because of its influence in genetic improvement through selection intensity. Gemeda et al. (2010), Kahsa (2009) and Zander et al. (2009) reported that choice experiments are important for identifying selection criteria in subsistence production systems and evaluated cattle and sheep trait preferences of pastoralists and smallholder farmers in Ethiopia. Previous breeding objective traits identification studies (Tadelle et al., 2012 and Berhanuet al., 2012) indicated that body size, body conformation, twinning, color and beauty related traits were considered as important traits of preference for breeding animals during group animal ranking experiments.

#### **Genetic Improvement and Conservation Strategies**

Livestock productivity can be increased through genetic improvement. Strategies for genetic improvement of livestock mainly involve the decision on the use of genetic variation within a breed (selective breeding) or between breeds (crossbreeding) (FAO, 2007b; Philipsson, 2000). The sheep breeding strategies adopted in Ethiopia over the last several decades largely focused on importing exotic breeds for cross-breeding (Markos, 2006; Solomon et al., 2013; Tesfaye et al., 2016). However, study of Solomon et al. (2013) and Tesfaye et al. (2010) indicated that the impact of decades of this program is negligible.

This has been due to poor involvement of stakeholders and implementing livestock improvement programs without taking into consideration the needs of farmers (Markos, 2006; Gemeda et al., 2010 and Tadelle et al., 2012). The distribution of the improved genotypes of these programs was indiscriminate and unplanned, resulting in failure of the breeding programs and threatened to dilute the sheep genetic diversity in the country (Solomon et al., 2011d). There are also well documented examples of unsuccessful projects using exotic breeds (Taberlet et al., 2011) in developing countries. Therefore, appropriate conservation-based breeding strategies are needed to avoid indiscriminate crossbreeding as well as to improve sheep production and productivity of the locally adapted breeds like Gumz sheep. Philipsson (2000) suggested that in order to increase livestock productivity in developing countries like Ethiopia, diversity within indigenous breeds should be efficiently exploited. Open nucleus schemes and community/village-breeding programs are being increasingly advocated for traditional production systems (Zewdu, 2008). Although the indigenous livestock genetic resources of Ethiopia have high within breed genetic variations (Solomon et al., 2008a) and desirable characteristics, there has been little effort to improve the genetic merits of the local livestock genetic resources using the within breed genetic variation. However, in Ethiopia, like in the other parts of the developing countries small flock sizes, lack of animal identification, lack of performance and pedigree recording, low level of literacy and organizational shortcomings and flock mobility have been major constraints that hinders within breed selection (Markos, 2006; Solomon and Tesfaye, 2009). To overcome these problems, nucleus breeding schemes have been suggested as tools for small ruminant genetic improvement programs in tropical countries (Markos, 2006; Mueller, 2006). The scheme could be utilized for conservation of genetic resources through improvement and proper utilization. This scheme can serve for both pure-breeding and crossbreeding, and dissemination of improved genetic materials allowing conservation and improvement of the indigenous sheep breeds. However, implementation of nucleus breeding schemes in low-input environments has proven to be difficult because of requirement of long term commitment of sponsors and involvement of farmers (Solomon and Tesfaye, 2009; Kosgey et al., 2008). Village based breeding schemes have also been suggested as an alternative to nucleus scheme. Recently, community based breeding strategy has been gained momentum for increasing productivity of indigenous sheep of Ethiopia (Solomon et al., 2013; Gemeda et al., 2010; Tadelle et al., 2012), as a means to improve the livelihood of smallholder farmers as well as to conserve the existing genetic diversity through utilization. Community (village)-based breeding schemes is also a recently suggested viable option for the genetic improvement programs of small ruminants in low-input, smallholder production systems in the tropics (Kosgey et al., 2008; Wurzinger et al., 2011).

#### A) Central Nucleus Breeding Schemes

Sheep production in developing regions is generally characterized by small flock size, uncontrolled mating, and the absence of pedigree and performance recording (Markos, 2006; Solomon and Tesfaye, 2009). These characteristics limited the implementation of effective genetic improvement programs. To overcome these problems, nucleus breeding schemes have been suggested for tropical countries, in which genetic improvement is centrally organized in a population maintained in research institutes or government farms. The open nucleus breeding scheme offers a simple procedure for producing and disseminating breeding stock of high breeding value. Based on breeding values for the breeding goal traits, ewes born to superior rams would be selected and bought for transfer to nucleus flock. Nucleus flocks will be set-up in the governmental breeding ranches. Subsequent genetic evaluations and selection for superior rams would be undertaken in the nucleus flocks where animals from different sources are evaluated in the same environment. Dissemination of superior rams to participating farmers will be made on cost recovery basis. Several studies indicated the significance of using open nucleus breeding scheme to increase the rate of genetic gain (Philipsson, 2000; Yapi-Gnoare, 2001). Markos (2006), has also been presented a plan for the indigenous Horro sheep open-nucleus breeding scheme in Ethiopia. This scheme could be utilized for conservation of genetic resources (including breeds, desirable genes, genotypes, etc) through improvement and proper utilization (Markos, 2006; Philipsson, 2000). This scheme can serve for both pure-breeding and crossbreeding and dissemination of improved genetic materials allowing conservation and improvement of the indigenous sheep breeds. On-station breed evaluation work of some well-known sheep types has been undertaken. These include the Ethiopian Agricultural Research Institutes (EARI) evaluation program on Afar, Black Head Somali (BHS), Menz and Horro sheep. Major emphasis in all of these studies was on meat production and wool (Menz sheep) production. Results in a nucleus flock of Menz sheep indicated that genetic progress of 0.29 kg and 0.34 kg (Solomon et al., 2014a) in a six month weight was achieved. Selection in the Horro sheep nucleus flock did not result in appreciable genetic improvement and the breeding program shows no progress (Solomon and Duguma, 2000; Solomon et al., 2007a). Whereas results of selection in the nucleus flocks of Afar and BHS sheep remain unreported.

#### B) Village based Breeding Schemes

A community-based breeding program refers to village-based breeding activities planned, designed, and implemented by smallholder farmers, individually or cooperatively, to effect genetic improvement in their flocks and conserve indigenous genetic resources (Sölkner et al., 1998). The design of these programs considers description of the production system, definition of the breeding objective traits of the farmer and assessment of alternative schemes (Mueller, 2006; Baker and Gray, 2004; Sölkner-Rollefson, 2003; Aynalem et al., 2008). Solomon et al. (2011c,d) showed that this new community-based or village centered approach in animal breeding can be used for smallholder system as this ensures the full participation of the farmers and is more sustainable in the long term as it is less dependent from external inputs. This program is also intended to overcome the problems of genotype-environment interaction, avoid the genetic lag between nucleus and village populations, and are appropriate for in situ conservation of indigenous animal genetic resources (Solomon et al., 2011d). Village based breeding scheme had been tried in a number of areas (for example, on Menz sheep in DebreBerhan and MehalMeda area, Horro sheep in Shambu areas, Afar sheep in Afar area and Bonga sheep in Bonga area) and has been found successful in improving the growth performance of the sheep (Gemeda et al., 2010; Tadelle et al., 2012). For instance, body weights of Menz sheep at birth, 3 and 6 months of age increased by 0.42, 2.29 and 2.46 kg, respectively, in the third generation over the base generations. However, genetic progresses in lamb survival and litter size were low on the local Menz sheep breeds (Tadelle et al., 2012).

#### CONCLUSION

It is clear that information in production systems, breeding objective traits and improvement breeding programs from numerous research projects have been reviewed. Currently available information could help decision-makers formulate in better way sustainable development programs for conservation and utilization of sheep genetic resources. While inventory of species and breeds, their population sizes and geographic distribution for the management of animal genetic resources remains poor. There are also gaps in research and development efforts. Thus, the next step should focus on designing of breeding strategies suitable for smallholder production system for sustainable management of diversity among promising and widely used local breeds.

#### **DECLARATIONS**

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#### **Authors' contribution**

I fully engaged in the reviewing processes of this paper.

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# PHENOTYPIC CHARACTERIZATION OF DONKEYS IN BENISHANGUL GUMUZ NATIONAL REGIONAL STATE

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ABSTRACT: Fifteen morphometric measurements and eighteen qualitative traits were recorded on 323 randomly sampled adult donkeys (123 jacks and 200 jennets) to meet an objective of characterizing a heterogeneous donkey population of three phenotypic types (Sinnar, the locals and their crosses) found in Benishangul Gumuz region of Ethiopia. The General Linear Model and non-parametric test (chi-square) procedures of SAS software were used for the analysis of the morphometric data and qualitative traits, respectively. Means were separated using the Tukey-Kramer test. The studied morphometric measurements were significantly affected by the phenotypic type and partially affected by sex and sample location /district. Sinnar donkeys were significantly bigger and heavier than the local and the crosses. However, for some of the morphometric measurements no significance difference was observed between Sinnar and crosses implying the presence of heterosis. Heart girth measurements for Sinnar, local and crosses were 110.61±0.436, 106.18±0.448 and 108.87±1.251, respectively. Body weight estimates of 127.26±1.277, 113.40±1.312 and 121.13± 3.665 kg were obtained for Sinnar, the local and the crosses. There was sexual size dimorphism and depending on the type of morphometric trait either jacks or jennets show significantly (P<0.05) higher values as compared to the opposite sex. Jacks had wider chest (22.61 vs 22.09 cm.), thicker (24.40 vs 23.24 cm.), and longer cannon bone (31.00 vs 31.32 cm) than jennets, while the jennets possess wider hip (33.00 vs 31.69 cm.) and longer body (90.49 vs 88.52 cm.), back (67.37 vs 66.17), ear (24.42 vs 23.90 cm) and heavier estimated weight (122.47 vs 118.71 kg) than the jacks. Limited location effect was recorded showing donkeys from Guba district were comparatively the largest. Majority of the studied donkeys possess white abdominal color, unpigmented hoof and muzzle, short and medium hair size, plain body color pattern with long dorsal stripe without leg stripe, straight face and sloppy rump profile, medium tail length and thickness at the base of the tail. Qualitative differences (P<0.05) were also observed among the class categories. Further molecular level studies could supplement the current study and provide more refined classification of the various genotypes in the studied area. Similarly, characterization of the local donkeys found in other parts of the region and the Abyssinian donkeys in the adjoining areas is required.

Keywords: Heart girth, Morphometric, Qualitative, Phenotypic types, Sinnar

# INTRODUCTION

Ethiopia is endowed with diverse domestic, aquatic and wild animal genetic resources. The diverse ecology Ethiopia has and its position as a route of entry to domestic animals from Asia to Africa has resulted in the presence of diverse animal genetic resources. Despite the presence of the resources adequate characterization work is lacking and the information on the state of the animal genetic resources is incomplete to support sustainable utilization and conservation of the resources (EBI, 2016). This is more so for the equine genetic resources of the country.

Donkeys, like other livestock species, have an important place in rural and urban communities in Ethiopia. Even if donkeys have not been serving as a food source to humans in Ethiopia due to religious and cultural taboos, they make significant economic contribution in all the regions. Donkeys specifically are important for transport of goods in urban, peri-urban and rural areas. In the latter case they also serve in transporting humans, threshing cereal crops and plowing of land pairing with oxen.

