BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

DEPARTMENT OF ANIMAL SCIENCES

GRADUATE PROGRAM IN FEEDS AND ANIMAL NUTRITON

EFFECT OF NUTRITIONAL FLUSHING WITH ENERGY AND PROTEIN LEVELS ON WEIGHT GAIN, REPRODUCTIVE CHARACTERISTICS AND SEMEN QUALITY OF MENZ RAMS IN CENTRAL HIGHLANDS OF ETHIOPIA

M.Sc. Thesis

By

Tesfa Getachew Belayneh

December 2022

Bahir Dar, Ethiopia

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Tesfa Getachew Belayneh

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (M.Sc.) in Feeds and Animal Nutrition

Major Advisor: Bimrew Asmare (Ph.D.)

Co-advisor: Wamatu Jane (Ph.D.)

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Bahir Dar, Ethiopia

THESIS APPROVAL SHEET

As members of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by **Mr. Tesfa Getachew Belayneh** entitled '**Effect of Nutritional Flushing with Energy and Protein on Weight Gain, Reproductive Characteristics and Semen Quality of Menz Rams in Central Highlands of Ethiopia.** We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (M.Sc.) in **Feeds and Animal Nutrition**.

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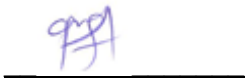
Shigdaf Mekuriaw (PhD)		**13 December 2022**
Name of External Examiner	Signature	Date
Fentahun Meheret (MSc, Assi. Prof.)		**13 December 2022**
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Name of Chair Person	Signature	Date

DECLARATION

This is to certify that this thesis entitled "Effect Of Flushing With Varying Energy and Protein Level on Reproductive Characteristics and Semen Quality of Menz Sheep Rams in Central Highlands of Ethiopia" submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in feeds and animal nutrition to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Tesfa Getachew (ID. No. BDU1300497) is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for an award of any degree or diploma to the best of our knowledge and belief.

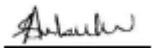
Name of the Student

Tesfa Getachew Belayneh Signature  Date 14-11-2022

Name of the Supervisors

1. Bimrew Asmare (Ph.D.)  14-11-2022

   Major Advisor Signature Date

2. Wamatu Jane (Ph.D.)  14-11-2022

   Co-advisor Signature Date

STATEMENT OF AUTHOR

I declare that this thesis is my original work and that all sources used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for an MSc degree at Bahir Dar University and is deposited in the libraries of Bahir Dar University, Debre Birhan Agricultural Research Center and ICARDA (International Center for Agricultural Research in the Dry Areas) in order to be made available to borrowers according to the rules of the library. I solemnly declare that this thesis has not been submitted to any other institution for award of any academic degree, diploma, and certificate. Short quotations from this thesis are permitted without special permission provided that source is acknowledgment. Requests for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the Head of the Department or the Dean of the School of Graduate Studies if, in their judgment, the proposed use of the material is in the interest of a scholarship. In all other cases, however, the author's permission must be obtained.

Name: Tesfa Getachew Signature ---  ---

Place: Bahir Dar University, Bahir Dar

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DEDICATION

I dedicate this thesis work to my beloved families, for nursing me with love, and for their dedicated partnership in the success of my life.

LIST OF ABBREVIATIONS/ACRONOMYS

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
ADL	Acid Detergent Lignin
AI	Artificial Insemination
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AV	Artificial Vagina
BW	Body Weight
CF	Crude Fiber
CP	Crude Protein
CPY	Crude Protein Yield
CSA	Central Statistical Agency
DM	Dry Matter
EE	Electro Ejaculation
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
ETB	Ethiopian Birr
FAO	Food and Agricultural Organization of the United Nations
FBC	Final Body Condition
FBW	Final Body Weight
FSC	Final Scrotum Circumference
FSH	Follicle Stimulating Hormone
GDP	Gross Domestic Product

LIST OF ABBREVIATIONS/ACRONOMY (Continued)

GLM	General Linear Model
GP	Gas Production
IBC	Initial Body Condition
IBM	Initial Body Weight
ICARDA	International Center of Agricultural Research in Dry Area
ILRI	International Livestock research institution
ISC	Initial Scrotum Circumference
IVOMD	Invitro Organic Matter Digestibility
LH	Luteinizing Hormone
ME	Metabolizable Energy
MJ	Mega Jule
NDF	Neutral Detergent Fiber
NIRS	Near-Infrared Reflectance Spectroscopy
OM	Organic- Matter
PA	Peasant association
PPRSE	Prediction Potential of Ram to Serve Ewe
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
SC	Scrotum Circumference
TDN	Total Digestible Nutrient

"Effect of Nutritional Flushing with Energy and Protein on Weight Gain, Reproductive Characteristics and Semen Quality of Menz Rams in Central Highlands of Ethiopia"

Tesfa Getachew[1], Bimrew Asmare[2], Wamatu Jane[3]

[1]Debre Birhan agricultural research center, Debre Birhan, P. O. Box 112**,** Ethiopia

[2] Bahir Dar University Department of animal sciences, Bahir Dar, P.O. Box 79, Ethiopia

[3]International Center for Agricultural Research in the Dry Areas (ICARDA) P.O. Box 5689, Addis Ababa, Ethiopia

ABSTRACT

Pre-breeding nutritional management of rams is critical to viable, sustainable, and profitable farms in the tropics. This study aimed to evaluate the effect of flushing with varying energy and protein levels on the reproductive characteristics and semen quality of Menz rams. Forty-nine Menz breeding rams, with an average age of 18.79±3.9 (Mean ± SD) months, were used. The rams were allowed to graze daily for 8 hrs on natural pasture and fallow lands. The study was conducted using a randomized complete block design. The treatments used were combinations of diets consisting of three energy levels above farmers' practice (High (50%), Medium (40%), and Low (30%)) 9.91, 8.14, 7.02 MJ ME/kg DM, respectively) and two protein levels (high and low; (12.5% and 8.17%), respectively). The Farmers' practice was grazing on natural pasture (energy and protein 4.7 MJ ME/kg DM and 6.65%, respectively) was used as a control. Semen was collected early in the morning (7:30 - 8:30 am) from each experimental ram every seven days by using an artificial vagina. Physical parameters such as semen volume, sperm concentration, sperm motility, color, viscosity, sperm morphology were determined in fresh semen. In addition to these body condition score, body weight, scrotal circumference, libido, and predictive potential of rams to serve ewe were collected during the experimental period. Body condition score, scrotal circumference, semen volume, spermatozoa concentration, and prediction potential of rams to serve ewe were greater ($P<0.05$) in the supplemented groups than in the control. There was no significant difference ($P>0.05$) in all variables within supplemented groups. Moreover, libido, the color of semen, semen viscosity, and sperm morphology were not significantly ($P > 0.05$) different between the control and supplemented groups. The body condition of rams was positively correlated with scrotum circumference ($r=0.57$), semen volume ($r=0.54$) and the potential of rams to serve ewe ($r=0.44$). The scrotal circumference was positively correlated with volume

(r=0.89), prediction potential of ram to serve ewe(r=04). Energy and protein supplementation above farmers' practice to Menz rams during the breeding season improve reproductive performance and semen qualities. Based on the cost of supplementary feed T6 and T3 were recommended to improve the reproductive performance, semen quality and potential of rams to serve ewes of breeding Menz rams. Since the study was conducted in one season (spring, harvest time) and the semen quality of rams is influenced by season, the similar feeding situation in different seasons of the year should be evaluated.

Keywords: *energy, flushing, rams, protein, semen quality, reproductive characteristics*

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CHAPTER ONE: INTRODUCTION

1.1. Background and Justification

Ethiopia has the largest livestock population in Africa; with 70 million heads of cattle, 42 million heads of sheep, 52 million heads of goats, 8 million heads of camels, 56 million chickens, 2 million heads of horses, and 10 million heads of donkey (CSA, 2021). Despite these enormous numbers, the contribution of livestock to the producers' income and national economy is very small. Due to its low productivity and production levels the supply of livestock products lags far behind the demands of the rapidly growing population (Asresie Alemu, 2015). The need to feed the growing global population requires more intensive and efficient agriculture and livestock production worldwide, especially in the tropical region (Renaudeau *et al*., 2012; Scholtz *et al*., 2013). Most sheep in Ethiopia are kept by smallholder farmers in the mid and highland areas (CSA, 2021). Although genetic merit, physical environment, diet and management affect sheep reproductive performance; diet is perhaps the most crucial in terms of its direct effects on reproductive performance. Pre-breeding management of rams is critical to viable, sustainable, and profitable farms, and proper nutritional management is critical for successful mating in flocks of sheep (Fernandez *et al*., 2004, Maquivr *et al*. 2021). Meeting nutritional requirement allows sheep to express their genetic potential, overcome the effects of the harsh physical environment and minimize the effects of poor management.

Menz sheep are one of the important sheep breeds identified in Ethiopia. It is a local breed reared in the North Shewa Zone of Amhara Regional State in Ethiopia. About 1.5 million, are indigenous to the highlands of Ethiopia and characterized as fat-tailed, medium-sized, predominantly black, brown, or white in plain and patchy coat color pattern, and are bred for meat and coarse wool (Solomon Gizaw *et al*. 2008a). Like most Ethiopian indigenous sheep breeds, the Menz breed has low production and reproductive performance; its birth, weaning, and yearling weights are 2.58, 8.99, and 19.94 kg respectively (Aschalew Abebe, 2020). Increasing current level of productivity is essential to provide meat for the ever-growing human population, and to increase export earnings and household income, thereby improving the living standards of smallholders (Solomon Gizaw 2002; Tibbo Markos 2006). Therefore,

the breed performance needs to be improved using different approaches such as community based breeding program (CBBP).

The Community-Based Sheep Breeding Program (CBBP) for the Menz sheep was initiated in 2009 to improve the productivity of the Menz sheep through within breed selection to improve smallholder farmers' income by selecting genetically superior breeding stock (Solomon Gizaw *et al.*, 2011; Mirkena *et al*., l 2012). Crop residues and post-harvest stubble grazing contribute up to 100% to the total dry matter intake of sheep (Alkhtib *et al*., 2017) especially in the dry season where natural pasture is poor in terms of productivity and quality. Crop residues are high in fibre and low in digestibility and nitrogen (Devendra and Thomas, 2002). Ethiopian sheep, fed primarily on crop residues and poor natural pasture do not receive maintenance requirements for protein and energy (Preston, 1995). Energy and protein supplementation has been found to improve ruminant utilization of poor-quality roughages (Lenné *et al*., 2003).