The donkey (*Equus asinus*) is indigenous to the African continent and its wild progenitor is usually considered to be the Nubian wild ass (Blench, 2000). Ethiopia possesses the largest donkey population in the world with 8,439,220 donkeys (CSA, 2017; FAO, 2015). Even if, donkey is the least studied species in the country, previously four types of donkeys were recognized; namely Jimma, Abyssinian, Ogaden and Sinnar based on their phenotypic and physical characteristics like average size and coat colour (Befikadu et al., 2015). However, more recent nationwide study identified six distinct domestic donkey populations namely Abyssinian, Afar, Hararghe, Ogaden, Omo and Sinnar (Kefena 2012, Kefena 2014; EBI 2016). The same study showed that, most of the variations in the parameters of morphological

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**RESEARCH ARTICLE** 

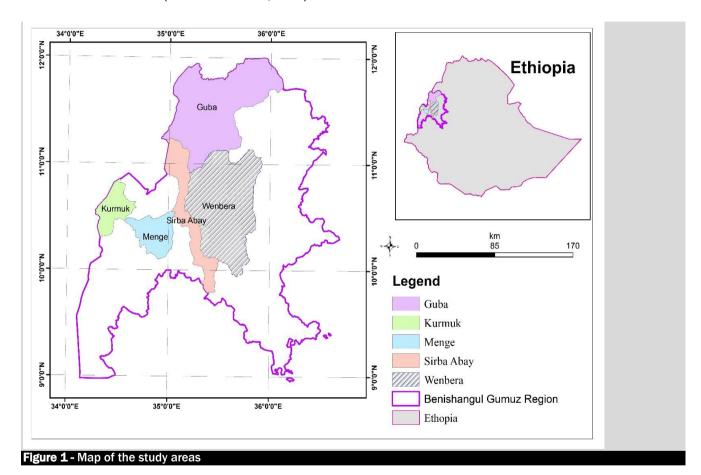
Supporting Information

characteristics vary with eco-geographical patterns and biophysical resources. Therefore, other morphometric variables and corporal indices need to be further incorporated and used to fully characterize and describe donkey populations in Ethiopia (Befikadu et al., 2015). Like all the other regions found within the country, Benishangul Gumuz region possess different donkey phenotypes and their crosses including the country's largest donkey type (Sinnar). Hence this study was aimed at revealing the phenotypic characteristics (qualitative and quantitative parameters) of the different donkey breeds found in the region to be used as input for a further conservation and sustainable utilization of the resources

#### **MATERIALS AND METHODS**

#### **Description of the study areas**

The study was conducted in Benishangul Gumuz Regional State. Benishangul Gumuz Regional State is one of the nine regional states which is located in the western part of Ethiopia between longitude 34° 10'N and 37° 40'E; and latitude 09° 17'N and 12° 06' N. It shares border with Amhara Region in the north and north east, South Sudan in the west, Gambella Region in the south, Oromia Region in the south east. The total area of the Region is approximately 50,380 square kilometers with altitude ranging from 580 to 2731 meters above sea level. About 75% of the Region is low land, 24% is semi-high land and 1% is high land. The capital city of the Region is Asossa, located at a distance of 659 kms west of Addis Ababa (Chekol and Getnet, 2010).



#### Sampling technique and sample size

The studied animals (adult donkeys for phenotypic characterization; morphometric and qualitative records) were sampled randomly from five districts (Guba, Menge, Wenbera, Sirba Abay and Kurmuk) within the region. Heterogeneous donkey populations of three phenotypic types (Sinnar, the local donkey and their crossbreds) were used for the qualitative records and morphometric measurements. A total of 323 full-mouthed adult donkeys (123 jacks and 200 jennets) composed of 157 Sinnar, 148 local and 18 crossbred donkeys were measured for linear and circular morphometric traits and described for the qualitative traits.

#### Measurement and data collection

The sample size determination and identification of traits for morphometric measurements and qualitative description were based on FAO guideline (FAO, 2012). Fifteen quantitative/ morphometric measurements (Heart girth, Height at wither, Height at back, Height at rump, Body length, Back length, Neck length, Head length, Canon bone length, Fore leg length, Hip width, Chest width, Chest depth, Canon circumference and Ear length) and 18 qualitative characteristics (coat hair size, body color pattern, body (coat) color, abdominal color, head color, ear tip color, tail switch color, hoof color, and muzzle color, ear shape, dorsal stripe, leg stripe, shoulder stripe, face profile, back profile, rump

profile, tail length and thickness at the base of the tail) were recorded from each individual.

Donkeys were carefully handled by trained laborers and made to stand squarely on flat grounds. Morphometric measurements and qualitative data recording were made by separate individuals. According to a study by Kosťuková (2015), who reported that the growth of donkeys terminates after the age of 5 years and all donkeys in the study were past this age. Body weight was estimated from the above measurements by the formula from Pearson and Ouassat (2000).

Live weight (kg) = (heart girth [cm] 2.12) x (body length [cm] 0.688)/3801

Breed / genotype	Jacks	Jennets	Total	Proportion
Sinnar	57	100	157	0.48
Local	58	90	148	0.46
Crosses	8	10	18	0.06
Total	123	200	323	1.00
Proportion	0.38	0.62	1.00	-

#### Statistical analysis

Data entry and management were done using Microsoft Excel. Analysis of data on quantitative measurements was carried out using the GLM procedure of SAS 9.0 software. Means were compared using Tukey-Kramer (SAS, 2002). Similarly, analysis of qualitative traits was carried out using the non-parametric test (chi-square) procedure of SAS 9.0 software. The model used for the analysis of quantitative data: Yijk =  $\mu$  + Ai + Bj +Ck + eijk, where Yijk is an observation,  $\mu$  is the overall mean, Ai is the fixed effect of sex, Bj is the fixed effect of the breed group, Ck is the fixed effect of district and eijk is the random error attributed to the nth observation. Interaction effects were found to be non-significant in most cases and were removed from the analysis model.

#### **RESULTS**

#### Morphometric measurements

The overall mean, standard error (SE), minimum and maximum value, and coefficients of variation (CV) of the collected morphometric measurements are presented in table 2. For all morphometric traits measured the coefficient of variation was within the range of 5.11 and 8.27%. Relatively higher coefficient of variation (13.11%) was calculated for estimated body weight implying higher variation in terms of body weight. The difference between the minimum and maximum value is sizeable in most cases. A range of 40 cm for height at wither, 37 cm for body length, and a range of about 104 kg for body weight were observed.

**Table 2 -** Overall least square mean , SE, CV, Minimum and Maximum of body measurements of the donkey populations in Benishangul Gumuz region.

Traits	Overall Mean ± SE	Minimum	Maximum	CV (%)
Hearth girth (cm)	108.7 ± 0.31	93.0	128.0	5.18
Height at wither (cm)	100.2 ± .030	79.0	119.0	5.31
Height at back (cm)	$102.0 \pm 0.31$	89.0	122.0	5.41
Height at rump (cm)	$102.3 \pm 0.30$	90.0	121.0	5.22
Body length (cm)	89.9 ± 0.30	68.0	105.0	6.00
Back length (cm)	67.3 ± 0.24	57.0	86.0	6.34
Neck length (cm)	49.2 ± 0.23	38.0	60.0	8.27
Head length (cm)	42.9 ± 0.12	36.0	50.0	5.11
Canon bone length (cm)	31.4 ± 0.12	26.0	39.0	6.92
Fore leg length (cm)	68.3 ± 0.26	54.0	86.0	6.92
Hip width (cm)	$32.6 \pm 0.11$	27.0	40.0	6.60
Chest width (cm)	22.3 ± 0.09	18.0	29.0	7.63
Chest depth (cm)	49.1 ± 0.15	42.0	59.0	5.65
Canon circumference (cm)	23.7 ± 0.09	20.0	29.0	6.70
Ear length (cm)	23.9 ± 0.10	19.0	28.0	7.27
Body weight (kg)	121.2 ± 0.93	77.8	181.6	13.82

SE=Standard error, SD=Standard deviation, CV=Coefficient of variation and cm=Centimeter.

**Table 3 -** Least square means (cm) and pairwise comparison of body measurements with standard error in each breeds/populations: 1) jakes.

Traits	Breed groups/ phenotypic types			
	Sinnar	Local	Cross	— p value
N	57	58	8	
Heart girth	110.4±0.72a	104.9±0.71 <sup>b</sup>	107.9±1.89ab	***
Height at wither	104.0±0.61a	96.5±0.60b	101.4±1.60a	***
Height at back	106.5±0.65a	98.8±0.64b	103.1±1.70a	***
Height at rump	106.2±0.67a	98.9±0.66b	102.3±1.74ab	***
Body length	90.9±0.70a	86.2±0.69b	86.9±1.82ab	***
Back length	67.9±0.52a	64.7±0.51b	65.9±1.36ab	***
Neck length	50.4±0.53a	46.9±0.53b	50.1±1.39ab	***
Head length	43.9±0.27a	42.2±0.26b	43.7±0.69ab	***
Canon bone length	33.0±0.30a	30.8±0.30b	32.3±0.78ab	***
Fore leg length	70.7±0.59a	66.4±0.58b	68.1±1.55ab	***
Hip width	32.0±0.26	31.4±0.26	31.0±0.68	NS
Chest width	23.0±0.21a	22.1±0.20b	22.0±0.53ab	**
Chest depth	50.1±0.33a	47.2±0.33b	49.9±0.86ª	***
Canon circumference	24.8±0.21a	23.9±0.20b	24.6±0.54ab	*
Ear length	24.0±0.25a	22.9±0.24b	25.8±0.64a	**
Body weight	126.3±2.14a	108.9±2.10b	116.4±5.57ab	***

In jacks, with the exception of hip width, donkey phenotypic type had a significant (P<0.01) effect on the measured traits and it is indicated that almost all body measurements were highest for Sinnar jacks (Table 3) followed by the crossbred type. In almost all cases the difference between the Sinnar and the crossbred jacks is not significant while the difference between the Sinnar and the local is significant in all cases.

The difference between the local and the crosses reached significance level only for height at wither and at back, chest depth and ear length. The Sinnar and crossbred jacks have larger chest depth and taller height at withers than the local jacks. Positive heterosis of more than one percent was calculated for height at withers, neck length, head length, canon bone length, chest depth and ear length. The highest heterosis of close to ten per cent was observed for ear length.

In jennets, significant (P<0.05) difference was observed between phenotypic types for all morphometric traits (Table 4). In all cases Sinnar jennets along with the crosses have significantly higher values than the local donkeys. No significant difference was observed between Sinnar jennets and the crossbreds. Despite the fact that the crosses would of various types (F1, F2, F3, back cross etc.) and blood levels heterosis of more than one per cent was calculated for body length, fore leg length, hip width, chest width, chest depth, ear length and body weight of jennets.

**Table 4 -** Least square means (cm) and pairwise comparison of body measurements with standard error in each breeds/populations: 2) jennets.

Traits	Breed groups/ phenotypic types			
	Sinnar	Local	Cross	p value
N	100	90	10	
Heart girth	110.8±0.53a	107.1±0.56b	109.8±1.66ab	***
Height at wither	102.6±0.6a	97.4±0.49b	100.7±1.43ab	***
Height at back	104.3±0.46a	98.9±0.49b	101.3±1.43ab	***
Height at rump	104.7±0.44a	99.6±0.47b	102.4±1.39ab	***
Body length	92.4±0.50a	89.7±0.53b	92.0±1.56ab	**
Back length	69.0±0.42a	66.8±0.45b	66.2±1.32ab	**
Neck length	50.7±0.39a	48.6±0.42b	48.9±1.22ab	**
Head length	43.7±0.20a	41.8±0.21b	43.2±0.62ab	***
Canon bone length	32.1±0.18a	30.4±0.20b	31.4±0.58ab	***
Fore leg length	70.1±0.42a	66.8±0.45b	69.2±1.33ab	***
Hip width	33.6±0.21 <sup>a</sup>	32.5±0.23b	33.4±0.67ab	**
Chest width	22.6±0.17a	21.6±0.19b	22.6±0.54ab	**
Chest depth	50.2±0.26a	48.2±0.28b	50.1±0.82ab	***
Canon circumference	23.6±0.15 <sup>a</sup>	23.0±0.16b	22.9±0.47ab	*
Ear length	24.6±0.16a	23.7±0.17b	24.4±0.50ab	**
Body weight	128.6±1.54a	117.1±1.64b	125.5±4.83ab	***

The combined data analysis has revealed that there is significant difference between the phenotypic types for all traits and Sinnar donkeys were superior in all cases but the differences between Sinnar and the crosses in some cases was not significant (Table 5). Sinnar donkeys and their crosses have significantly higher (P<0.05) chest depth, ear length, canon bone length and height at withers. The three types were significantly different from each other for height at back and rump. Positive heterosis as calculated from the combined data was found to be more than one per cent for head length, canon bone length, chest depth and ear length. The highest (4.22%) was calculated for ear length.