1.2. **Statement of the Problem**

To accelerate the CBBP for Menz sheep, the artificial insemination (AI) technique has been recently implemented by Debre Birhan agricultural research center. Artificial Insemination is among the tools in animal breeding programs to improve genetically and economically important traits in animals (Morrell, 2011; Tsuma *et al*., 2015, Durrant B.S., 2009). Artificial insemination, following oestrus synchronization, can be performed in Menz sheep and can be used as an alternative strategy to ram mating (Shanbel Besufkad *et al*., 2021) and could allow the rate of genetic improvement to be accelerated by increasing the number of offspring from a desirably fewer best ram. A $PGF_2\alpha$-analogue (PGF)-based protocol with 2 injections 11 days apart, preceded by a careful selection of non-pregnant ewes for cervical fixed-time AI, is a feasible reproductive management for sheep breeding programs in Ethiopia. Therefore, the production of quality semen plays a significant role in increasing the success rate of AI as well as the sustainability and profitability of the tool in sheep production.

Rege *et al*.(2000) reported that semen volume (ml) and motility (scale 1-5) of Menz breeding rams were 0.3 &1.06, 0.47&1.62 and 0.58 &2.65 on 6, 9 and 12-month of age respectively. This result indicates that the semen volume and other related parameters of the breed need

quality improvement through nutritional management. Flushing is a temporary but purposeful increase in the plane of nutrition around breeding season. Increasing the availability of energy and protein improves reproductive efficiency in rams and rams should be flushed (4-6 weeks) before and during mating (Allaoui *et al*., 2018). Therefore, the optimal dietary supplementation of energy and protein should be determined above farmers' practices that maximize the reproductive performance of rams. However, to date, the effect of flushing with different energy and protein levels on the reproductive performance of rams has not been well documented.

Therefore, this study was conducted with the following objectives:

1.3. Objectives

1.3.1. General objective

The general objective of this thesis was to evaluate the effect of flushing with different energy and protein levels on the reproductive performance of Menz rams in the central highlands of Ethiopia.

1.3.2. Specific objectives

- ✓ To evaluate the effect of flushing with different energy and protein levels on body weight and body condition of Menz rams in the central highlands of Ethiopia
- ✓ To evaluate the effect of flushing with different energy and protein levels on scrotal circumference, and libido characteristics of Menz rams in the central highlands of Ethiopia.
- ✓ To evaluate the effect of flushing with different energy and protein levels on semen quality of Menz rams in the central highlands of Ethiopia.
- ✓ To evaluate the effect of flushing with different energy and protein levels on the potential of Menz rams to serving ewes in the central highlands of Ethiopia.

1.4. Research Questions

The research questions to be addressed in this study were:

- Does flushing with varying energy and protein levels affect body weight and body condition of Menz rams in the central highlands of Ethiopia?
- Does flushing with varying energy and protein level affect semen quality of Menz rams?
- Does flushing with varying energy and protein level affect the potential of rams to serve ewes?

CHAPTER TWO: LITERATURE REVIEW

2.1. Over View of Sheep Production in Ethiopia

Ethiopia with the largest population of sheep and great variation in climate and topography represents a good reservoir of sheep genotype. The Ethiopian sheep population is estimated to be 42 million. About 25- 27% of Ethiopia's sheep are located in the lowlands which are arid and semi-arid areas and about 73 to 75% are located in the mid and highland areas (CSA, 2021).

Sheep production in Ethiopia is based on indigenous breeds except for less than 1% exotic sheep group of mainly Awassi-Menz and Dorper crossbreds. However, comparing the presence of a large sheep population similar to other tropical countries, present production levels are far below their potential and productivity per sheep is very low. This is mainly due to low genetic potential for functional traits as compared to improved tropical and temperate breeds (Tsegaye Tekleab *et al*., 2013). Increasing the current level of productivity is essential to provide meat to the ever-increasing human population, and to increase export earnings and household income thereby improving the living standard of smallholders (Solomon Gizaw 2002; Tibbo Markos 2006).

2.1.1. The sheep production system in Ethiopia

Mammo and Wude (2012) reviewed that the common feature of all production systems used in Ethiopia is mainly the extensive type, characterized by small flock sizes and the flock being periodically devastated by poor management. According to Solomon *et al*. (2008), sheep production systems are classified into five based on the degree of integration with crop production and contribution to livelihood, level of input and intensity of production, agro-ecology, length of the growing period, and relation to land and type of commodity to be produced.

Highland sheep-barley production system

This production system is found in the highlands above 3000 m.a.s.l. where the major crops grown are barley and pulses. Cropping intensity in these areas is generally low and sheep are

the dominant livestock species with sheep flock sizes ranging from 30 to several hundred heads. There is, therefore, a clear possibility of establishing more formal sheep production enterprises using appropriate technology packages in this production system.

Mixed crop-livestock production system

This production system is found in areas where the altitude ranges between 1500 and 3000 m.a.s.l. Crop and cattle production are the dominant agricultural practices and sheep and goats are kept meeting small and immediate cash needs. Sheep are more dominant than goats in this production system. Sheep in this system experience year-round nutritional stress due to increases in cultivated land area that results in very high grazing pressure, excessive soil erosion, and soil nutrient depletion. There is a need to intensify production in these areas because of the high population density.

Pastoral and agro-pastoral sheep production systems

These production systems are found at altitudes below 1500 m.a.s.l. mainly integrated with large livestock (camels, cattle, goats, and donkeys) population and with little or no crop agriculture due to low rainfall. Agro- pastoral sheep production systems are also found below 1500 m.a.s.l. in areas with higher rainfall to support short-season crops compared to the pastoral system.

Ranching sheep production systems

These can be undertaken in high land and arid/semi-arid either intensive or extensive, in which it is possible to produce sheep that are more uniform and targeted to satisfy the increasing export and domestic market. This production system could be specialized to produce weaned lambs for fattening or finishing by other production systems.

Urban and peri-urban sheep production system

This system can be an important entry point for poverty alleviation and employment for the household family members (FAO, 2009). Sheep keeping in urban areas has little difference in management practices and flock productivity from those in rural areas (Barbara *et al*., 2006). In line with (FAO 2007a) reported that crop production and livestock production in urban

production systems tend to be taken up by separate households, and mixed crop-livestock systems tend to be less common than in rural agriculture.

According to Solomon Gizaw *et al*. (2008a), urban and peri-urban sheep production systems involve the production of sheep within and at the periphery of cities including Addis Ababa. In most cases, the types of sheep available from this system are for local consumption, being well-finished and fatty animals demanded by the local Ethiopian market. Solomon Gizaw *et al*. (2008a) also reported that the general characteristic of small-scale urban sheep production is small-scale sheep fattening to generate cash income or household consumption.

2.1.2. Sheep Breeds in Ethiopia

Ethiopia is the home to the most populous and diversified indigenous sheep breeds. There are about 14 traditionally recognized sheep populations in Ethiopia, furthermore, the sheep populations are classified into 9 genetically distinct breeds and 6 breed groups (Gizaw 2008b). Table 1 shows the classification of the sheep population. They are found in different ecological zones of the country.

Table 1 Classification of Ethiopian sheep breeds

Breed group	Breed	Tail type/shape	Fiber type
Short fat-tailed	Semien	Fatty and short	Fleece
Short fat-tailed	Sekota, Farta, Tikur, Wollo, Menz		Fleece
Washera	Washera	Fatty and short	Hair
Thin tailed shape	Gumuz, Begait	Thin and long	Hair
Long fat-tailed	Horro	Fatty and long	Hair
Arsi	Arsi-bale, Adilo		Hair
Bonga	Bonga	Fatty and long	Hair
Fatty rumped shape	Afar	Fat rump/fat tail	Hair
Black –Head-Somali	Black –Head-Somali		Hair

Source: Solomon Gizaw *et al*. (2008b)

Menz sheep is a local breed reared in the North shewa zone of Amhara regional state of Ethiopia. Numbering about 1.5 million, are indigenous to the highlands of Ethiopia and characterized as fat-tailed, medium-sized (30-35 kg adult weight), predominantly black, brown, or white in plain and patchy coat color pattern, and are raised for meat and coarse wool (Solomon Gizaw *et al*. 2008a). Menz sheep is a coarse-wool type of sheep and inhabits mainly the cool highlands in central Ethiopia within an altitude range of 2 500 and 3 000 m. They are among the few coarse woolen and fat-tailed sheep breeds in Ethiopia (Tibbo Markos *et al*., 2006).

2.2. Reproductive performance of rams

The reproductive performance of the animals is one of the most important traits, due to its impact on the overall profitability of the flock. Fertility is a particularly crucial trait; it affects the production and economic efficiency of ovine industries. Previous reports suggest that only 70–75% of the males in a flock are reproductively optimal at the beginning of the breeding season (Van Metre D.C *et al*., 2012). Therefore, the management of males that are going to be used during the breeding season presents several challenges, not only in terms of the selection and acquisition of animals, but for the general management of males in the pre-breeding, mating and post-mating seasons (Beltman M. 2013). Management of the males is necessary to ensure the success of the flock productively and economically and to maximize the longevity of the animals.

2.3. Factors Affecting the Reproductive Performance Spermatogenesis, Semen Quantity, and Quality of rams

2.3.1. Age and frequency of semen collection

The residual standard deviation in volume, mass sperm motility, and semen concentration tended to increase with age in rams, but to a lesser extent than the means. Semen quality traits (dead and abnormal sperm) showed an increasing tendency with age (Rege *et al*., 2000). Cloete *et al*. (2000) concluded that it is clear that Dorper ram lambs can fertilize ewes at an early age. Benia A R *et al*. (2018) reported that the volume of ejaculate was not by age. The mass motility showed significant variation between age groups and higher motility was observed in adults than in young rams. The sperm concentration varied significantly with the

age category and higher motility was observed in adults than in young rams. But the result shows that the number of straws per ram is not significantly influenced by age. The scrotal circumference is directly proportional to the body weight, and age of the animal, so mature Awassi rams are heavier and have a greater scrotal circumference than their younger counterparts (Salhab *et al*., 2003; Tabbaa *et al*., 2006). The sperm motility decreases by 19-36% with successive and frequent ejaculation (Kaya *et al.*, 2002).