Comparison of jacks and jennets have shown that for some of the morphometric variables there is sexual size dimorphism where either the jack or jennet could show higher values as compared to the other sex (Table 6). Jacks have shown significantly (P<0.05) higher values for height at back, cannon bone length, chest width and cannon bone circumference while jennets have significantly higher values for body length, back length, hip width, ear length and body weight. There was no significant (P>0.05) difference between the sexes for heart girth, height at withers, height at rump, neck length, head length, fore leg length and chest depth.

The donkeys were sampled from five locations / districts to consider if there are environmental differences; however, location effects were limited to few of the recorded traits. Body length, back length, neck length, head length, fore leg length and ear length are the traits which have shown significant (P<0.05) fluctuations as the sampling location / district differs. Based on this, foreleg length of donkeys from Wenbera district and head length of donkeys from Sirba Abay district were the shortest among the donkey populations from the other districts. More or less, the results show measurements of donkey populations from Guba district were comparably the largest showing above mean performances.

**Table 5 -** Least square means (cm) and pairwise comparison of body measurements with standard error in each breeds/populations: 3) all sexes.

	Br€	Breed groups/ phenotypic types				
Traits	Sinnar	Local	Cross	p value		
N	157	148	18			
Heart girth	110.6 ± 0.44a	106.2 ± 0.45b	108.9 ± 1.25ab	***		
Height at wither	103.2 ± 0.37a	97.1 ± 0.38 <sup>b</sup>	101.0 ± 1.07 <sup>a</sup>	***		
Height at back	105.3 ± 0.38a	98.9 ± 0.39°	102.2 ± 1.10 <sup>b</sup>	***		
Height at rump	105.3 ± 0.38a	99.4 ± 0.39°	102.4 ± 1.09 <sup>b</sup>	***		
Body length	91.5 ± 0.41 <sup>a</sup>	88.0 ± 0.43 <sup>b</sup>	89.7 ± 1.19 <sup>ab</sup>	***		
Back length	68.5 ± 0.33a	65.9 ± 0.34 <sup>b</sup>	65.9 ± 0.96b	***		
Neck length	50.5 ± 0.32a	47.8 ± 0.33 <sup>b</sup>	49.4 ± 0.91ab	***		
Head length	43.9 ± 0.16 <sup>a</sup>	42.0 ± 0.16b	43.5 ± 0.46a	***		
Canon bone length	32.5 ± 0.16 <sup>a</sup>	30.6 ± 0.17 <sup>b</sup>	31.9 ± 0.47a	***		
Fore leg length	70.3 ± 0.35a	66.7 ± 0.36 <sup>b</sup>	68.8 ± 1.04 <sup>ab</sup>	***		
Hip width	32.8 ± 0.17a	31.9 ± 0.17b	$32.3 \pm 0.48$ ab	**		
Chest width	22.8 ± 0.14a	21.9 ± 0.14b	$22.4 \pm 0.39$ ab	***		
Chest depth	50.2 ± 0.21 <sup>a</sup>	47.8 ± 0.22 <sup>b</sup>	50.0 ± 0.60 <sup>a</sup>	***		
Canon circumference	24.2 ± 0.12a	23.5 ± 0.13b	23.7 ± 0.35 <sup>ab</sup>	**		
Ear length	24.3 ± 0.14a	23.4 ± 0.14b	24.8 ± 0.39a	***		
Body weight	127.3 ± 1.28a	113.4 ± 1.31 <sup>b</sup>	121.1 ± 3.67ab	***		

Table 6 - Least square means and pairwise comparison of body measurements with standard error in each sexes.

Body variables	Se	X	n volue
bouy variables	Jacks	Jennets	p value
N	123	200	
Heart girth	108.2±0.59	108.9±0.53	NS
Height at wither	100.7±0.51	100.1±0.46	NS
Height at back	102.9±0.52	101.4±0.47	**
Height at rump	102.7±0.51	102.1±0.46	NS
Body length	88.5±0.56	90.9±0.51	***
Back length	66.2±0.45	67.4±0.41	**
Neck length	48.9±0.43	49.6±0.39	NS
Head length	43.3±0.22	42.9±0.20	NS
Canon bone length	32.0±0.22	31.3±0.20	**
Fore leg length	68.7±0.48	68.5±0.43	NS
Hip width	31.7±0.23	33.0±0.21	***
Chest width	22.6±0.18	22.1±0.17	**
Chest depth	49.2±0.28	49.5±0.26	NS
Canon circumference	24.4±0.17	23.2±0.15	***
Ear length	23.9±0.19	24.4±0.17	**
Body weight	118.7±1.73	122.5±1.56	*
N= Number of observations, *p<0.05, **p<0.01, **	*p<0.0001, NS=Not Significant		

**Table 7 -** Least square means (cm) and pairwise comparison of body measurements with standard error in each districts.

Traits		Sam	pled Location /Dis	trict		n volue
iraits	Guba	Menge	Wenbera	Sirba Abay	Kurmuk	p value
N	58	84	89	59	33	
Heart girth	108.7±0.81	108.8±0.64	108.5±0.68	109.7±0.77	107.1±0.97	NS
Height at wither	100.2±0.69	100.4±0.55	99.9±0.58	101.8±0.66	99.8±0.83	NS
Height at back	101.9±0.71	101.7±0.56	101.9±0.59	102.8±0.68	102.4±0.86	NS
Height at rump	102.7±0.70	102.1±0.56	101.8±0.59	103.1±0.67	102.1±0.85	NS
Body length	89.0±0.77ab	89.8±0.61ab	88.5±0.64b	91.3±0.73a	90.0±0.93ab	*
Back length	66.8±0.62ab	66.4±0.49b	66.4±0.52b	68.4±0.59a	65.9±0.74b	*
Neck length	49.8±0.59ab	48.1±0.47b	49.0±0.49ab	49.9±0.56a	49.3±0.71ab	*
Head length	43.3±0.30a	43.6±0.24a	43.0±0.25a	41.9±0.28b	43.7±0.36a	***
Canon bone length	31.8±0.30	31.2±0.24	31.5±0.25	31.7±0.29	32.1±0.36	NS
Fore leg length	69.5±0.65a	69.1±0.52a	66.1±0.55b	69.2±0.62a	69.1±0.79a	***
Hip width	32.5±0.31	32.6±0.25	32.5±0.26	31.6±0.30	32.5±0.37	NS
Chest width	22.7±0.25	22.5±0.20	21.9±0.21	22.3±0.24	22.3±0.30	NS
Chest depth	49.0±0.39	49.9±0.31	49.2±0.32	48.9±0.37	49.5±0.47	NS
Canon circumference	23.7±0.23	23.9±0.18	23.8±0.19	23.7±0.22	23.9±0.27	NS
Ear length	24.8±0.25a	23.8±0.20b	23.9±0.21b	24.3±0.24ab	23.9±0.31ab	**
Body weight	120.2±2.48	121.1±1.88	119.4±1.98	124.8±2.25	117.5±2.85	NS
N= Number of observations	, *p<0.05, **p<0.01	., ***p<0.0001, NS:	=Not Significant			

#### **Qualitative characteristics**

The qualitative characteristics of the studied donkey population under the effect of breed, sex and source of the animals is presented in Table 8 and 9. Genotype and environmental factors like sex, animal source and district affects the qualitative characteristics of the studied donkeys. The donkey populations studied possess the following qualitative characteristics; white abdominal color, unpigmented hoof and muzzle, short and medium hair coat cover, plain body color pattern with long dorsal stripe (leg stripe in some cases), straight face and sloppy rump profile, medium tail length and thickness at the base of the tail. The studied qualitative characteristics were affected by different genetic and environmental factors. Breed group has a significant effect on the studied traits except on coat description, abdominal color, face profile and tail length while sex affects few of the qualitative characteristics recorded like coat description, back profile and tail thickness at the base. Similarly source of the animal affects most of the traits except body color pattern, ear shape, leg strip and rump profile.

The results showed dorsal body color of the studied donkey populations was affected (P<0.05) by breed group of the donkeys. Based on this result, dorsal body color of Sinnar donkeys were 18% brown, 17% dark brown, 15% white, 13% light red, 8% dark gray, 8% gray dun and 21% others. While the locals were 24% gray dun, 23% bay dun, 16% dark gray dun, 13% gray/roan, 9% brown and 15% others. Similarly, the crossbreds were 22% bay dun, 22% dark gray dun, 17% light red, 11% black, 11% dark brown and 17% other colors. The head color of the Sinnar donkeys was 31% white, 11% brown, 10% light red, 10% dark brown, 9% black and 29% others. However, the head color of the local donkeys was 21% gray dun, 18% bay dun, 16% dark gray dun, 14% gray/roan, 10% brown and 21% others. The crossbreds were also 28% dark gray dun, 17% white, 11% light red, 11% dark brown, 11% gray/roan and 5% others in head color. The ear tips color of Sinnar donkeys was 47% dark brown, 21% black, 10% brown, 9% light red and 13% others, while the local donkeys were 63% dark brown, 28% black and 9% other colors. Similarly, the crossbreds were 44% dark brown, 39% black and 17% other in colors of their ear tips. The dominant tail switch color of the studied donkeys was black, with dark brown; almost all (89%) of the crossbreds possess black tail switch color, similarly most (81%) of the locals tail switch color was black with 16% dark brown. The results also showed that 60% of the Sinnar donkeys had black tail switch color with 20% dark brown and 20% other colors.

The study revealed that local and cross donkeys possess plain body color pattern while some of the Sinnar donkeys were shaded. Almost 70 percent of the Sinnar donkey breeds ear shape was round edged while most of the local and crossbreds ear was straight edged in shape. Almost all local (93%) and crossbreds (83%) possess long dorsal stripe while this feature was observed on half of the Sinnar donkey breeds. 40 % of the local donkeys had leg stipe, however, it was not seen on most of the Sinnar and its crossbred donkeys. Most local donkeys and the crossbreds had either short or long shoulder stripe, while, it was absent on more than half of the Sinnar donkeys. Almost 50% of the Sinnar donkeys back profile was convex, however, in most of the crossbreds and local donkeys it was hollow.

The results also showed that most of the short coat hair size was possessed by jacks while the jennets had medium coat description. Most of the jacks back profile was hollow while the jennets were hollow. Most of the jacks had small to medium thickness at the base of their tail, however it was medium to large for the jennets. Purchased animals had short coat hair size while the born ones had medium coat description.

There was no dorsal stripe on half of the purchased donkeys while half of them possess long. On the other hand, most of the donkeys born on-farm possess long dorsal stripe. Similarly, there was no shoulder stripe on more than half (52%) of the purchased donkeys while there was either short or long shoulder stripe for the donkeys born there. Most of the purchased donkeys back profile was convex while half of the borne ones possess hollow back profile. Short and medium tail length was observed on the donkeys born there while the purchased donkeys tail length was medium to long.

Animals were sampled from different locations/districts to have a representative picture of the study area (Benishangul Gumuz region). Most of the qualitative characteristics of the studied donkey populations were significantly (P<0.05) influenced by location /district. Based on this, ear shape, leg stripe, shoulder stripe, back profile, rump profile, tail length and thickness at the base of the tail are some of the traits which show significant differences among districts. The results show most of the donkeys from Kurmuk district had round edged ear shape while straight edged ear shape was seen on most of the donkeys sampled from Menge district. Most of the donkey populations from the sampled locations do not possess leg stripe while 50% of the donkeys from Guba and Kurmuk districts had a leg stripe.

The results also revealed that most of the donkeys from Sirba Abay district had a hollow back profile while straight back profile was observed on above half of the donkeys from Guba district. On the other hand, above half of the donkeys from Menge district had convex back profile.