In the thought of Gundogan (2007), the frequency of ejaculation had a considerable effect on the biological parameters of semen quality (volume, concentration, motility, normal and abnormal sperm). The interval between collected ejaculations was another factor (pruned *et al*., 2005). Stanimir Yotov *et al*. (2011) stated that sperm volume and concentration in semen samples decreased gradually with an increase in ejaculation frequency. They also concluded that the frequency of ejaculation and the period of semen collection had an impact on sperm motility in extended and short-time stored semen from Pleven blackhead rams. In contrast, Nel-Themaat *et al*. (2006) observed higher motility in the second ejaculate compared to the first, collected at a 10-min interval in Gulf Coast Native rams. Kistanova *et al*. (2007) reported a negative correlation between sperm volume and sperm concentration and a trend toward increased motility in three ejaculates consecutively obtained at a 30-min interval from Il-de-France rams.

2.3.2. Season

Benia *et al*. (2018) reported that the volume of ejaculate was highly affected by season. It was more important in spring and autumn than in winter and summer. The mass motility, sperm concentration, and the number of straws per ram were not significantly affected by seasonal factors. Hafez and Hafez (2000) indicated that the ram does not show a restricted mating season, but the sexual activity is highest during the autumn and declines in late winter, spring, and summer. Rams show seasonal fluctuations in semen qualities and scrotal circumference. The magnitude of these seasonal effects is not so marked as to prevent rams from being used for breeding purposes throughout the year. Semen of superior quality and quantity has however been collected in late summer and throughout autumn (Kafi *et al*., 2004).

Shenkutie Goshime *et al*. (2018) study result indicates that the color of the semen of the Menz rams differed across seasons with the semen of lighter color recorded during the long and short rain seasons. Abnormal spermatozoa of Menz rams were higher during the long rainy season, while no differences across seasons were observed in volume and motility of semen in Menz, Dorper, and Awassi breeds. Seasonal variations contribute to differences in semen volume, sperm motility, scrotal circumference, and testosterone level. However, the semen of Suffolk and Lincoln rams and those raised in Hungary are suitable for AI throughout the year (Oláh *et al*., 2013; Benmoula *et al*., 2017). Oláh *et al*. (2013) conducted a study on seven breeds of sheep, including the Ile de France ram and Barbados Blackbelly ram. Other studies reported a higher scrotal circumference in autumn for bucks (a breed of goats) (Al-Ghalban *et al*., 2004; Kridli *et al*., 2007) and Karakul rams (Kafi *et al*., 2004). Kafi *et al*. reported that the rams produced superior quality semen throughout late summer and autumn, and therefore could be utilized for AI.

Belkadi S. *et al*. (2017) reported that volume, mass motility, live sperm, and scrotal circumference were significantly influenced by seasonal factors, and higher during spring. Whereas the sperm concentration was higher during autumn compared to spring. They also reported that season influenced significantly the percentage of abnormal sperm, especially during winter but had no influence on the weight of rams. The seasonal hormonal activity was high with 4.89±2.06 ng/mL and 3.09±1.35 ng/mL of testosterone in mating seasons (spring and autumn, respectively), knowing that the sexual season is not marked too much in these latitudes.

an average volume range of 0.5 to 2 ml of ram semen, and a higher mean semen volume was recorded among Persian Karakul rams in the autumn, which correlated with the maximum sexual activity of the animals. This shows that semen volume was found to be influenced by the season of the year, achieving a lower mean value in summer in both Najdi and Naimi rams compared with spring, autumn, and winter. It remained significantly high in spring (Menchaca *et al*., 2005 and Safdarian et al., 2006). This is contrary to the study on Chios and Friesian rams in Greece, in which it was observed that semen volume improved in autumn and summer, and decreased in spring (Karagiannidis *et al*., 2000).

Al-Anazi *et al.* (2017) reported that significant differences were observed in the scrotal circumference among various seasons. The highest production of semen was recorded mainly in spring, whereas the lowest was in summer. The pH of the semen was slightly alkaline and significantly lower in autumn than in spring. Furthermore, the highest value of the total number of sperm per ejaculate was observed in spring for both breeds. The results indicated that mass motility increased significantly in autumn compared with winter, spring, and summer. Progressive motility was significantly lower during the months of summer and spring. However, no significant differences were observed between autumn and winter. Hence, the presence of significant seasonal variations in semen quantity and quality of Naimi and Najdi rams suggests the viability of increased utilization of rams in spring and autumn for semen collection and reproductive practices.

Moreover, monthly variations were detected in reproductive and semen characteristics, such as sperm concentration and motility, ejaculate volume, relative testes volume, and serum testosterone levels, which were found to be higher in autumn (Gundogan & Demirci, 2003).

2.3.3. Nutrition

The onset of puberty, expression of libido, testicular function, and endocrinology are all affected by the energy and protein content of the diet. The normal developments of ram lambs rely on an adequate plane of nutrition. The rate of sexual development is highly dependent on the growth rate of the animal. Depending on the breed and sensitivity to photoperiodic stimulation, the body weight at puberty may vary from 40 to 70% of adult body weight. Dietary changes have a minimal effect on the reproductive organs of the adult male than on the growing immature animal. However, Rae *et al*. (2002) concluded that pre-natal under-nutrition in sheep has an effect on male reproductive development and adult function, but it resulted in a reduced ovulation rate in the female progeny. Severe feed restrictions may even result in permanent damage to gonadal and neural tissue.

Spermatogenesis and libido in males may be impaired by carbohydrate, protein, and metabolism of nucleic acid and their deficiency (Mitchell *et al*., 2003, Alejandro *et al*., 2002). A reduction of androgen secretion and semen quality can be caused by a restriction of feed intake. Such effects are temporary, as re-feeding previously underfed adult animals usually

restores the reproductive function. Certain components of the diet can be affected differently by the production and/or release of luteinizing hormone (LH) and Follicle-stimulating hormone (FSH), as some nutritional regimes imposed on animals can alter the volume of ejaculates and androgen activity without necessarily affecting spermatogenesis. Hafez and Hafez (2000) suggested the effects of nutritional restrictions on fertility be more notable in the female than in the male animal. Barth *et al.* (2008) found that medium or high level of nutrition does not influence the overall percentage of morphologically normal spermatozoa. If rams are intensively fed, their testicular measurement may seem acceptable, but the ram's reproductive potential may be hampered by excessive scrotal fat deposition, particularly in the neck of the scrotum (Fourie *et al*., 2003).

2.3.3.2. The energy content of the diet

Shadnoush *et al*. (2003) found that feeding ram lambs 10 % less energy than the recommended level resulted no significant difference in carcass weight, eye muscle area, wholesale cuts, and chemical composition, compared to lambs that received a standard level of energy. Schwalbach *et al*. (2006) also reported that rams fed high energy (9.39 MJ ME/kg DM) and low energy (6.52 MJ ME/kg DM) diet recorded were not significant differences for the qualitative semen parameters (Overall motility, Forward progression, Sperm concentration, and Live sperm) considered (both fresh and post-thawed semen). However, Nel *et al*. (2004) reported dietary energy level to have a significant effect on the growth performance and carcass characteristics of Dorper sheep. Therefore, the selection of breeding rams based only on scrotal and testicular measurements is not sufficient. If rams are intensively fed, their testicular measurement may seem acceptable, but the ram's reproductive potential may be hampered by excessive scrotal fat deposition, particularly in the neck of the scrotum (Fourie *et al*., 2003). Obesity and overfeeding reduce libido and sexual activity in rams, particularly during warm weather.

2.3.3.1. The protein content of the diet

Hafez and Hafez (2000) concluded that diets high in protein were not essential for optimal sperm production in the ram. Values of live weight, testicular diameter, testicular circumference, testicular length, and testicular volume of ram lambs supplemented with an 18% CP diet were higher than the 12% CP supplemented group Özkan Elmaz *et al*. (2007).

However higher motility, density, and semen volume value have been obtained from the group low protein diet (12%cp) compared to the high protein diet (18%) during the entire research period. It is assumed that the results that have been obtained are because a high protein diet results in the excess fat being stored in the scrotum, thus the thermoregulation mechanism in the testis and the spermatogenesis collapse. Özkan Elmaz *et al*. (2007) reported that feeding with a high protein diet harmed semen characteristics by impairing the thermoregulation mechanism and spermatogenesis in testicles because of excessive fat accumulation in the scrotum. Lindsay *et al*. (1984) demonstrated that the feeding of high levels of protein increase testicular weight, diameter, and volume, whereas low levels of protein had little or no effect on these testicular parameters.

Fernandez *et al*. (2004) have reported that the testosterone hormone concentration had fluctuations for the Assaf race rams which were fed with low, medium, and high protein diets. The result shows that diets with equal energy levels but different protein levels have different effects on the live weight, testicular parameters, testosterone hormone concentration, and sperm parameters of the ram lambs. It has been determined that the live weight and testicular parameters of the ram lambs that were fed with high protein diets during the pubertal period have been affected positively.

2.3.3.2. Vitamin and mineral content of the diet

Dietary vitamin A or B-carotene deficiency leads to testicular degeneration in all farm animals as the release of pituitary gonadotropins is suppressed by a vitamin A deficiency (Hafez & Hafez, 2000). In addition, testicular degeneration is responsible for reduced production and inferior quality sperm. However, in ram vitamin A deficiency may retard the development of the testes and impair the production of spermatozoa. Vitamin A deficiency induces lesions in the testes that are reversible. Injections of gonado-tropic hormones or vitamin A will restore spermatogenesis, except if there is permanent damage to the testis. Vitamin E is also important for normal reproduction, but its role in the fertility of male farm animals is poorly studied (Hafez & Hafez, 2000). Yue D *et al*., (2010) result demonstrated that supplementing Vitamin E can have a positive role in improving semen quality via protecting testicular cell membrane and mitochondria from antioxidant abilities.

Hafez and Hafez (2000) observed an improvement in sperm production and fertility following supplementary feeding of Cu, Co, Zn, and Mn. The concentration of Cu in the semen is known to be rather high. In the spermatozoa, the Cu is localized mainly in the tail and the middle piece, but its biochemical importance remains to be clarified.

Kendall et al. (2000) investigated the effect of Zn, Co, and Se soluble glass bolus, on the trace element status and semen quality of ram lambs grazing on pastures that were not considered to be deficient in any of either element. A significant increase in erythrocyte glutathione peroxidase activity in seminal fluid and sperm motility, the proportion of live sperm, and the proportion of intact sperm membranes were found. Plant oestrogens exert adverse effects on the male accessory sex organs. Many chemicals, rare earth salts, and ionizing radiations interfere with spermatogenesis in a variety of mammalian species, but their contributions to male infertility remain to be clarified. Selenium (Se) is a trace mineral element with antioxidant activity that together with vitamin E performs essential functions to prevent cellular damage (Mahmoud *et al*., 2013). In rams, Se and vitamin E improved semen quality, associated with a higher testosterone concentration and increased activity of the GSH-Px enzyme in blood serum (Mahmoud *et al*., 2013). During the breeding season, rise of sexual activity in rams increased their nutritional requirements for semen production in a short period (breeding season), which can induce Se deficiency and cause greater oxidative stress, with lower semen production and quality (Ahsan *et al*., 2014; Zubair *et al*., 2015). Soluble bolus with sodium selenite increase Se concentration in serum and GSH-Px enzyme activity in a sustained manner; in addition, they improve the mobility and viability of the ejaculate in Hampshire and Suffolk rams (Carrillo-Nieto *et al*., 2018).

2.3.4. Genotype

The reproductive tracts of the rams as that of other mammals comprise several anatomical structures which are the penis, epididymis, testis, vas difference, and other accessory glands. Differences in fertility have been reported between rams within a breed and among rams of the same ages and across breeds. The semen quality parameters, color, volume of ejaculation, the concentration of spermatozoa, sperm motility, sperm viability, and spermatozoa morphology varied across the breed of Menz, Awassi X Menz, and Dorper rams (Shenkutie *et al*., 2018). The differences in semen quantity and quality have also been reported to vary

across breeds managed under similar conditions (Salhab *et al*., 2003; Al-Samarrae, 2009; Martinet *et al*., 2013; Casaco, 2010; Babiker, 2010). In line with the above fact, the semen characteristics of Karradi rams are superior to their Arabi counterparts in terms of volume, mass motility, individual motility, concentration, and viability of semen (Al-Samarrae, 2009).

It has also been reported that differences in the semen quantity, quality, and scrotal circumference vary across breeds and also among rams of the same age within breeds (Mohammed *et al*., 2006). According to Al-Samarrae, (2015) seminal characteristics of Karrabi rams were superior when compared to Arabi rams in the aspect of ejaculation volume, mass motility, individual motility, sperm concentration, and viability. Another report showed that differences were reported in quality and quantity of semen; Canadian rams had higher seminal volume semen per ejaculation when compared to those of the Finnish Landrace (Babiker, 2010). It has also been reported in a study by Kridli (2006) that semen motility was lower and abnormal spermatozoa was higher among the Awassi rams when compared to the Romanov x Awassi and Charolaise x Awassi crossbreds. Findings by Milosevic & Stojkovic (2012); Rodríguez-Martínez, (2013) indicated that testicular size among the rams corresponds with the ovulation of the ewes within a particular breed.

2.3.5. Semen collection techniques

Semen quality and quantity evaluation is a means to evaluate the process of spermatogenesis, and the potential fertility of the male requires the collection of a semen sample. The semen collection method or technique used may have a major effect on the quality of the sample (VandeVoort, 2004). For this reason, a short description of the most important semen collection methods currently used in rams is evaluated (Matthews *et al*., 2003).

The artificial vagina semen collection method

A basic model Salisbury *et al*. (1978) found the artificial vagina (AV) to eliminate several disadvantages above the collection of semen from the natural vagina. The artificial vagina is easy to use, the semen collected is fairly clean and the ejaculate is similar to the natural sample. The AV consists of a rigid cylinder of rubber, PVC, or other material and a thin-

walled rubber tube. A water-tight jacket is formed inside the cylinder by turning back both ends of the thin-walled rubber tube over the outer cylinder on both sides.

The jacket is filled with water hot enough (45-55 ºC) to bring the inside temperature of the artificial vagina to a few degrees above the normal body temperature through a screw-plug hole. According to Donovan *et al*. (2001), warm water simulates the thermal and mechanical stimulation of the vagina over the glans penis. Into one end of the artificial vagina, a graduated, glass semen collection tube of a slightly smaller diameter than the cylinder is fitted. A female of the same species (preferable in oestrus) is placed in a neck clamp and the male is allowed to mount. When the male mounts, the penis has manually deviated from the natural vagina into the AV, where the male ejaculates naturally. The major disadvantage of this method is that the rams have to be trained in advance for this method of semen collection (Matthews *et al*., 2003).

Matthews *et al*, 2003 study results showed that the artificial vagina collection method produces better semen samples with a higher concentration and percentage of live sperm than electro-ejaculation. No differences in sperm morphology were found between the two semen collection methods.

The electro-ejaculation method of semen collection

Gunn (1936), in Australia, was the first to use the electro-ejaculation (EE) semen collection method. The method consisted of stimulating the spinal cord between the 4th lumbar and the first sacral vertebrae by placing one electrode in the rectum and the other in the back muscle. Bypassing a few 5 to 10-second rhythmic electric stimuli through the electrodes, an ejaculation was produced, and the semen was collected in a glass tube. The animals experienced no harmful effects, no loss of condition, no change in disposition, and no special disinclination to further application of the treatment. However, during the application of this method, the electric current produced general tetanic contractions of all body muscles, and a slight and temporary motor inability of the hindquarters and hind limbs, at the end of the treatment.

According to Matthews *et al*. (2003), semen collected with the aid of an AV produced a higher sperm concentration, but the similar volume and morphological results, when compared to that collected by an EE. Carter *et al*. (1990) also compared the EE with the AV method for semen collection in rams. The repeatability of the volume of the ejaculate obtained, sperm concentration, total sperm number, percentage of normal sperm, and wave motion were slightly higher when using the artificial vagina technique. The major advantage of using EE is that no training is required by the ram using this method of semen collection.

2.4. Semen Evaluation

Sperm cells are unique among cells in their uniformity and function. Mature sperm are terminal cells, the end products of complex developmental processes that cannot undergo further division or differentiation. Examination of semen is the standard method of evaluating the potential fertility of breeding males, other than directly evaluating their ability to produce a pregnancy (Hafez & Hafez, 2000). The qualitative characteristics of the semen include the motility and the morphology of the spermatozoa. These characteristics, as well as the color and smell of the semen sample, should be evaluated as soon as possible after collection. No single characteristic can accurately predict the fertility of a semen sample; however, examining various physical characteristics of sperm can determine the potential fertility.

High-quality ram semen is grouped as semen with motility of higher than 85 % and with less than 10% abnormal sperm (Hafez & Hafez, 2000; Gil *et al*. 2003). Gil *et al*. (2003) also classified sperm motility higher than 70% to be normal. Fertilizing ability, however, does not only rely on these two parameters alone. The total number of live sperm per insemination is more important than the percentage of abnormal sperm. The limiting factor in semen fertility is the inability of a single sperm to penetrate the zona pellucida of the ova (Hafez & Hafez 2000). The normal concentration of an ejaculate varies from 3.5 - 6 X 109 sperm/ml in the ram (Hafez & Hafez, 2000). Gil *et al*. (2003) however, considered a concentration of 2.5 x 10 9 sperm/ml to be normal and acceptable.

2.4.1. **Semen's appearance and volume**

According to the study of Benia A R *et al*. (2018), the sperm collected had a vicious and whitish appearance and this can be affected by age and seasonal factors. Ram semen varies from milky-white to pale creamy color (Bag *et al*., 2002). Hafez and Hafez (2000) report the correlation between color and concentration of the semen ejaculate. The presence of blood in the semen is indicated by a pink color of the semen (contamination) and can be due to injury or disease of the penis or reproductive tract. A grey or brown semen color indicates contamination or infection of the ram's reproductive tract. Urine can be present when an electro-ejaculator is used and this is indicated by a yellowish discoloration of the semen, often diluted and with a strong odor. Contaminated semen samples should be discarded. Semen volume varies according to the method of collection. Larger volumes usually result from EE, compared to the AV method of semen collection. However, Matthews *et al*. (2003) reported no significant differences between semen collected from Dorper rams by AV or EE in terms of volume. A significantly higher concentration and better percentage of live sperm cells were recorded using the AV.

Hafez and Hafez (2000) indicated that ram age and condition, season, the skill of the collector, and frequency of collection affect ejaculate volume. False mounts may increase ejaculate volume when an AV is used for semen collection. The ejaculate volume ranges between 0.5 and 2 ml in mature rams, and 0.5 and 0.7 ml in young rams. The ejaculate volume will decrease if a ram is collected three or more times per day or for lengthy periods. Gil *et al*. (2003) report shows that using the AV to collect semen from rams, regarded a volume of between 0.75 and 2ml to be normal.

2.4.2. Concentration of spermatozoa

Hafez and Hafez (2000) reported that the accurate determination of the concentration and volume of an ejaculate determines the number of insemination doses and consequently the number of females that can be inseminated with an ejaculate. Sperm concentration in the ejaculate is recorded by using a haemacytometer, a colorimeter, or a spectrophotometer. The haemacytometer is a microscope slide with precisely scored chambers (volume). A semen sample of the ejaculate is diluted in a fixed ratio with water to kill the sperm cells and thus render them immobile. The number of sperm cells lying in a chamber is counted under the microscope and multiplied by the dilution factor used (Loskutoff & Crichton, 2001). This

technique is very accurate but time-consuming. The spectrophotometric or colorimetric methods can be used instead. The advantage of these methods is that they are accurate and fast to implement. A spectrophotometer, calibrated at 550nm, is preferred for determining sperm concentrations. Photometers are not accurate with contaminated semen samples, and the addition of cloudy extenders before estimation of concentration can also influence the results (Hafez & Hafez, 2000).

2.4.3. Sperm motility

The semen sample can also be evaluated according to the wave motion and thus referred to as mass motility. The normal semen of rams exhibits a wave-like motion when examined for motility under a microscope. Experienced inseminators can observe wave motion in the collection glass with a naked eye, but accurate assessment requires the use of a microscope. An estimate of the motility of sperm is made based on the vigor of the wave motion or the overall sperm activity if wave motion is not present. This is assessed on a 0 – 5 scoring system, where 5 is a very good wave motion and 0 is when the sperm is motionless. Hafez and Hafez (2000) reported sperm motility assessment to involve the subjective estimation of the viability of the sperm and the quality of motility.