Traits	Bree	Breeds/populations		Sex		Animal source	
Traits	Sinnar	Local	Cross	Jacks	Jennets	Born	Purchased
N	157	148	18	123	200	232	91
Coat hair size (Chi-square)		NS			***		**
Short	48	39	39	63	31	38	57
Medium	47	53	61	36	60	55	38
Long	5	8	0	1	9	7	5
Body color pattern (Chi-square)		***			NS		NS
Plain	75	100	94	89	87	90	81
Shaded	25	0	6	11	13	10	19
Ear shape (Chi-square)		***			NS		NS
Rounded	69	22	28	48	43	42	53
Straight	31	78	72	52	57	58	47
Dorsal strip (Chi-square)		***			NS		***
Absent	50	7	17	29	27	22	45
Long	49	93	83	70	72	77	55
Short	1	0	0	1	1	1	0
Leg strip (Chi-square)		**			NS		NS
Present	19	39	17	29	27	30	22
Absent	81	61	83	71	73	70	78
Shoulder stripe (Chi-square)		***			NS		***
Absent	52	9	17	30	31	22	52
Long	21	46	44	32	35	39	21
Short	27	45	39	38	34	39	27
Face profile (Chi-square)		NS			NS		*
Convex	27	38	33	36	31	37	22
Straight	72	61	67	63	68	62	78
Concave	1	1	0	1	1	1	0
Back profile (Chi-square)		**			*		**
Hollow	32	47	61	32	45	47	24
Straight	19	23	0	20	20	20	19
Convex	49	30	39	48	35	33	57
Ramp profile (Chi-square)		**			NS		NS
Flat	22	36	39	37	25	33	22
Sloppy	65	59	61	56	65	59	68
Roofy	13	5	0	7	10	8	10
Tail length (Chi-square)		NS			*		*
Short	25	30	33	26	30	32	18
Medium	50	40	22	37	48	43	46
Long	25	30	45	37	22	25	36
Tail base thickness (Chi-square)		*			***		*
Narrow	33	17	17	34	19	20	36
Medium	44	56	55	59	45	53	44
Wide	23	27	28	7	36	27	20

<b>T</b>			Location / distric	ct		
Traits	Guba	Menge	Wenbera	Sirba Abay	Kurmuk	Chl-square
N	58	84	89	59	33	
Coat hair size						NS
Short	53	39	43	44	39	
Medium	40	54	50	51	61	
Long	7	7	7	5	0	
Body color pattern						NS
Plain	84	90	89	83	91	
Shaded	16	10	11	17	9	
Ear shape						***
Rounded	43	26	52	47	73	
Straight	57	74	48	53	27	
Dorsal strip						NS
Absent	26	32	34	17	27	
Long	74	68	64	83	73	
Short	0	0	2	0	0	
Leg strip						***
Present	43	27	11	31	42	
Absent	57	73	89	69	58	
Shoulder stripe	<u> </u>					**
Absent	33	35	35	15	30	
Long	22	39	25	56	27	
Short	45	26	40	29	42	
Face profile						**
Convex	26	27	48	20	39	
Straight	74	70	52	80	61	
Concave	0	3	0	0	0	
Back profile						***
Hollow	16	30	40	76	45	
Straight	55	13	24	0	0	
Convex	29	57	36	24	56	
Ramp profile				<u> </u>		**
Flat	21	32	27	42	24	
Sloppy	77	55	66	54	55	
Roofy	2	13	7	4	21	
Tail length			•		21	***
Short	21	11	29	44	55	
Medium	45	55	38	37	39	
	45 34	34	33	19	39 6	
Long Tail base thickness	34	34	33	19	0	***
	31	26	21	3	33	000
Narrow		36				
Medium	48	44	59	49	49	
Wide N= number of observations, *	21	20	20	48	18	

#### DISCUSSION

#### Effect of breed group

The results revealed that there were differences among the studied breeds/populations indicating Sinnar donkeys were significantly bigger than the local donkeys while some similarities were observed with the crosses which might be due to heterosis effect. Heterosis of various magnitude was calculated for the various traits studied. However, as the crosses are of diverse type (F1, F2, back cross etc.) and blood level it will be difficult to interprete the heterosis effect obtained in this study in both sexes. The big body size, height and length in the measured traits of Sinnar indicated that the breed is highly adaptable to the hot environment with good reproduction ability (Beja-Pereira et al., 2004; Marshall 2007; Rossel et al., 2008). These results are in line with the results of Kefena et al., (2011) and Tsega and Lemma (2015) who reported Sinnar donkeys are the tallest of all donkey populations in Ethiopia and they are also an excellent desert adapted animals used for riding and breeding. Therefore, due to these special characteristics of the Sinnar donkey, Kefena et al. (2011) has reported that its ancestral trunk might be different from the rest of the donkey breeds/populations but this needs to be supported by further genetic studies. Some of the characteristics of desert adapted donkeys include fairly bigger body sizes and similarities in coat color patterns among donkey populations of arid and semi-arid lowlands (Beja-Pereira et al., 2004; Marshall, 2007; Rossel et al., 2008). Differences in breed groups in this study were in line with the results of Kosťuková et al. (2015). The possible cause of differences among the studied breed groups might be due to the differences in domesticated ancestors, ecology and biophysical resources for the overall body build and local environment and history (Beja-Pereira et al., 2004; Kefena et al., 2014; Gubitz et al., 2000). The local breeds were smaller and lighter than the Sinnar donkeys which is suitable for different purposes which is in line with the

results of Sargentini et al. (2018) who reported that the Amiata donkeys' biometrics (small-medium sized) was suitable for different purposes.

The dominant body colors of Sinnar donkeys from this study were white and light colors with short to medium hair while, most of the locals and crossbreds possess gray dun and bay dun with medium hair size. These results are in line with the results of study by Tsega and Lemma (2015) in Gondar, Ethiopia, who reported Sinnar donkeys possess white body color with short hair and crossing them with Abyssinian donkeys often results in hairier, longer, saggy and dark colored hybrids.

#### Effect of sex

Eight of the fifteen morphometric measurements were significantly affected by sex; jacks were dominant over the jennets in four traits (height at back, chest width, canon bone length and canon circumference) while the jennets were dominant over jacks in five traits (body length, back length, ear length, hip width and body weight). These results were in line with the results of Kosťuková et al., (2015) and partially with the results of Folch and Jordana (1997) on Catalonian donkeys. The magnitude of sexual dimorphism in the current study is quite higher than what has been reported for Catalonian donkeys where only Eight out of twenty-six morphometric measurements showed significant differences between sexes (Folch and Jordana, 1997). The report of Koubek (1933) also showed that male donkeys have lower heart girth and stronger shin than the jennets. Jacks in this study were stronger (wide chest and thick canon) than the jennets which is in line with the results of Andersson (1994 cit. in Purzyc et al., 2007) who reported that, there are specific physiological and biochemical processes in jacks, which results in them being stronger than the jennets. In line with the current study the chest circumference and hip width in female individuals is mainly influenced by physiological processes during gestation and by metabolic traits that differ from those in males (Koubek, 1933). Similarly, height at wither and at rump were not significantly different among the sexes while the jennets had shorter height at back than the jacks which might be due to the fetus load which pull their belly down during the pregnancy period. On the other hand, it might also be due to their loss of strength to hold up the load they carry in comparison to jacks.

#### **Effect of location**

Location affects only six traits (body, back, head, neck, ear and fore leg length) out of the eighteen morphometric measurements. In the study by Tsega and Lemma (2015) on Sinnar donkeys in Gondar, Ethiopia, differences in morphometric measurements and qualitative characteristics were recorded among different sampling location due to differences in ecological selection regimes, history or both. However, such huge significant differences were not recorded in the present study which may be due to the closeness of the locations sampled. However also, the donkeys from the locations/ districts which are on the border with South Sudan shows some dominance over the others as these districts may serve as the gate ways of the Sinnar donkeys to the region. Most of the qualitative characteristics of the purchased donkeys approaches the characteristics of Sinnar donkeys, showing most of the farmers were purchasing Sinnar donkeys preferring their heaviness. The white color and short hair size of the Sinnar donkeys might help them in physiological adaptation of the hot environments.



Local Male Sinnar Male Cross Male



Local Female Sinnar Female Cross Female

#### CONCLUSION

The donkeys in Benishangul Gumuz region were characterized based on FAO guidelines. Accordingly, three phenotypic types were covered; the Sinnar, the locals and their crossbreds. Significant differences were recorded among the three breed groups on the studied morphometric measurements and qualitative characteristics. Based on these results the Sinnar donkeys were the tallest, widest and strongest with some similarities with their crosses. Some sex effect was observed on the overall performances while, its effect was limited on the interaction effect with the breed groups. However, jacks and jennets were dominating each other on equal basis. Jacks had wide chest, thick canon and long height at rump and cannon bone, while the jennets possess wide hip and long body, back, neck and ear. Significant differences were observed between sampled location for some of the studied traits. Qualitative differences were also recorded among the studied class categories. Further molecular level studies are required to characterize the differences among the studied donkey breed groups. Similarly, characterization of the local donkeys found in other parts of the region and the Abyssinian donkeys in adjoining areas is required.

#### **DECLARATIONS**

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#### **Authors' Contribution**

AM contribute on data analysis and the write up of the manuscript, AA, MM, FG conceived the study and collect data, SA review the manuscript, AH and YE contribute on entering data. All authors read and approved the final manuscript.

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### **Conflict of interests**

The authors have not declared any conflict of interests.

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# EFFECT OF PHASE FEEDING ON BROILER PERFORMANCE

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ABSTRACT: This study was conducted to assess the effects of phase feeding on growth performance of broiler chicks. The experimental work consisted of a 7-week trial feeding, in which three different feeding programme. Three experimental diets formulated to contain different levels of metabolizable energy (ME) and crude protein (CP); namely a broiler starter diet containing approximately 3072 Kcal ME/kg and 23.9% crude protein, a grower diet containing 3118 Kcal ME/kg and 21.8% crude protein, and a finisher diet containing 3200 Kcal ME/kg and 18% crude protein. The results diets not reveal any significant differences in productive parameters, but indicated marked trends in differences among the experimental treatment. These differences showed that the highest feed consumption and live weight gain were attained by the group of birds fed the starter diet throughout the experimental period. The starter diet followed by the finisher diet for four weeks resulted in the lowest total feed consumption, reasonably high body weight gain, lowest feed conversion ratio and highest dressing percentage. It can, however, be considered under the conditions of the present experiment that the three experimental feeding programmes supported similar productive performance of broiler, indicating a slight economic advantage of feeding the starter diet followed by the finisher diet.

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RESEARCH ARTICLE

Keywords: Broiler chicken, Growth performance, Phase feeding

#### INTRODUCTION

Feed constitutes approximately 60-70% of total broiler production costs (Saveewonlop, 2019). The broiler industry has been developed into the most efficient animal production sector. This development has been brought about through development and advancement of research in the fields of poultry science and related subjects. The most significant of these developments were in the fields of genetics and nutrition, which lead to the breeding of commercial broiler chicks capable of rapid growth and efficient utilization of feed (Anonymous 2013; Mehmood et al., 2014). Since feed cost constitutes the major cost item of producing broiler meat, considerable research effort was concentrated in improving nutrition and efficiency of feed utilization of the broiler chicks. The broiler chicks are known to be exacting in their nutrient requirements for rapid growth and development, and that they need to be supplied with adequate diets capable of satisfying these requirements in a utilizable form (Skinner-Noble et al., 2001).

The nutrient requirements of boiler chicks have been almost precisely established and the capacity of various feed ingredients to supply these nutrients has been identified, and consequently the formulations of adequate diets became possible. The broiler chick has some extremely high nutrient requirements, particularly during the early stages of its growth. The capacity of the digestive system of the chick is very limited during this period, and it became important to feed them diets highly concentrated in metabolizable energy and protein of high quality, beside high levels of vitamins and essential minerals during the early stages of the growing period. Such diets have been commercially established and used world- wide. These high density broiler diets were recommended to be fed as a sole diet for the whole 6-7 weeks growing period (Skinner-Noble et al., 2001).