The light microscope at low magnification (x200 to 400 magnifications) is most commonly used to analyze sperm motility. Fresh, raw, and extended semen can be used to evaluate sperm motility. High sperm concentrations in a fresh semen sample can hamper the assessment of motility, making it difficult to discern individual motility patterns. This limitation can be overcome by using an aliquot of diluted semen (concentration x106 sperm/ml) in a good-quality semen extender. Sperm performance in its accessory gland fluid (seminal plasma) can be evaluated if raw sample semen is used. Sperm motility is extremely susceptible to environmental changes (such as excessive hot ambient temperatures) thus it is necessary to protect the semen from harmful agents or conditions before evaluation. An experienced person and a properly equipped microscope are necessary for a reliable estimation of semen motility. A drop of extended semen is placed on a glass slide and a glass cover slide is placed over the drop and then observed using a microscope with a built-in warmer stage and phase-contrast optics. Parameters of sperm motility include the following (Hafez & Hafez, 2002): Percentage of sperm motile (normal is 70 to 90% motile sperm)

Percentage of sperm, progressively motile (normal is 70 to 90% motile sperm) Sperm velocity (based on a subjective scale of 0 to 4) Longevity of sperm motility in fresh semen sample (at room temperature of 20 to 25 ºC), and in extended semen (at room temperature, or refrigerated temperature – 4 to 6 ºC).

Sperm motility in diluted semen samples is a long semi-arc pattern. The degree of motion is used to score a sample. Sperm motility can be influenced by many factors. In ram semen, initial motility of 90 % is acceptable for further processing and freezing (Bag *et al*., 2002). Loskutoff and Crichton (2001) recommended the evaluation of motility in 200 individual sperm and the use of a calculated mean, using the following criteria: 0 = no movement, 1 = head movement only (no forward sperm progression), 2 = slow forward sperm progression (usually with labored head movement), 3 = fast forward sperm progression, 4 = faster forward sperm progression, and 5 = fastest, linear forward progression. The semen extender used may slightly alter the motility, usually by increasing velocity measures.

2.4.4. Sperm morphology

Sperm evaluation should be made as soon as possible after the ejaculates have been collected from a male. Care must be taken to prevent cold shock during and after collection until the cells are fixed on the microscope slide. The cold shock will coil the sperm tails and make it impossible to distinguish artifacts due to handling from abnormal cells produced by the testes. Freshly ejaculated sperm cells are readily broken at the neck and can produce a high proportion of tailless sperm heads. Hafez and Hafez (2000) stated abnormal sperm cells are present in every semen sample. Morphologic abnormalities of sperm have the greatest relationship to fertility in livestock, with heat stress causing high percentages of damage to sperm. Periods of high ambient temperature together with high humidity may render a male infertile for up to 6 weeks, and many abnormal sperm cells may appear in ejaculates collected during the recovery period. Providing adequate shade and clean, cool water can minimize the effect of heat stress. The ram's fertility is questionable when 20 % or more cells are abnormal in a semen sample. Semen with more than 15% abnormal sperm should not be used for artificial insemination (AI).

Gil *et al*. (2003) regarded semen with less than 10% abnormalities as normal in sheep. Seasonal variations influence the percentage of abnormal sperm, with the number being the highest in spring, declining as the breeding season advances. An eosin-nigrosin stain can be used to evaluate sperm morphology. Stained thin semen smear slides are examined with the help of high microscopic magnification (x1000). At least 150 spermatozoa must be examined. Abnormal sperm are classified into the following 5 categories (Hafez & Hafez, 2000) Loose sperm heads abnormal sperm heads/abnormal sperm tail formations, abnormal sperm formation with a proximal cytoplasmic droplet, and abnormal sperm tail formation with a distal cytoplasmic droplet.

2.5. Effect of Nutritional Flushing on Reproductive Characteristics and Semen Quality of Rams

Flushing is increased energy and protein availability, in the ration, before breeding has a determining effect on the fertility of rams, by conditioning their quality of semen, sexual behavior, and body condition at mating (Martin *et al*., 2010).

Scrotal measurements, semen volume, total sperm production, and percentage of sperm were showed an increase during the flushing period and confirmed that a strong relationship between nutrition and reproductive variables of ram (Allaoui *et al*., 2018). Ghorbankhani *et al*. (2014) reported that semen quality parameters (except percentage of abnormal sperm and semen pH), serum testosterone concentration and testicular circumference were positively influenced by nutritional state. Nutritional flushing of rams with higher concentrate supplementation resulted in improved body weight gain, feed intake, sperm production, and semen quality in Sardinian rams (Tufarelli *et al*., 2011). Feeding rams at maintenance requirements negatively affected sexual behavior by impairing anogenital sniffing, flehmen, penis erection, lateral approaches, reaction time and libido score (Lassoued *et al*., 2013).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Description of the Study Area

The experiment was conducted on-farm in Menz Mama District (Figure 1), selected purposively, where the Menz sheep Community-Based Breeding Program (CBBP) is implemented. Menz Mama District is one of the North Shewa Districts located in north of Debre Birhan, the capital city of the North Shewa Zone. It is 256 km, 640 km, and 124 km away from Addis Ababa, Bahir Dar, and Debre Birhan respectively and also has a human population of 106, 805. The district covers 67,072 ha of land and lies at an altitude of 1590-3413 meters above sea level. It receives an average annual rainfall of 896 mm with minimum and maximum temperatures of 15 to 20 ^{o}C, respectively. The rainfall and temperature of the study area for the year 2021 are presented in table 2.

The area is characterized by a crop-livestock mixed farming system, and sheep are the dominant livestock in the area. The livestock population of the district is 91803 heads of cattle, 132264 heads of sheep, 37235 heads of goats, 112340 heads of chicken, 19703 heads of donkey, 2404 heads of mule, and 1605 heads of horse (Abiro Tigabie *et al*., 2021). Sheep farming in the Menz area is for income generation, followed by meat, manure, coarse wool, and as means of saving, in that order (Getachew Tesfaye, 2008). The main feed resources available in the area include crop residue, hay collected mainly from natural pasture and sometimes from fallow lands, grazing of natural pasture, Cut and carry of crop weeds, improved forages (Oats, Vetch, and tree lucerne), crop aftermath locally called ‘qarmiya’, Industrial by-products such as ‘fagulo’ (oilseed cake) and ‘frushka’ (wheat bran) and local beverage residue ‘attela’ (Abiro Tigabie *et al*., 2021).

Table 2 Meteorological data of the study area

	2021											
Factors	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Max (T)	19.7	19.8	20	19.8	20.9	19.4	17.1	16.7	17.8	17.5	18.5	19.2
Min(T)	6	6.4	7.3	7.9	8.9	8	8.7	8.9	7.9	6	5.3	4.5
Rainfall(mm)	8	38.1	0	111.3	66.3	77.8	396.3	305.8	87.6	0.7	1	0

T= Temperature
Source: National meteorology agency (2021) Addis Ababa, Ethiopia

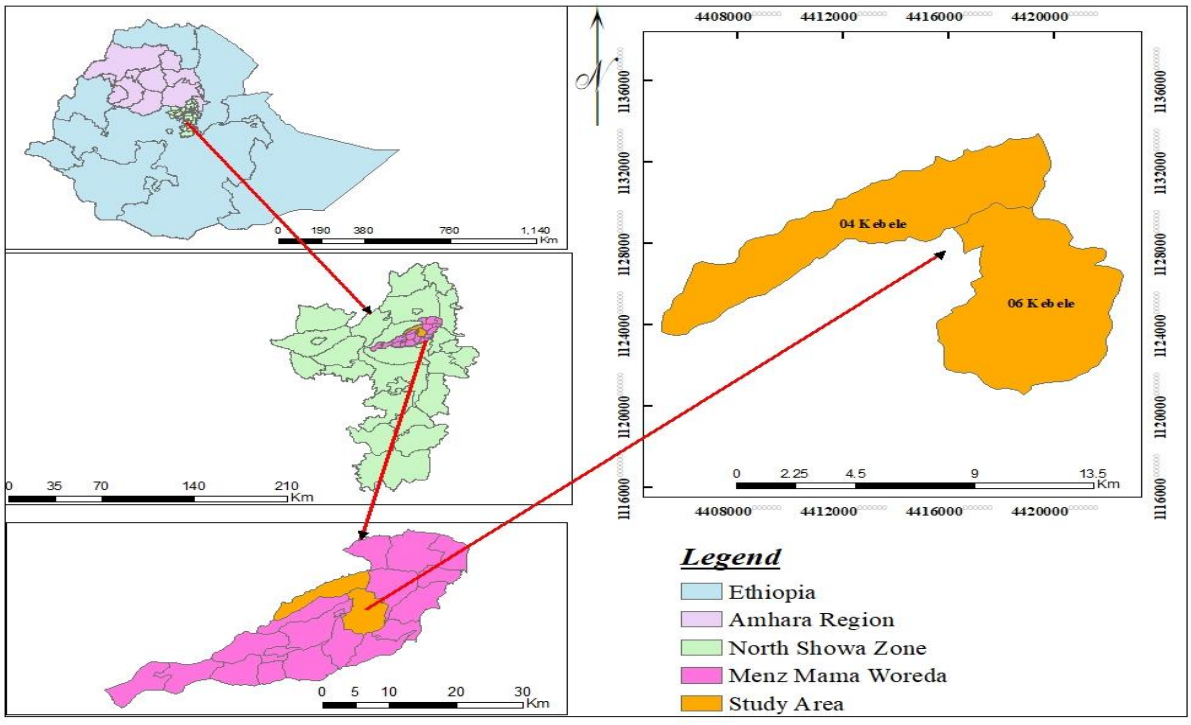

Figure 1 Map of the study area

3.1. Experimental Animals and their Management

Forty-nine Menz breeding rams, with an average age of 18.79±3.9 (Mean + SD) months, were selected from volunteer farmers and used for the experiment. Rams were selected based on different criteria such as, health, body conformation, generally rams selected by the community and breeders for breeding purpose. The rams were periodically treated against internal and external parasites using anti-helminthes Albendazole (7.5 mg/kg weight, orally ingested) and Ivermectin (0.2 mg/kg weight, administered through subcutaneous injection), respectively (DACA, 2013). The feeding and other management activities were monitored daily with the help of enumerators. Supplementary feed was measured in an individual plastic bag and distributed to farmers during the data collection day for seven 7 days of consumption and continued until the end of the experiment. Mating of rams was controlled by using the tying apron technique. The rams were allowed to graze for 8 hrs per day on natural pasture and fallow land and the supplement were divided into two halves and supplemented according to the treatments at 10:00 am and 4:00 pm. The experiment was carried out from September 7th, 2021, to October 5th, 2021, after a 15-day adaptation period for the feed.

3.2. Experimental Design and Treatments Layout

The experiment was conducted using a randomized complete block design (RCBD) with seven replications and six treatments (T1-T6). Farmers' practice (C) was the control. Experimental rams were blocked based on age and body weight, and rams from each block were randomly assigned to one of the treatments. All rams were grazed for eight hours and supplemented with the respective treatments. The treatments and experimental diets are presented in Table 3. Treatments with CP above 12 and below 8.5 were considered high and low protein, respectively (Özkan Elmaz *et al*. (2007).

Table 3 Experimental treatment arrangement

Treatments	Oat grain (g)	Wheat bran (g)	Maize bran (g)	Broken lentil grain (g)	Natural pasture	Level of energy (MJ ME/kg DM)	Crude protein (%)
T0/FP/C					Grazing 8hr	4.7	6.65
T1	500	0	0	0	Grazing 8hr	9.93 (50%)	8.4 (L)
T2	0	0	500	0	Grazing 8hr	7.01 (30%)	8.01 (L)
T3	0	500	0	0	Grazing 8hr	9.89 (50%)	12.77 (H)
T4	300	0	0	150	Grazing 8hr	7.03 (30%)	12.1 (H)
T5	0	0	300	150	Grazing 8hr	8.08 (40%)	8.1 (L)
T6	0	400	0	0	Grazing 8hr	8.2 (40%)	12.2 (H)

FP/C = Farmer practice/ Control, T = Treatments, H= High, L= Low ME=Metabolizable Energy

3.3. Data Collection and Analysis

3.3.1. Body weight and body condition

The initial and final body weight measurements of the experimental rams were taken in the morning after overnight fasting using a 50 kg clock-faced salter balance. This was done by taking the average of two consecutive days of fasting weight. Average daily weight gain (ADG) was calculated as the difference between final and initial body weight divided by the number of days. The body condition score of the rams were taken using 1 to 5 scales (ESGPIP, 2008).

3.3.2. Reproductive Performance

Reproductive characteristics of rams that include scrotum circumference and libido character were measured. Scrotal circumference (SC) was measured by firmly pulling the testes down into the lower part of the scrotum and placing a measuring tape around the widest point starting from the beginning of the experiment up to the last. Libido was assessed based on (Evans and Maxwell, 1987) at semen collection and scored on 1 to 5 scales (1= very poor and 5=excellent).

Table 4 Libido characteristics measurement scales and description

S.no	Category	Grade	Description
1	5	Excellent	When the ram is anxious to mount the teaser ewe such that the staffs are unable to hold it still
2	4	Very Good	The ram immediately mounts the teaser ewe, but the staff able to control it
3	3	Good	The ram when brought to the teaser sniffs around it and after 1-2 minutes it starts to mount
4	2	Poor	The ram sniffs the teaser for a few minutes and mounts it within 3-4 minutes and collapses the artificial vagina, repeats mounting during which it ejaculates
5	1	Very poor	no interest in sniffing or mounting

Evans and Maxwell, 1987

3.3.3. Semen Quality

Semen quality such as ejaculation volume, the color of semen, sperm mass motility, the concentration of spermatozoa, and morphology of spermatozoa were evaluated. After training the rams for artificial vagina (AV) Semen was collected weekly from every ram, early in the morning (7:30 – 8:30 am) by using AV (water, 40 ºC) and we placed it in a neck clamp. Semen volume was measured using a graduate tube and recorded for every ejaculation. Sperm mass motility was measured using a phase-contrast microscope and scored from zero to five based on the intensity of wave motion as described by Evans and Maxwell (1987).

Table 5 Sperm mass motility measurement scales and description

S.no	Categories	Grade	Description
1	0	Zero	All spermatozoa are immotile (motionless)
2	1	Very poor	Very few spermatozoa are active about 10% (weak movement around)
3	2	Poor	Some movement of semen is visible about 20-40% of spermatozoa are live but poor motility
4	3	Fair	Small, slow-moving wave movement 40-70% of sperm cells are active
5	4	Good	Dense, vigorous wave movement 75-90% of sperm cells were active
6	5	Very good	Cloudy, dense, and rapidly moving waves more than 90% of spermatozoa are active

Evans and Maxwell (1987)

The proportion of morphologically normal and abnormal spermatozoa was also determined by A drop of Eosin, four drops of Nigrosin and a drop of semen were placed on a clean, grease free slide then mix the semen first with eosin and then immediately with Nigrosin stain. Again the mixture was taken on the edge of a slide and pulled across the top of another slide leaving a smear, thereafter it was air dried. 200 numbers of spermatozoa were counted under oil immersion at a magnification of 100X in different areas of smear and classify them as normal, head abnormal, mid piece abnormal and tail abnormal sperm (Rege *et al*., 2000)

$$\text{Head abnormal sperm percentage} = \frac{\text{Total head abnormal counted}}{\text{Total numbers counted}} * 100$$

$$\text{Midpiece abnormal sperm percentage} = \frac{\text{Total midpiece counted}}{\text{Total number counted}} * 100$$

$$\text{Tail abnormal sperm percentage} = \frac{\text{Total tail abnormal counted}}{\text{Total number counted}} * 100$$

Both the viscosity and color of the semen were identified by physical observation. Viscosity was observed in a laboratory environment by first allowing the semen to liquefy. When the

liquefaction was completed, the sample was allowed to drop by the force of gravity through a pipette. The length of the thread was measured as the semen dropped. The normal semen was left with a very small trailing thread and semen with abnormal viscosity left a thread more than 2 cm long. Viscosity or consistency was measured using 4 grades (4= very thick, 3=thick, 2=thin, and 1=very thin) while the color was measured using 5 grades (5=thick cream, 4=creamy, 3=thin creamy,2=milky, and 1=watery).

3.3.4. Potential of rams to serve Ewe (PRSE)

It was measured within one mating cycle. The predictions were made by multiplying the value of semen volume, sperm concentration, and sperm motility and then dividing by the artificial insemination dosage (Kurnia *et al*., 2020). The dosage for artificial insemination was according to Menchaca *et al* (2005) for fresh semen cervical insemination.

$$\text{PRSE} = \frac{\text{Semen volume} * \text{sperm concentration} * \text{sperm motility}}{\text{AI dosage (200000000 spermatozoa per 0.25 ml straw)}}$$

3.3.5. Chemical analysis of the Experimental feeds

The chemical analysis was performed in the animal nutritional research laboratory, of the International Livestock Research Institute (ILRI) in Addis Ababa. After oven-drying at 100^{o}C for 24 h, samples were ground to pass through a 1-mm sieve mesh. The samples were analyzed using conventional wet chemistry. Dry matter (DM) and crude protein (CP) were analyzed according to the methodology of AOAC (2000). Dry matter was determined by oven drying at 105^{o}C overnight (method 934.01). Ash was determined by burning in a muffle furnace at 500^{o}C overnight (method 942.05). Nitrogen content was determined by Kjeldahl method using Kjeldahl (protein/nitrogen) Model 1026 (Foss Technology Corp.), (method 954.01). A conversion factor of 6.25 was used to convert nitrogen to crude protein. Neutral detergent fiber, acid detergent fiber (ADF) and lignin were determined as described by Van Soest & Robertson (1985). Neutral detergent fiber (NDF) did not involve use of heat stable amylase and the result was expressed exclusive of residual ash. Acid detergent fiber was expressed without residual ash. Lignin was determined by solubilisation of cellulose with sulphuric acid. *In vitro* organic matter digestibility was measured in rumen microbial inoculum using *in vitro* gas production technique. The buffer solution was prepared according

to the method described by Menke & Steingass (1988). Rumen fluid was collected prior to morning feeding using a vacuum pump from three ruminally cannulated cows fed a total mixed ration of grass hay (790 g/kg), wheat bran (203 g/kg), salt (3.2 g/kg) and a mineral and vitamin mixture (4.6 g/kg) on a DM basis. Use of cows was assessed and approved by the Environmental and Occupational Health and Safety Unit of ILRI. The rumen fluid from the cows was composited (1:1, v/v), filtered through four layers of cheesecloth, and added to the buffer solution (1:2, v/v), which was maintained in a water bath at 39^{o}C under continuous flushing with CO2. The buffered rumen fluid (30 ml) was pipetted into 100 ml syringes containing 0.2 g of sample and immediately placed into a water bath at 39^{o}C. Gas production was recorded after 24 hours of incubation and used to calculate IVOMD according to Menke et al. (1979) equations as follows:

IVOMD (g/kg) = 14.88+0.889GP+0.45CP+0.0651XA

Where GP: 24 h net gas production (ml/200 mg); CP: Crude protein (g/kg DM); XA: Ash content (g/kg DM).

$$ME(MJ/kg) = TDN(\%) * 0.15104$$ (Nsahlai et al., 2004)

3.3.6. Cost of supplementary feeds

The cost of supplementary feeds per each treatment was taken and computed by multiplying the actual intake per day with the prevailing prices.

3.3.7. Statistical Analysis

The collected data were managed and organized with MS-Excel (2010). The statistical analysis was performed according to the general linear model of the SAS 9.0 program (SAS, 2002). Mean comparison was carried out using Tukey's HSD test at 5% level as described by Steel and Torrie (1980) for variables whose F-values indicated significant differences at $P<0.05$. The correlation between reproductive characteristics and semen quality was performed using the Pearson correlation test (Petrie and Watson, 1999). Data were reported as mean ± S.E.M. Statistically significant was declared when $P < 0.05$.