Further research development indicated that the nutrient requirements of the broiler chicks vary with its advancement of growth according to the increase in body weight and nature of growth. The growth curve established that young broiler chicks during the first 3-4 weeks of life are at their most active phase of growth, and the nature of growth during this period is mostly in the form of tissue protein and very little fat (Skinner-Noble et al., 2001). Accordingly, it has been indicated to vary the composition of broiler diets during the growing period according to the actual nutrient requirements for the changing nature of growth. In this context, different feeding programmes with varying dietary nutrient composition have been developed for the growing broilers as a means for economy of feed and efficient nutrient utilization, and ultimate reduction of the cost of feeding. In this respect, different diets of variable nutrients composition have been advocated for phase feeding of broilers, based on increasing the energy content of the diet and reducing its crude protein content with the advancement of age (Roush et al. 2004). It is intended in this study to assess the effect of phase feeding using different feeding programmes, on the production performance and carcass yield of broilers.

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<sup>&</sup>lt;sup>™</sup>Supporting Information

#### MATERIALS AND METHODS

#### **Experimental diets**

Three types of starter, grower and finisher broiler diets were formulated from the local feed ingredients and imported super concentrate which used as complement dietary protein, amino acids, vitamins and minerals. The composition of the super concentrate was described in table 1. The diets were formulated according to the recommended nutrient composition of the diets commonly used in phase feeding of broiler chicks. In this respect, the starter diet was relatively high in metabolizable energy (ME) and crude protein content, which were reduced in the grower diet, the protein content was further reduced in the finisher diet with increasing the ME content. The formulation of the experimental diets is shown in table 2. The calculated and proximate analyses were shown in tables 3 and 4, respectively. Each diet was analyzed for its content of dry matter, crude protein, ether extract and crude fiber according to the procedure of AOAC (1980) for proximate analysis. The metabolizable energy (ME) content of the diets were calculated according to the equation of Lodhi (1976) based on the determined proximate analyses values of the diets. The equation is as fallow:

M.E (Kcal kg-1= (1.549+0.102(CP) +0.275(EE)+0.148(N.F.E)-0.034(C.F) X239.

The calculated analyses of the diets were made according to the tables of NRC (1994) for ingredients composition.

Table 1 - The chemical composition of the super concentrate used in the formulation of the experimental die
(LNB concentrate 5%)

Analysis	Min.
Crude protein %	40.00
Metabolizable Energy kcal/kg	2100
Crude fiber %	2.00
Calcium %	10.00
Phosphorus total %	4.00
Lysine %	12.00
Methionine %	3.00
Methionine + Cysteine %	3.20

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<b>Table 2 -</b> The formulation	at the exherim:	antal Alleis inel	rrent as teni
	OF CHE CAPCITIES	Jiitai aicts (pci	cont as rea.

Ingredient	Experimental diets	Starter	Grower	Finisher
Sorghum		60.0	67	76.41
Groundnut meal		18.0	14	10
Sesame meal		14.85	11.8	6
Super-concentrate		5.0	5	5
Crushed oyster shell		1.5	1.5	1.5
Common salt		0.3	0.3	0.3
Methionine		0.1	0.15	0.21
Lysine		0.25	0.25	0.25
Vegetable oil		-	-	0.33
Total		100.0	100.0	100.0

Table 3 - The calculated analyses of the experimental diets (percent as fed)

Experimental	diete			
Items	Starter	Grower	Finisher	
Metabolizable energy (kcal/kg)	3072	3118	3200	
Crude protein	23.9	21.8	18.9	
Lysine %	1.34	1.25	1.20	
Methionine %	0.60	0.60	0.59	
Calcium %	1.4	1.3	1.2	
Av. Phosphorus %	0.69	1.39	0.313	

 Table 4 - Proximate analysis of the experimental diets (percent)

	Experimental diets	Starter	Grower	Finisher
Items		Starter	GIOWEI	i illisilei
Dry matter %		9486	90.45	93.87
Crude protein %		22.25	20.98	19.58
Crude fiber %		6.19	6.02	6.03
Ash %		8.57	13.87	6.64
Fat %		6.94	6.45	5.72

#### **Birds and management**

A total of 120 unsexed day-old broiler chicks of commercial strain (Hubbard) were purchased from a local hatchery (Coral CO.) The birds were reared on deep litter in an open side house; the experimental house was apportioned in to 9 pens of equal area  $(1 \times 1 \text{ m})$ . The pens were arranged in three rows, each row containing three pens, each pen was equipped with a tube feeder and a fountain waterer. Upon arrival, the chicks were immediately given sugar in drinking water to reduce the transportation stress. At two weeks of the age the chicks were vaccinated against Newcastle Disease.

#### **Experimental procedure**

Birds were randomly assigned to the experimental pens at the rate of 10 chicks per pen. The three experimental treatments were randomly assigned to each row of pens (block), thus giving a Randomized Complete Block Design arrangement. Treatment 1 consisted starter diet from day-old. Birds were fed starter diet for the first two weeks followed by the grower diet for 2 weeks and then the finisher diet to the end of the experiment (7 week). Treatment 3 consisted starter diet from day old to 3 weeks of age followed by the finisher diet to the end of the experiment. Feed consumption and live weight weekly determined for each pen, and mortality was recorded as it occurred. At the end of the experiment the birds were fasted overnight except for water. Three birds from each replicate (pen) were randomly selected, wing tagged and individually weighed. They were then slaughtered by jugular severing and left for an appropriate time to bleed. The birds were eviscerated and hot dressed carcass weight was recorded. The data for weekly feed consumption, live weight gain, feed conversion ratio and dressing percentage were collected. The data was analyzed statistically for treatment differences by analysis of variance for the randomized complete block design according to Steel et al. (1980).

#### **RESULT AND DISCUSSION**

Performance results did not show significant differences in total feed consumption, live weight gain or feed conversion efficiency among the experimental birds. These results are in line with several reports on the effects of phase feeding of broiler chicks by Warren and Emmert (2000); Pope and Emmert (2001); Skinner-Noble et al. (2001); Pope et al. (2002); Roush et al. (2004); N. Saveewonlop (2019). They indicated that phase feeding can support similar feed intake growth, carcass yield and feed efficiency to that of feeding a single starter diet based on NRC recommendations. Despite the insignificant differences in performance among the different feeding programmes, the results revealed certain trends in insignificant differences in performance among the experimental feeding programmes. The highest feed intake and live weight gain were attained by feeding the single starter diet throughout the experimental period, while feeding the starter diet accompanied by the grower and finisher diets resulted in the lowest body weight gain; and feeding the starter diet followed by the finisher diet to the end of the experimental period resulted in the lowest feed intake and lowest feed conversion ratio. These trends in performance generally follow the pattern of nutrients intake, resulting from the differences in feed consumption of the experimental diets. In this respect, the intake of all essential nutrients was highest under programme I, where the chicks were fed the nutrient dense starter diet during the whole experimental period. This was followed by the birds reared on programme 2, and was least on phase feeding programme 3. No differences in feed intake, body weight gain, or feed conversion efficiency were observed among the experimental birds during the three weeks starting period. This can be attributed to the fact that all the birds during this period received the same starter diet and almost consumed the same amounts of feed and nutrients. The amount of feed consumed during this period was only a small fraction of the total feed consumed by the birds over the 7 weeks' growth period. Consequently, the highest amount of feed and nutrients were consumed during the grower and finisher periods, thus having a greater impact on body weight gain and feed utilization during the later phases of the growth period. These effects were clearly marked in the works of Warren and Emmert (2000), Skinner-Noble et al. (2001), Roush et al. (2004), Tremarin & Sakamoto (2017), and Saveewonlop (2019). These workers indicated that body weight and feed conversion were not sensitive to the grower diet feeding in a three- diets phase feeding programme.

Treatments	1	2	3	SE
Experimental parameters	_	2	3	JE
Number of bird/treatment	30.0	30.0	30.0	-
Initial body weight (g/bird)	40.90	43.60	42.30	-
Total feed consumption	3306.60	3080.6	3021.6	90.68 NS
Final live body weight (g/bird)	1390.0	1317.0	1349.0	95.87 NS
Live body weight gain (g/bird/7)	1349.1	1253.4	1306.7	39.96 NS
Feed conversion ratio (g feed/ g)	2.45	2.45	2.31	0.055 NS
Dressing percentage	68.30	69.80	72.7	2.92 NS
Cost of feed consumed (SD)	291.80	297	296.1	– NS

grower diet + finisher diet; 3= starter diet + finisher diet.

Table 6 - Total nutrients consumption of the experimental birds (g/bird/ 7week)

	Treatments	1	2	3
Items		_	2	3
ME Kcal		10132	9575	9587
Crude protein		788	669	566
Lysine		44	38	35
Methionine		19	18	17
Calcium		48	42	37
Av. Phosphorus		11	10	9

Values are means of 30 birds/treatment; NS= not statistically significant, SE= Standard error of means; 1= starter diet; 2= starter diet + grower diet + finisher diet; 3= starter diet + finisher diet.

On the other hand, increasing the starter diet feeding period had the strongest effect on increasing body weight and decreasing feed conversion ratio; while decreasing the feeding period of the finisher diet increased the body weight and decreased the feed conversion ratio. It has also been indicated that broiler performance under phase feeding programmes is influenced by the length of the growing period and the proportion of time that the starter, grower and finisher diets are fed, as well as the stage of growth at which these diets are introduced. The absence of significant differences among the results of the present study indicates that the three tested feeding programmes were capable of supporting similar productive performance of broilers. This is irrespective of the marked differences in nutrients intake, particularly crude protein and lysine. This, however, did not reflect any differences in the cost of feed consumed under the different feeding programmes, due to the unexceptionally high prices of grain sorghum in the particular season, which was used at high percentages in the grower and finisher diets this result similar with those reported by Ajighjigh (2018). Under normal price conditions, the cost of feeding will be lowest in the programme which consisted of feeding the starter diet followed by the finisher diet. The same programme seemed to be more appropriate for supporting broiler production in the light of the attained lowest feed consumption, reasonably high live weight gain and lowest feed conversion ratio among the experimental treatments.

#### **CONCLUSIONS**

The absence of significant differences among the results of the present study indicates that the three tested feeding programmes were capable of supporting similar productive performance of broilers. This is irrespective of the marked differences in nutrients intake, particularly crude protein and lysine. This did not reflect any differences in the cost of feed consumed under the different feeding programmes. It can be considered that the conditions of the present experiment though limited, reflects the advantage of phase feeding of broiler chicks, particularly the programme of feeding the starter diet followed by finisher diet.

# **DECLARATIONS**

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#### **Authors' contributions**

All authors were contributed equally.

#### **Competing interests**

The authors declare that they have no competing interests.

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# ASSESSMENT OF DAIRY VALUE CHAIN SUSTAINABILITY, CONSTRAINTS AND OPPORTUNITIES IN AKSUM, CENTRAL TIGRAY, ETHIOPIA

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ABSTRACT: Describing the sustainability of dairy value chain and showing the clear gaps of the sector is important for bringing continual improvement that can support the livelihoods of dairy farmers and the wellbeing of environment. To facilitate a balanced practice on the three pillars of sustainability (people, planet and profit), a regular updating of the existing situation of dairy production is crucial. Therefore, the objective of this report was describing the sustainability of dairy value chain in Aksum district, and shows clearly the gaps of the sector that needs urgent action for continual improvement. Desk study for gathering secondary information was employed. Different analytical tools were used to analyze and present the result. In Aksum, milk is produced by mainly smallholder farmers, dairy cooperatives and few commercial medium scale farms. The production system is operated by gender inclusive system which is in line with sustainable dairy. The production potential of milking cows is very poor that is mainly due to poor genetic makeup and management system. The common feed is roughage that causes to the low production performance (poor economic viability) and high greenhouse gas emissions from enteric fermentation (risky for environment). The highest value addition, risks and costs are belonged to producers but the high share of margin is for processors and retailers. Therefore, this unfair share of profits, costs and risks is not a good sign of sustainable dairy development. Hence, an attempt to improve the dairy sector should consider the three pillars of sustainability.