The model used for the analysis of the data was:

$Yijk = \mu + \alpha i + \beta j + \varepsilon ijk$

Where:

Yij = response/dependent variables

μ = overall mean

$\alpha i = i^{th}$ treatment effect (T1, T2 …Tn)

βj = block (age) effect

$\varepsilon ijk = i^{th}$ random error

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Chemical analysis of the experimental feeds

The DM, CP, ash, NDF, ADF, and ADL contents of the experimental ingredients and grass hay are given in Table 6. Natural pasture had lower CP and higher NDF and ADF content than the supplemented feeds. Sheep require at least 7% dietary crude protein for maintenance (Pugh *et al*., 2020), therefore, the CP content of the natural pasture in this study met the maintenance requirement of Menz sheep. The CP content of natural pasture in the present study result was comparable with Kitaba A. and Tamir, B. (2007) 6.8% after 30 days of harvest, Gizachew, Lemma. and Smit, G.N. (2012) 5.9% in wet season and Bogal Solomon *et al*. (2008) 6.2% at the end of rain season. Although maize bran and oats grains were lower and broken lentil screening was higher in CP content, the four supplemental diets are generally rich in protein.

The crude protein content (23.85%) of the broken lentil screening was lower than the value reported by Wude Tsega (2017) who reported that the crude protein content of lentil screening was 28.32%. The difference might be the lentil genotype, processing methods and screening system. The ADL and ash content were higher in broken lentil screening this might be due to contamination of dusts during lentil screening process. Wheat bran and broken lentil screening had equal metabolizable energy 10.4 and 10.7 MJ/kg respectively. The metabolizable energy content of maize bran (13.7 MJ/kg of DM) in the current study was in the range of (Singh et al., 2013) who reported that maize bran has moderate level of crude protein (8.6%-17.6%). Invitro Organic matter digestibility (IVOMD) is the proportion of organic matter in the feed that apparently digested in the total of ruminant digestive tract. Organic matter digestibility can use to measure the energy available and to estimate the protein microbial synthesis in the rumen (Al-Arif *et al*., 2017).The lowest IVOMD was recorded from natural pasture (49.76%). This result was comparable with Kitaba A. and Tamir, B. (2007) 51.2% and 41.1 % at 90 and 120 days of harvest and Feyisa Tesfaye et al. (2022) 46.9-72.9%.

Table 6 Chemical composition of the experimental feeds on DM basis

Treatment feeds	DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
Wheat bran	92.18	5.51	15.83	44.30	13.59	3.60	10.7	69.22
Broken lentil screening	92.53	12.17	23.85	34.42	27.96	8.95	10.4	62.80
Maize bran	91.71	1.48	11.9	44.88	14.23	4.21	13.7	61.65
Oats grain	92.65	5.17	10.2	51.10	24.18	4.73	11.7	55.06
Natural pasture	93.84	10.52	6.87	66.99	43.88	7.52	6.8	49.76

ADF=Acid Detergent Fiber; ADL=Acid Detergent Lignin; CP=Crude protein; DM=Dry Matter; NDF=Neutral Detergent Fiber; OM= Organic Matter; IVOMD = Invitro Organic Matter Digestibility

4.2. Body weight change and body condition score of rams

In the present study, initial body weight (IBW), final body weight (FBW), average daily body weight gain (ADWG), and initial body condition score were similar ($P > 0.05$) between supplemented and control groups (Table 7). The current result shows that short-term flushing (4 weeks) of Menz rams with different energy and protein level feed had no effect on body weight change compared to the farmer's practice (natural pasture grazing). This might be due to the experiment duration (short period, 28 days) and season when the experiment was conducted. The current experiment was conducted during the spring season/harvest season/ in Ethiopia season when quality pasture was available in the study area. However, final body condition varied significantly ($P<0.05$) between the treatment and control groups except treatment 2 and 6. Rams under treatment 1, 3, 4, and 5 had a greater body condition score (*P*-value 0.049) than the control (T0) group and rams in Treatment 2 and 6 tended to be better than the control group . But there was no significant difference ($P>0.05$) among supplemented group on the final body condition score of Menz breeding rams (Table 7).

The body condition scores (BCS) of the supplemented groups in the current study were comparable with the values reported by Shenkutie Goshimie *et al*., (2018) for Menz breeding rams under on-station management supplemented with commercial concentrate. Furthermore,

body condition is directly related to reproductive performance (Maquivar *et al*., 2021). BCS is more related to the reproductive performance of a ram. The observed mean BCS of (3.745) for different levels of energy and protein supplemented group was greater than the value recommended for breeding rams (3.5; on a scale of 1 to 5; NADIS, 2022). This suggests that supplementation of grazing rams with protein and energy supplementation prior to breeding enhances BCS.

Table 7 Body weight change and body condition score of Menz rams fed on grazing natural pastures and supplemented with different levels of energy and protein.

Treatments									
Parameters	T1	T2	T3	T4	T5	T6	T0/Control/FP	P-value	SEM
IBW (kg)	26.98	24.62	27.07	25.2	26.22	22.9	26.87	0.25	2.8
FBW (kg)	29.88	27.00	30.4	28.78	29.3	27.24	27.7	0.4	3.44
ADG(g/d)	138.1	107.74	99.21	170.75	74.29	160.95	138.1	0.34	3.2
IBCS	2.81	2.78	2.77	2.84	2.70	2.71	2.71	0.16	0.45
FBCS	3.78^{a}	3.57^{ab}	3.91^{a}	3.78^{a}	3.83^{a}	3.6^{ab}	2.8^{b}	0.049	0.12

a, b, c LS MEANs with different superscripts in the same row differ significantly; (**) = P<0.05; ADG=average daily gain; FBW=final body weight; IBW=initial body weight; IBCS= Initial Body Condition Score, FBCS= Final Body Condition Score; SEM= Standard error of mean, FP= farmers practice, T1 =FP+9.93(50%)ME +8.4%CP, T2 = FP+7.01(30%)ME +8.01%CP, T3 = FP+9.89(50%)ME +12.77%CP, T4 = FP+7.03(30%)ME +12.1%CP, T5=FP+8.08(40%)ME +8.1%CP, T6=FP+8.2(40%)ME +12.2%CP

4.3. Reproductive characteristics of Menz rams

Table 8 shows scrotum circumference (SC) and libido of Menz breeding rams supplemented with different energy and protein levels. The result of the present study shows that a 50% energy supplementation above farmers' practice with both 8.4% and 12.77% CP resulted in a significantly larger (P<0.05) scrotum circumference of Menz breeding rams compared to the control. The results of the present study also showed that the above maintenance energy and protein supplemented groups (30% and 40% ME) had no significant difference (P>0.05) with 50% energy supplementation and also with the control/farmers practice on scrotum circumference of Menz breeding rams. However, supplemented and control group were similar (P > 0.05) in libido characteristics. In contrast to the current result, A Jibril *et al*. (2011) reported that Yankasa rams supplemented with 12.11% CP had significantly higher

(P<0.05) scrotal circumference than those supplemented with 17.11 % CP. The greater SC for lower-level supplementation might be due to the optimum utilization of dietary protein (Negesse *et al*., 2001) and the utilization of Adenosine Triphosphate (ATP) to convert excess ammonia to urea.

Scrotal circumference is an indicator of male fertility and serving capacity. Rams with normal, large scrotal circumference produce more and higher quality semen than rams of the same age and breed with small diameter. Daughters from sires with a larger testicle circumference are more fertile than females sired by males with a smaller circumference (Rekik M., 2016). The result of the supplemented group in the present study were in line with (Rekik M., 2016) who stated that for Ethiopian sheep breeds (Horro, Bonga and Menz), average scrotal circumference increases from 25 cm at 1 year of age to nearly 30 cm at 4 years of age. For larger breeds like Awassi, target scrotal circumference is 36-38 cm. In the present study, lack of difference among energy and protein supplemented groups might be the requirement for protein and energy might be satisfied at the lower level of supplementation for scrotal growth. The present study result is also in line with Ghorbankhani *et al*. (2015) on Sanjabi ram lambs, positive attributes of quality feed is characterized by improving reproductive organs, and especially the testicular circumference as this trait is associated with semen quality.

Table 8 Reproductive Characteristics of Menz breeding rams fed on grazing natural pastures and supplemented with different levels of energy and protein feed

	Treatments								
Parameters	T1	T2	T3	T4	T5	T6	Control/FP	p-value	SEM
ISC (cm)	26	25	25.42	25.14	25	24.21	24.57	0.78	1.8
FSC (cm)	28.14^{a}	25.71^{b}	27.9^{a}	26.9^{ab}	26.9^{ab}	26.4^{ab}	25.35^{b}	0.02	1.47
Libido (1-5)	4.7	5	5	5	5	5	5	0.11	0.31

a, b, c LS Means with different superscripts in the same row differ significantly; (**) = P<0.05; ISC *= Initial Scrotum circumference, FSC= Final Scrotum circumference, SEM= Standard error of mean, FP= farmers practice, T1 =FP+9.93(50%)ME +8.4%CP, T2 = FP+7.01(30%)ME +8.01%CP, T3 = FP+9.89(50%)ME*

+12.77%CP, T4 = FP+7.03(30%)ME +12.1%CP, T5=FP+8.08(40%)ME +8.1%CP, T6=FP+8.2(40%)ME +12.2%CP

4.4. Semen quality of Menz breeding rams

Semen quality evaluation parameters such as ejaculation volume, mass sperm motility, sperm cell concentration ($1X10^9$), color, and consistency (viscosity) were presented in Table 9. There was a difference greater ($P<0.05$) in semen volume and concentration of spermatozoa for breeding rams supplemented with different levels of energy and protein than in the control group.

Semen volume and concentration of spermatozoa was similar ($P < 0.05$) among different levels of protein and energy supplemented groups (T1-T6). Moreover, sperm mass motility, color, and viscosity of semen were similar ($P > 0.05$) among the treatments and control groups. The semen volume in this study was greater than the value of 0.15-0.65 ml reported for Ethiopia highland sheep rams (Gebresilassie, 2012). However, the semen volume of the supplemented group observed in the present study was comparable with Shenkutie Goshimeie *et al*. (2018) who reported that a mean semen volume of 0.7 ml value reported for Menz ram supplemented with commercial concentrate on station at Debre Birhan research center and Tejaswi *et al*. (2016) in NariSuvarna rams (0.71 ± 0.15 ml) which was within the physiological range for rams as stated by Nasrin et al. (2012).

The semen volume result of this study is comparable with M Gunawan *et al*. (2020) who reported that the semen volume of Garut Ram rams was 0.7 ml. In contrast, the semen volume observed in the present study is lower than the value reported by (Azizunnesa et al., 2013) for volume of semen for Bangladeshi ram which was 1.4 and 1.2 ml for concentrate supplemented and control groups respectively. The difference in the results of this study may be due to the influence of genetics, environmental factors and feeding practice.