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#### INTRODUCTION

Sustainable dairy farming refers to a system based on three pillars, which are environmentally sound, socially responsible and economically viable. Sustainable dairy farming requires a commitment to continuous improvement, which means that performance gaps are identified and addressed, and actions taken must maintain an appropriate balance among the three interdependent pillars of sustainability (Allan et al., 2017). General improvement of crop and livestock production practices can also contribute to climate adaptation due to improved resilience of animals and increased farm productivity and income; which means all pillars of sustainable farming would be considered (Marion et al., 2016).

Dairy farms in Ethiopia known by low milk production on average about 1.7 liters per cow/day (Yilma et al., 2011) and poor reproductive performance. Therefore, improving general dairy management, such as breeding, feeding and animal health, will lead to increased efficiency of dairy production (economic viability) and a decrease in greenhouse gas (GHG) emissions to the environment (Gerber et al., 2013). In Ethiopia's Livestock Master Plan, crossbreeding with exotic dairy breeds, and Al and synchronization, combined with better feed and health services is already in the development roadmap for dairy farming (ILRI, 2015). This is a good kickoff of sustainable dairy development in the country. Domestic demand of milk is projected to grow by 47% towards 2020 (GTP II, 2015). This can offer chances for the dairy farmers to increase their income from selling dairy products and enhance their livelihoods.

Efficient milk production is a key to sustainable development of dairying. In Tigray regional state, there are about 862,441milking cows. The average lactation performance of the cow was 415 and 1712 litres of milk for local and crossbreed cows respectively (CSA, 2016). Milk is a vital cash income sources for household consumption expenses. There are many opportunities for the development of sustainable milk production including the presence of suitable agroecology for the realisation of potential dairy production and the enormous market potential for dairy products (Haregeweyni, 2015).

However, information is scant on the existing situation of dairy production from the three pillars of sustainability point of view; which is important for researchers and policy makers to set out improvement considering people, planet and profit. Therefore, this study focused on reviewing and analyzing of different secondary information to describe the

Supporting Information

magnitude of dairy production sustainability and clear gaps of the sectors in Aksum district which needs urgent action for continual improvement.

#### **MATERIALS AND METHODS**

#### **Description of study area**

The study was conducted in central Zone of Tigray regional state. Aksum is the capital city of the zone and located in Northern Ethiopia. Aksum is situated 1024 kilometres far from Addis Ababa the capital city of the country (Figure 1). The altitude of the zone is mainly falls within the category of 2000 to 3000 meters above sea level. A Large part of the district receives mean annual rainfall ranging from 400 to 800 mm. The mean monthly maximum and minimum temperature of the zone are 30°c and 10°c, respectively. The district has the largest human population in the region. Tigray region has a total of 4,791,341 cattle, and 18% are milking cows (CSA, 2016).

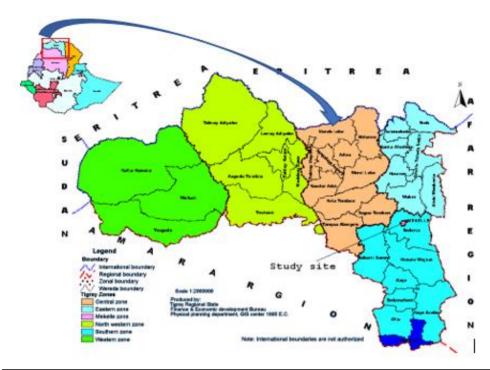


Figure 1 - Map of Central Zone of Tigray (Aksum)

#### **Data collection**

This study was mainly done based on the secondary information from others work such as articles and institution papers from an internet search. Besides, personal experience and practical field visit were employed to generate the information.

#### **Data analysis**

Both qualitative and quantitative methods of data analysis were employed. For qualitative data, PESTEC (Political, Economical, Social, Technological, Ecological and Cultural) and SWOT (Strength, Weakness, Opportunity and Threat) analytical tools were used to analyze and describe the strength and weakness of dairy value chain in the area. Problem tree was also applied to identify the core problem, its causes and effects of milk production. Narrative description was used to assess the sustainability profiles of dairy production from People, Planet and profit point of view (3p profile). For quantitative information, different economic parameters have used to find out the distribution of added value and share of margins among dairy value chain actors. An economic parameter like gross margin was used to analyse the benefit share and added value of dairy chain actors in the area. The gross income was estimated by subtracting the cost price of the product/unit from the sale price (revenue) of that product. Or in short: Grossincome = Revenue – Variablecost (KIT and IIRR, 2008). A gross margin (GM) shows the percentage of the actor's revenue that is gross profit per unit of produce and was calculated as follows:

$$GM = \left(\frac{Grossincome}{Saleprice(revenue)}\right) * 100 \text{ (KIT and IIRR, 2008)}$$

Added value is the difference between the price the actor pays for the produce, and the price she or he sells it for. It was calculated as follows.

AddedValue = Pricereceived by actor - Pricepaid by actor (KIT and IIRR, 2008)

Like gross margins, the size of the value share also reflects the number of costs and risks appear in the product flow by that actor. Value share was estimated by using the following formula:

$$ValueS \square are = \left(\frac{Addedvalue}{Finalretailprice}\right) * 100$$
 (KIT and IIRR, 2008)

#### **RESULTS AND DISCUSSION**

#### Description of dairy value chain actors

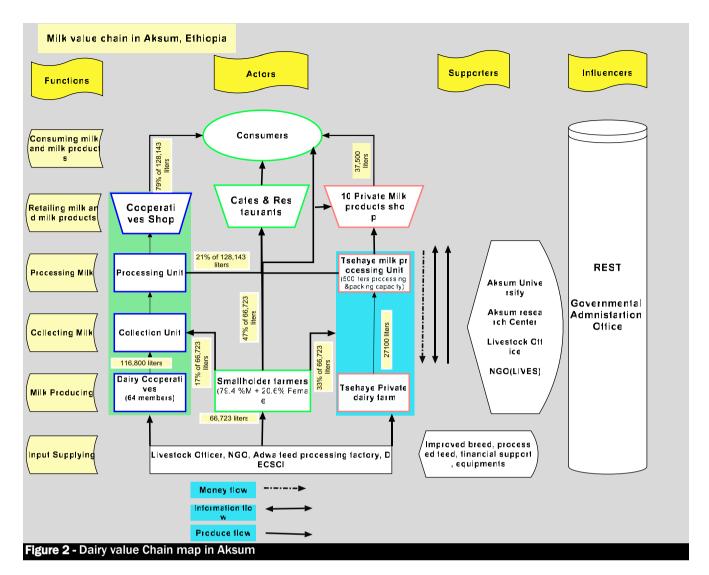
In central zone of Tigray, milk production is mainly carried out by many smallholder farmers, few medium scale commercial farmrs and cooperatives. Dairy cooperatives in Aksum and Adwa town was composed from some smallholder farmers and college graduated youths (Misganaw et al., 2017). Tsehaye private dairy enterprise is one of the commercial dairy farm and act as producers, milk collectors from the nighboures, processors and wholesaler/retailr (Figur 2). Private milk and milk products shops are also play an important role in the distribution of milk to the consumers. The detailed description of available dairy chain actors is depicted in table 1.

## Available supports for dairy sector in Aksum

All milk producers are fully dependent on the support of districts livestock office and some private veternary clinics. Besides, Dedebit Credit and Saving Institution (DCSI), Livestock and Irrigation Value Chains for Ethiopian Smallholders project (LIVES), Aksum University (AKU) and Aksum Research Center (ARC) are also involved the support of milk production in Aksum. Most of these supporters provide inputs and skills that help to realize sustainable dairy development in the area. The detail functions of supporters were presented in table 2.

Table	1 - Key actors play role in mil	k production and marketing				
No.	Actors	Functions	Remark/Output			
1	Smallholder farmers	Producing milk	Produce and supply fresh milk to collectors, retailers and consumers			
2	Dairy cooperatives	Producing, collecting and processing and then retailing milk products	Produces Pasteurized milk, Yoghurt & cheese, and distribute it to consumers and hotels			
3	Tsehaye dairy enterprise	Producing, Collecting and Processing milk	Pasteurized milk, cheese, sometimes cream & supplied to retailers			
4	Private milk products shops	Selling milk and milk products	Receive processed milk from processors &			
5	Cafes and Restaurants	Selling milk and milk products	fresh from farmers, &sell it to consumers			
6	Consumers	Consuming fresh and processed milk	Purchasing milk & milk products from different retailers			
Source	Sources: (Aksum University, 2016; Misganaw et al., 2017; Yaynishet et al., 2018)					

No.	Supporters	Functions	Remark/Outputs
1	Aksum Livestock Office	Providing extension services	<ul> <li>✓ Train and advice farmers, cooperatives and retailers.</li> <li>✓ Transfer information and technology,</li> <li>✓ ProvidingAl and health service through experts</li> </ul>
2	Dedebit credit and saving institute (DECSI)	Providing financial source	They offer credit for  ✓ farmers to purchase breeds, drugs, ✓ Cooperatives and retailers as initial capital
3	NGO (LIVES, ILRI)	Providing training and some inputs	<ul> <li>Organizing farmers in a cooperative, providing technical skills and some processing equipment's as starting point</li> </ul>
4	Aksum Research centre	Disseminating research findings to stakeholders	✓ Support producers and processors through research findings on constraints, quality assessment, breed improvement strategy
5	Aksum University	Providing training, involved in research and community service.	<ul> <li>✓ Support dairy farmers in providing practical based training</li> <li>✓ Monitor and give professional feedbacks to processors and retailers</li> </ul>
6	Adwa Feed Processing Factory	Supplying formulated feeds	✓ Formulate feeds for different class of dairy and distribute it to the dairy farmers
7	Relief Society of Tigray (REST)	Infrastructure development	It's local NGO that control all foreign aid of the region and influences value chain through infrastructure development
8	Aksum governmental administration	Policy, rules and regulation	Try's to control everything and influence the milk value chain



#### Sustainability profile (3P)

Sustainability measure is an essential and newly adapted parameter for successful and continual operation of a business. Any business will be sustainable if it is environmentally sound (Planet), socially responsible (People) and economically viable (Profit). Hence, sustainability of milk production in Aksum is examined according to people, planet and profit profiles, and discussed as follows.

#### Socially responsible (People) dairy production

The social responsibility component of sustainability in dairy production includes elements such as: improving and maintaining the societies and communities where dairy food products are produced; good working and social conditions are valid regardless of gender, age, personal preferences, or conviction; guaranteeing food safety and public health, and improving and safeguarding animal health and welfare (Allan et al., 2017). Besides, farmers' cooperation to improve their bargaining power and existence of long-term relations among chain actors are important indicators for sustainability. These elements were used as a reference to evaluate and assess the sustainability of milk production in Aksum districts from the perspective of people profile (Table 3).

A) Gender Involvement: Dairy cooperatives in Aksum and Adwa are principally established from many jobless youth females, and few college graduated males (Misganaw et al., 2017). The role of women was high in the collection of milk, processing and retailing functions in their cooperative. Similarly, the ten private milk products shops in Aksum and Adwa towns owned by eight women and only two men (LIVES, 2015). But, in the case of smallholders, the contribution of women is limited to activities performed around the home. Therefore, activities like cleaning dairy shade, caring dairy cow and calves, milking, milk container cleaning and milk quality control, milk processing, butter selling were mainly performed by females while selling milk, buying and selling dairy cows and feed collection and animal breeding were also given principally for males (Zemeda, 2015). Similarly, (Gebrekidan et al., 2012) reported that in central zone of Tigray the highest participation of adult female family members was observed in milking, making and selling dairy products and calf caring in the urban areas. Besides, (Haregewoyni, 2015) also indicated that, men and women are moderately involved in feed collecting, feeding, health follow up, cleaning and herding. Therefore, this active involvement of gender in dairy production and marketing has a positive contribution for improvement and sustainability of dairy sector in the area.

- B) Chilled labor: Family children boys were mainly involved in herding, watering and barn cleaning in both urban and peri-urban areas of Aksum district. Family children girls had less involvement in the farms and none of the hired children girls were involved in any dairy activities (Gebrekidan et al., 2012). In contrary, (Haregeweyni, 2015) reported that in Central zone of Tigray, women and female child are highly involved in milking (72.5%), processing (75%) and selling of dairy products (54%). Therefore, child labor is common in both urban and peri urban dairy production and marketing system in the study area. However, sustainable dairy operation should be free from child labor.
- C) Farmers' cooperative: Dairy Cooperatives play a significant role in ensuring sustainable supply of raw milk to the dairy industry by coordinating the flow of milk from their members and assisting them by providing the required dairy farm inputs (FAO, 2011). Farmers' cooperation is the main tool of empowering the bargaining power of dairy farmers in Aksum and there are two female-dominated milk cooperatives in the town (Misganaw et al., 2017) which has its own positive contribution for sustainable dairy production. Some of these contributions are creating fair value share through improving their bargaining power, maintaining products quality, reducing market barriers and spoilage, improving productivity.
- D) Job creation for local community: Dairy production is therefore an important source of self-employment, especially for rural households. A significant proportion of dairy operators also hire long-term or casual labour, which creates employment among some of the poorest segments of society, including landless households in rural areas (Staal et al., 2008). In central zone of Tigray, income and employment opportunity are common under market-oriented dairy production system. The type of labour that employed in dairy producer around Aksum district is herder, milkier, cleaner and processer. The availability of employment creation for herding of local and cross breed cow owners was 5% and 11%, respectively. The average amount of salary payment for one hired labor in Aksum was 3906 Ethiopian Birr (ETB) per year (Haregeweyni, 2015). Therefore, the dairy sector in Aksum is giving only a limited number of employment opportunities for local communities with very low salary rate. Thus, this practice contributes negatively for the sustainability of dairy production in the area. Because the communities in general and youths in particular are not well benefited from the dairy sector through obtaining decent job and income; sense of ownership and support will be reduced and they try to devastate or rob the resource.
- E) Guaranteeing food safety and public health: In central zone of Tigray, milk production and prestige value were the main purpose of keeping dairy cattle both in urban and peri-urban areas (Gebrekidan et al., 2012). Dairy products are an essential component of the diet in the area. Fresh milk, yoghurt, butter, buttermilk, cheese and whey are among the common milk products produced and consumed by the local communities. About 51% of the communities prefer to consume milk either after boiling or souring, but, the rest proportion consumed raw milk which is not safe for health and consumption. The major proportion (75%) of produced butter is sold and the remaining proportion is used for various purposes like cooking and cosmetic. The income generated from the sale of milk products is used to purchase farm inputs, food item, education materials for their children and health services for the household (Haregeweyni, 2015).

Sustainability indicators of People profile	Current situation in dairy sector	Remarks for sustainable development
Gender involvement	Better gender involvement	It should be kept the contribution of gender for sustainable development
Chilled labor	High chilled labor	Using chilled labor must be stopped in dairy sector
Farmers' Cooperative	Positive contribution	Even if their power and capacity is weak, a good start is observed (gender inclusive jobless youths are emerging to dairy sector in the form cooperatives)
Job creation for local community	Minimal and insignificant	The dairy sector can generate more jobs for the local community and has to be pay a fair wage in order to be sustained
Guaranteeing food safety and public health	Has potential risks	Especially in peri-urban areas, half of the community consumes milk in a raw state. Therefore, strict quality measurement and awareness should be made

There are no any legally registered standard quality measures for milk and milk products in Aksum. Traditionally, the communities have a mechanism to check the quality of milk products. This measurement is dependent on sensory reflections. Color (pure white), Smell (not offensive or attractive odour), and taste are the major intrinsic fresh milk quality attributes. The textureis also considered as intrinsic quality for yoghurt and cheese along with the others. Shelf life for milk by-products released from processing units is even considered as intrinsic quality attributes in the area. Packing materials for yoghurt, pasteurized milk and cheese is the extrinsic quality attributes used to check the safety of dairy products in Aksum. Even for milk and milk products, there are no international quality management systems in the country. But, the Ethiopian Food, Medicines and Healthcare Administration and Control Authority (EFMHACA) is the National Regulatory Body of Ethiopia which is under the Ministry of Health. The Authority is responsiblefor ensuring the quality, safety and efficacy of medicines and foods. This authority takes a sample of milk and milk products randomly at producers, processors and retailers, and then checks it in a laboratory. Later, the feedback and corrective measures are addressed through extension officers.

#### **Environmentally sound (planet) dairy production**

An environmentally sound dairy production system is characterized by the adoption of practices and technology for more efficient use of natural resources per unit of animal food produced. Increasing production and improve environmental impacts through reducing emission intensity of greenhouse gas and decrease air, water, and soil pollution (ammonia, nitrate, phosphorus) are the basic elements of environmentally sound dairy production. Implementing practices to improve air quality; manage manure in ways that ensure recycling of nutrients and energy, and minimize release of gases with high global warming potential, and applying efficient grazing systems are also considered as environmentally friend practices (Allan et al., 2017).

With regard to the environmental aspect, the analysis demonstrates that the most serious impact in terms of CO<sub>2</sub> equivalent per unit of product is due to the emissions of methane in the enteric fermentation of animals; these emissions are directly connected to the type of diet fed to cattle. Besides, the production of feed and fodder, either self-produced by the companies themselves or purchased from external suppliers have also contribution for the rise of emissions (Lucio et al., 2016). In order to improve these aspects in terms of sustainability, among others, two key factors have to be considered in conventional livestock breeding such as management of feeding practices and annual milk production per hectare and per cow which means efficiency of production (Dillon et al., 2010 and 0 Brein et al., 2015).

- A) Efficient use of resources and milk production: In Central zone of Tigray, the average milk yield potential of local and cross breed cows was reported approximately 2 and 7 liters/day per cow respectively. The overall average lactation length of local and crossbred cows was 193 and 233 days, respectively (Haregeweyni, 2015). Both the milk yield and lactation length are under the standard performance of dairy cows. However, the cows will consume feed throughout the year and contribute greenhouse gas emissions. According to Mulugeta (2015), the daily feed intake of local cows was ranged from 7.8 to 9.4 kg in dry matter (5.28 to 7.07 roughage and 2.68 to 2.74 concentrate feed). Hence, we can conclude that dairy animals in Central zone of Tigray are not as such efficient producers and there is a probability of high greenhouse gas emission per unit of products. About 99% of dairy farmers have access to get Artificial insemination service and the average conception efficiency was 85.5% (Haregeweyni, 2015). Possible reasons for these low performances of dairy animals in central zone of Tigray are poor production genetic potentials of the animals and weak management system such as feeding, health follow-up, housing and the like factors.
- B) Efficient grazing system: Grazing was practiced by small farmers and mostly for local animals in peri-urban areas, though there was a practice to some extent in urban areas. Majority of the urban dairy producers rely on zero grazing but smaller proportions were used roadsides, hillsides and vacant plots for grazing to their dairy cattle (Gebrekidan et al., 2012). Tigray region is a model for the country in the conservation practices and wise use of natural resources; and Aksum is the one that found at the center of the region and known by conservation of natural resources that mainly practicing intensive or semi-intensive dairy farming in urban and peri-urban area. This practice supports the sustainability of dairy farming since damage of natural vegetation and soil compaction minimized. So that, lands that are not suitable for crop production are used for animal grazing and the possible competition of land between dairy and crop production will be reduced.
- C) Feed types: The main feed resources available for smallholder dairy producers are grass and hay (Zemeda, 2015). The average amount of grass and hay produced in the dairy farmers from own lands were 184.75quintal /year. Whereas the average amounts of grass and hay purchased from other farmers were 276.5quintal/year (Haregeweyni, 2015). Likewise, Gebrekidan et al. (2012) reported that, the major sources of feed for milking cows in Central zone of Tigray were hay, crop residues, grazing, crop after math and non-conventional feedstuffs (like: 'Atela', kitchen waste and weeds). Concentrate feeds were rarely supplied to dairy producers by the private feed traders in Aksum and Adwa. Therefore, dairy cows in Aksum is mainly dependent on roughage feed stuffs that are not easily digested and converted to animal products. The less digestibility of the feed would increase the time of enteric fermentation and methane gas production and emission. The production efficiency of the animals will be reduced and their contribution for global warming raised; these features negatively treat the sustainability of dairy production in the area.
- **D) Waste management:** In central zone of Tigray, wastes from dairy farm such as manure, urine, wastewater, and feed leftover were removed either manually as was the case in small and medium farms or through concrete drainages in the case of large farms. Urban farmers were obliged to pile the cow dung outside of the farm which caused a nuisance to the area, including the risk of local pollution due to nutrient leaching. But in the peri-urban areas, due to alternative uses of manure as organic fertilizer, waste disposal was not well thought-out as a serious problem (Gebrekidan, 2014).

Manure from the urban areas is also supplied to a limited extent to the peri-urban areas, particularly to crop producing farms. Hence, urban to peri-urban linkage is evolved informally and this should be recognized and strengthened to promote nutrient recycling and benefit both urban and peri-urban producers (Gebrekidan, 2014). On the other hand, waste disposal and its valorization thorough renewable energy production technologies (in particular the anaerobic digestion of sewage) allow for not inconsiderable reductions in the Carbon Footprint of milk production. Otherwise, pilling of manure hips outside of the dairy farm is also responsible for phenomena such as the eutrophication and acidification of waters along with GHG emissions (Lucio et al., 2016).

Table 4 - Summary of dairy production sustainability from planet profile					
Sustainability indicators of Planet profile	Current situation in dairy sector	Remarks for sustainable development			
Efficient use of resources and milk production	Very poor production and reproduction performance	Upgrading the genetic makeup of the milking cows could help to them utilize resources efficiently			
Efficient grazing system	Little dependency on grazing, urban producers uses zero grazing system	Efficiently utilizing road and hill side grazing good practice and must be maintained			
Feed types	Roughage feeds are common diet	Instead of fully depending on less digestible feeds that increase emission and reduces production, using concentrate feeds has double advantage			
Cultivation of forage	Negligible due to land shortage	Cultivating forages through intercropping system has paramount important for sustainable dairy production			
Waste management and nutrient recycling	Poor manure handling system in urban but used it as organic fertilizer in peri-urban	A promising start was observed between dairy producers and crop cultivars. Scaling up of this practices and linkage must be considered for sustainable development			

## Economically viable (profit) dairy production

Food production must be economically viable: the producers and other food chain stakeholders must be able to prosper and sustain investment, while consumers need access to quality food in affordable prices. From an economic standpoint, sustainable dairy farming systems are therefore characterized by elements such as: enabling economically viable food production along the food chain, while accomplishing social and ecological goals; ensuring that farm operations obtain a fair share of the profits achieved in the food chain; supporting the ability of dairy producers to invest in sustainability improvements; adopting innovation and approaches that help farmers deal with market volatility and hence the prices of products and inputs (Allan et al., 2017). Economic viability was assessed by determining fixed and variable, direct and indirect costs, with regard to all factors involved in the milk production process (Lucio et al., 2016). However, due to information limitation; fixed cost is not accounted in the economic analysis of present study.

# A) Revenue and variable cost of milk production

The average cost of production per liter of milk in Aksum was reported to be 6.5 Ethiopian Birr (ETB). As indicated from Figure 3, the revenue generated by milk producers from a litre of milk was 12.5 ETB. Estimation of the average variable cost includes cost of milk production, cost of transportation, labour, electricity, water, detergents and gevernment tax, and it was 8.7 ETB for a liter of milk (Haregeweyni, 2015; Misganaw et al., 2017). Therefore, based on this information producers earn a gross income of 3.8 ETB from a liter of milk.

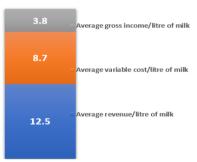


Figure 3 - Average revenue and gross income of milk producers in Aksum

## B) Cost and selling prices of milk and milk products

C) For the producers, cost price includes the costs of feed, hired labor, transport and opportunity cost. For the processors and retailers, the cost of milk purchased, labor and transport costs were included. On top of these, processors have an

additional expenserelated to processing and packing. Producers sell a litre of milk in 12.5 ETB to processors and retailers. Then the processor processed it into at least three by-products (fluid milk, cheese and yoghurt/curd) and sells it by (18.85, 68.3 and 20.5 ETB/unit respectively) on to retailers (Table 5). Retailers purchase fresh milk directly from producers and the processed products from processors. Finally, the retailer sells these dairy products to consumers.

Items (measured ETH Birr/liter)	Producers	Processors	Retailers
Total Cost price/unit			
Fresh milk	8.7	-	12.5
Pasteurized milk	-	15.75	15.75
Cheese/kg (4-liter milk = 1kg cheese)	-	57.49	65.3
Curd/Yoghurt	-	16.1	17.5
Sale Price/unit			
Fresh milk	12.5	-	16.8
Pasteurized milk	<del>-</del>	18.85	25.75
Cheese	<del>-</del>	68.3	72.35
Yoghurt	_	20.5	21.65

#### D) Gross margins and value share

Based on the above data (Table 6), gross margin and value share were calculated and presented as follows. Gross margins show the percentage of the actor's revenue that is profit. It's calculated as the gross income divided by the revenue and multiplied by 100 (KIT and IIRR, 2008). We can see that producer have a gross margin of 30.4% from pasteurized milk sales, while the retailers have highest gross margin 51.5% from this product (Table 6).

Value share is calculated by the actor's added value divided by the final retail price and then multiplied by 100(KIT and IIRR, 2008). Note that the percentage sum of value shares equals to 100%, but the gross margins do not. In the fluid milk, producers earn 48.5% of the final retail price, while the processors receive 24.3% and the retailers gain 27.2%. The value share of producers is high (69.1%) in cheese product, but it is low for final retailers (5.6%). In yoghurt, the trend is the same ascheese; producers have a high-value share.

Like gross margins, the size of the value share also reflects the number of costs and risks appeared in the chain by that actor. Also, the distribution of value share tells us something about the type of product. When the consumer buys a product as it produced, such as fresh milk, then there has been little value added (only transport). So that, it expected that producers have the highest value share (74.4 %) than retailers (25.6 %) (Table 6). Therefore, processors and retailers are taking the profit/advantage on the expense of producers. This type of unfair share of values is not a good sign of sustainability. Because, the major value is added by producers and the highest costs and risks of milk production are laid on producers.

Products	Actors	Cost price	Sale price (revenue)	Gross income	Added value	% Gross margin	% Value share
Freeh Mills	Producers	8.7	12.5	3.8	12.5	30.0	74.4
Fresh Milk	retailers	12.5	16.8	4.3	4.3	25.6	25.6
Pasteurized milk	Producers	8.7	12.5	3.8	12.5	30.4	48.5
	Processors retailers	12.5 12.5	18.75 25.75	6.25 13.25	6.25 7	33.3 51.5	24.3 27.2
Cheese	Producers	34.8	50	15.2	50	30.4	69.1
	Processors retailers	50 65.3	68.3 72.35	18.3 7.05	18.3 4.05	26.8 9.7	25.3 5.6
Yoghurt	Producers	8.7	12.5	3.8	12.5	30.4	57.7
	Processors	16	20.5	4.5	8	22.0	37.0
	retailers	17.5	21.65	4.15	1.15	19.2	5.3

#### Constraints for sustainable dairy production

In the vicinity of cities or large towns of Ethiopia, milk producers have a ready market for their liquid milk. However, in rural areas outlets for liquid milk are limited due to lack of accessible infrastructure that links producers with traders and consumers. For example, a study in Tigray by the Regional Bureau of Agriculture (2006) has shown that about 45,000 litres of fresh milk/per-day is remained to be wastage due to lack of access to the market. Hence, the government should take the lead in building infrastructure and providing technical service to the dairy sector (Tsegaye, 2010).

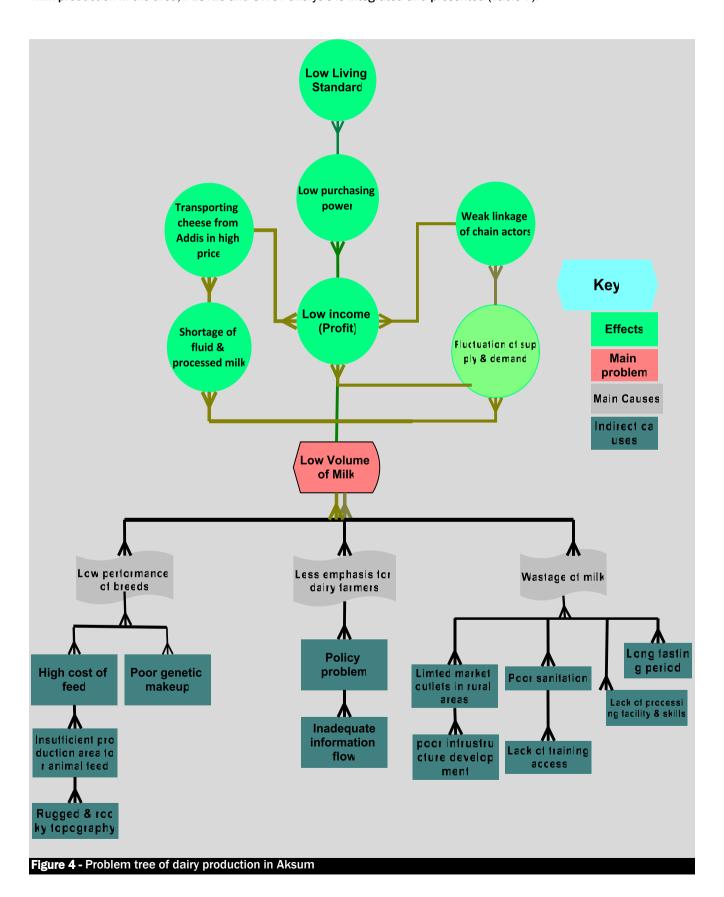
The significant constraints of dairyproduction in the Central zone one Tigray (Aksum) are high cost of inputs, low volume of milk, lack of training access and low household income/low purchasing power. Unavailability of cooling facilities for milk storing, long distance and rugged topography to reach the market, fluctuation of supply and demand, low breed performance and limited transport access were also reported by (Haregeweyni, 2015) as the major hindering factors for sustainable dairy production in Aksum district. Also, the high barging power of milk traders, weak relationship of the dairy cooperative with its members, extended fasting period of Ethiopia Orthodox Church are also identified as the significant factors affecting sustainable dairy production.

Moreover, the lack of milk processing facilities and skills, insufficient production area, poor sanitation, unpredictable marketing system, shortage of water are treating sustainability of the dairy sector negatively (Misganaw et al., 2017). There is also a problem of linkages among dairy value chain actors, and inadequate information on how to improve animal breeding, marketing, and processing aspects that critically affect dairy production in the area. To analyze these factors, Problem tree was used to identify the main problem, its causes and possible effects on milk production in Aksum district (Figure 4).

#### Opportunities for sustainable dairy production

Dairy production in Ethiopia is expected to increase rapidly in response to the fast-growing demand for milk and milk products resulting from growing human population and rising consumer income. Provided that, appropriate interventions are made along the dairy value chain and given the considerable potential for smallholder income and employment generation from high-value dairy products (Tegegne et al., 2013). Other opportunities for the development of sustainable dairy production in Aksum are the presence of suitable agro-ecology for undertaking of potential breeds, growing of different crops and forages. Presences of supporting institutions responsible for technology adoption of artificial

insemination, estrus synchronization and health services; the huge market potential of dairy products is also considered as an opportunity (Gebremedhin and Dawit, 2013). As Aksum is the center of tourists, the hotels and restaurants require a massive amount of fluid milk and processed dairy products to satisfy tourists demand (Getachew et al., 2016). As a result, different cheese and butter types are regularly transported from Addis Ababa to Aksum town (Misganaw et al., 2017). Also, the existence of many cultural and religious holidays that creates high consumption of animal products are also favoured for sustainable milk production. To analysis and summarize the different constraints and opportunities of milk production in the area, PESTEC and SWOT analysis is integrated and presented (Table 7).



PESTEC	SWOT					
1 LOILO	Strength	Weakness	Opportunity	Threat		
Political	- Presence of supporting institutions	- Insufficient production area - Lack of infrastructure - Inadequate data and flow of information	- Considerable employment generation	-Policy decision based on scant information -Transporting cheese from Addis Ababa		
Economical	<ul> <li>Fast-growing demand for dairy products</li> <li>Contribution to poverty reduction</li> <li>Cash income sources</li> </ul>	- High cost of inputs - Low household income - Low volume of milk	- Rising consumers income - High tourist flow to the area	- High inflation rate		
Social	High barging power trader	- Weak coordination of dairy cooperative     - the problem of linkages among chain actors     -lack of training access	- Contribution to nutrition and health	- Rapid increasing of human population		
Technological	- Provision of AI and Health services	Unavailability of cooling facilities     lack of processing equipment's	- Availability of breeding technologies			
Environmental	- Suitable agroecology for forage development	- Poor sanitation	Presence of suitable agro-ecology to bring potential breeds	- Rugged and rocky topography		
Cultural	- Celebration of holidays by consuming dairy products	Use of child labour     Religion prevents     consumption of Animal     products during fasting time	- Existence of many religion holidays	Long fasting period		

#### CONCLUSION

Milk production in Aksum is operated by a different segment of smallholder farmers, dairy cooperatives and few commercial private enterprises. The services and inputs provided by dairy sector supporters are overlapping one over the other. Involvement of chilled labor, principally utilizing of roughage feeds and unfair share of margins along the chain actors are main indicators against sustainable dairy production system. Many constraints that hinder the sustainability of milk dairy production in Aksum are identified, and organized with the opportunities and strengths in PESTEC-SWOT integrated matrix. As indicated from problem tree, the main problem for sustainable dairy production is low volume of milk due to poor breeds' performance and wastage of milk due to the lack of market outlets to the consumers and less government emphasis for smallholder dairy farmers. The owner of this problem is the government office that involved in breed improvement and infrastructure development. The effect of the problem is shortage of produces in the market, unpredictable marketing system and results to low incomes of farmers which enforces them to live in a low living standard.

#### Recommendations

Depending on the analysis of the report, the following recommendations are suggested for the leading supporters of dairy sector

#### For livestock office

The local and central livestock office should present most productive breeds that could be easily adapted and effective to the area. This could help to minimize carbon footprint of milk, increase income of producers and improves efficient utilization of resources.

Facilitate the possible ways realization of infrastructure development to link the rural producers with urban consumers through extending necessary infrastructure like accessible road.

Advising producers not to use child labor and children should be sent to school instead of giving task and duties in dairy farming that impedes their mental development.

Facilitate dissemination of market information to producers and providing trainings to improve their bargaining power and the share of margins.

#### For Aksum research center

Bringing and testing efficiency of modern breeding technologies (like, estrus synchronization, sex determination, multiple ovulation and embryo transfer) that speed up the improvement of genetic potentials of dairy farmers, and possible ways to change dairy wastes to worth should be considered

## For Aksum university

Providing training on proper husbandry practices, production of quality milk, feeding and health aspects of dairy cows.

Create awareness how to contribute and lead sustainable dairy production farming from the three pillars perspective

#### **DECLARATIONS**

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#### **Author's contribution**

Biruh T. and Demeke H. participated in the design of study and Girma G. critically revised the manuscript for important intellectual contents

#### **Competing interests**

The author declares that they have no competing interests.

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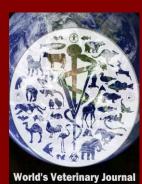
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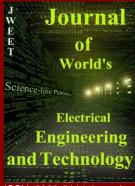
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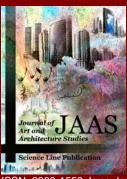
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