Semen volume is also affected by semen collection methods and collection frequency (P. Jiménez-Rabadán *et al*., 2016) and the current collection method was using an artificial vagina and every seven days of frequency. Semen volume is one of the important factors in

semen evaluation and reproductive performance in males (Ax *et al.,* 2000). Therefore, the current result revealed that supplementation of protein and energy above farmers practice improves semen volume even under grazing on good pasture.

The result of this study confirmed that sperm production, as well as the total number of spermatozoa per ejaculate, can be influenced by an improved diet. The result of the ejaculation volume result is consistent with the study of (Kheradmand *et al*., 2006, and Fernandez *et al*., 2004) on Bakhtiary rams (Iran) and Assaf rams (Spain) respectively. The result of this study confirmed that sperm production, as well as a total number of spermatozoa per ejaculate, can be positively influenced by an improved diet.

The mean sperm cell concentration of flushed and controlled group in this study (1.45 and 1.11 respectively) were lower than the value reported by Khalifa *et al*. (2013) which corresponds to 2000-3500 x 10^6 sperm/ml and Marti *et al*. (2011), Azizunnesa *et al*. (2014) which correspond to 4.8-5.4 x 10^9 of sperm/ml on Bangladesh rams. The lower concentration in the present study might be attributed to age (Alexopoulos *et al*., 1991), frequency of ejaculation (Kaya *et al*., 2002), and/or breed. The standard concentration of ram spermatozoa per ml varies from 1.6 – 6.0 x10^9 sperm/ml with an average value of 3.6 x 10^9 sperm/ml (Moss *et al*., 1988) indicating the values reported in the present study were within the normal range. A higher number of sperm/ml enables the production of a higher number of insemination doses, ultimately creating the possibility of inseminating many ewes (Robinson *et al.,* 2006). Therefore, supplementation of Menz rams with energy and protein above farmers practice improves the reproductive performance of breeding rams, however, increasing the level of supplementation didn't bring any advantage related to sperm concentration.

The mass motility of spermatozoa provides strong evidence for sperm maturation during ejaculation and a fairly reliable indication of sperm viability (Grahman *et al*., 1980). Mass sperm motility is a convincing indicator of fertility in sheep (David *et al*., 2015). The mean mass motility (grade 4) observed in this study is comparable with the finding of previous reports T.Ahemen *et al*. (2011) in West African dwarf rams (Nigeria), Azizunnesa *et al*.

(2013) in Bangladsh rams, Khalifa *et al*. (2013) in Chios rams (Greece), Nel-Themaat et al. (2006) in Gulf Coast native rams, Kumar et al. (2010) in Bharat Merino rams and Malpura rams and Talbi *et al*. (2010) in Boujaad rams and Vishal (2014) in Nellore Jodephi rams and he mass motility of the Begait sheep rams was not significantly different between supplemented treatments (Michael Yirdaw, 2018). As observed in the present study, different levels of protein supplementation (12 to 17% CP) did not affect motility (Jibril *et al*. (2011). In contrast, some studies have reported a comparatively higher sperm mass activity of Santa Ines sheep fed diets containing different levels of whole cottonseed in Brazil (Cunha *et al*., 2012) with fortnight semen collection frequency. The discrepancy among studies might be attributed to the frequency of semen collection Kaya *et al.* (2002). In this study semen was collected every seven day interval.

The color of semen is an indicator of spermatozoa concentration and injury or infection in the reproductive tract of animals. The presence of blood or pus flakes may indicate infection in the reproductive tract (Nabil *et al.,* 2006). The color in the present study ranged from creamy white to thin creamy. The result in the present study was within the physiological range for rams as compiled by (Nasrin *et al*. 2012, Gunawan *et al*., 2020). There was no significant difference (P>0.05) observed among the treatments in semen color and all semen colors had creamy color which is an indicator of healthy semen (Ax *et al*., 2000; Tejaswi V *et al*., 2016). The creamy semen color observed in the present study was an indicator of a high concentration of spermatozoa in the semen that can be used for serving and conception of a large number of ewes (Tejaswi V *et al*., 2016). The results of semen color are in agreement with the observations of Moghaddam et al. (2012) in Arkhar Merino × Moghani (AM × MG) and Baluchi × Moghani (BL× MG) rams and Malejane *et al*. (2014) in Dorper rams. The viscosity value observed in the present study was thick and it was within the range of values reported for ram semen (Ax *et al*., 2000). The semen viscosity result of this study is comparable with the result of Gunawan *et al*. (2020) who reported that the semen viscosity of Garut Ram was medium-thick.

Table 9 Semen qualities of Menz sheep breeding rams fed on grazing natural pastures and supplemented with different levels of energy and protein feeds

	Treatments								
Parameters	T1	T2	T3	T4	T5	T6	Control/FP	p-value	SEM
volume (ml)	0.81^{a}	0.66^{a}	0.73^{a}	0.70^{a}	0.76^{a}	0.74^{a}	0.37^{b}	0.0003	0.02
Mass motility (1-5)	4.71	4.71	4.00	4.14	4.4	4.5	4.14	0.34	0.71
Concentration ($X10^{9)}$	1.44^{a}	1.40^{a}	1.38^{ab}	1.46^{a}	1.38^{ab}	1.66^{a}	1.11^{b}	0.045	0.08
Color	CW	CW	CW	CW	CW	CW	CW	-	-
Viscosity(1-4)	3	2.87	2.66	2.85	3	3	2.57	0.23	0.35

a, b,c LS Means with different superscripts in the same row differ significantly; (**) = P<0.05; SEM= Standard error of Mean, FP= farmers practice, T1 =FP+9.93(50%)ME +8.4%CP, T2 = FP+7.01(30%)ME +8.01%CP, T3 = FP+9.89(50%)ME +12.77%CP, T4 = FP+7.03(30%)ME +12.1%CP, T5=FP+8.08(40%)ME +8.1%CP, T6=FP+8.2(40%)ME +12.2%CP Color (1-5 grades): 1 = watery, 2 = milky, 3 = thin creamy, 4 = creamy; 5= thick creamy, Mass activity (1-5 grades): 1= no perceptive motion, 2 = weak motion without forming any waves, 3 = small, slow moving waves, 4 = vigorous movement with moderately rapid waves and eddies, 5 = dense, very rapidly moving waves. Viscosity (4=very thick, 3=thick, 2= thin, 1= very thin).CW=Creamy White

4.5. Sperm Morphology

The result of sperm morphology of Menz breeding rams was presented in Table 10. This result shows that there was no significant difference (P>0.05) between different levels of energy and protein supplemented group and farmers' practice on sperm morphology. The result was similar to the finding of A. Jibril *et al*. (2011) who reported that graded levels of protein in diets had no significant effect (P>0.05) on overall sperm morphology of Yankasa rams. The mean percentage normal morphology of this finding (91.59) shows that the normal morphology of sperm is higher than Asaduzzaman *et al*. (2021) the normal morphology of fresh diluted sperm was 85.27%. The findings in this study were consistent with those of Barth et al. (2008) who found that medium or high level of nutrition does not influence the overall percentage of morphologically normal spermatozoa.

The normal sperm morphology in this study was comparable with the finding of Azizunnesa *et al*. (2014) who reported the normal sperm count was within the range of 86-98%. In contrast, this study result shows higher normal spermatozoa than Malama *et al*. (2013) who found 78.48% normal spermatozoa per ejaculation. Breeding rams should have more than 70% morphologically normal spermatozoa per ejaculation (Kasimanickam *et al*.,

2007). The percentage of abnormality result in this study was comparable with the result of Gunawan, *et al*. (2020) who reported that spermatozoa abnormality of Garut rams was 7.1%. The percentage of morphologically normal spermatozoa was affected by diet. Prolonged feeding of poor-quality diets hinders the function of the epididymis resulting in subnormal levels of testosterone production and an increased proportion of cell abnormalities and semen from most males contains some abnormal spermatozoa (David *et al*., 2007; Tufarelli *et al*., 2011).

Table 10 Spermatozoa morphology of Menz breeding rams fed on grazing natural pastures and supplemented with different levels of energy and protein feeds

	Treatments								
Parameters	T1	T2	T3	T4	T5	T6	T0(control	P-value	SEM
Morphology									
Normal (%)	94.42	91.71	91.83	90.85	92.8	88.3	91.28	0.83	6.3
Abnormal (%)	5.57	8.28	8.16	9.14	7.2	12.16	8.71	0.55	4.0
Types of abnormality									
Head	0	0	0	0	0	0	0		
Tail	5	7.85	6.5	8.28	6.8	5.16	8.0	0.63	4.00
Mid-piece	0.57	0.0.57	1.66	0.85	0.4	1.2	0.85	0.64	1.12

a, b,c LSMeans with different superscripts in the same row differ significantly; (**) = P<0.05; SEM= Standard error of Mean, FP= farmers practice, T1 =FP+9.93(50%)ME +8.4%CP, T2 = FP+7.01(30%)ME +8.01%CP, T3 = FP+9.89(50%)ME +12.77%CP, T4 = FP+7.03(30%)ME +12.1%CP, T5=FP+8.08(40%)ME +8.1%CP, T6=FP+8.2(40%)ME +12.2%CP

4.6. The potential of Menz rams to serve ewes

The result of the potential of Menz rams to serve ewes fed on grazing natural pastures and flushed with different levels of energy and protein feeds was presented in Figure 2. The result shows that there is a significant difference (P<0.05) between supplemented (T1-T6) and control on the potential of Menz rams to serve ewes. However, there was no significant

difference (P> 0.05) among the supplemented groups, indicating a lower level of protein and energy supplementation can be used for flushing Menz rams. This parameter was highly dependent on the measure of semen volume, sperm motility, and sperm concentration (Kurnia *et al*., 2020), which determines the amounts of motile sperm per-ejaculate and prediction in serving females to be higher in supplemented groups than in the control/farmers' practice. Supplementation of protein above maintenance plays a role in regulating and stabilizing plasma membrane in the process of spermatogenesis (Cheah and Yang 2011). Also increasing the maturation of sperm cells, the capability of serving ewes in rams was better in the protein supplemented group than in the control. In agreement with the current study, supplementation of protein and energy above farmers practice improved the potential of rams to serve ewes (Kurnia *et al*., 2020). The result of the above parameters further indicated that nutritional flushing of Menz breeding rams with energy and protein supplementation make suitable for the AI accelerate the Menz sheep genetic improvement by selection through CBBP approach.

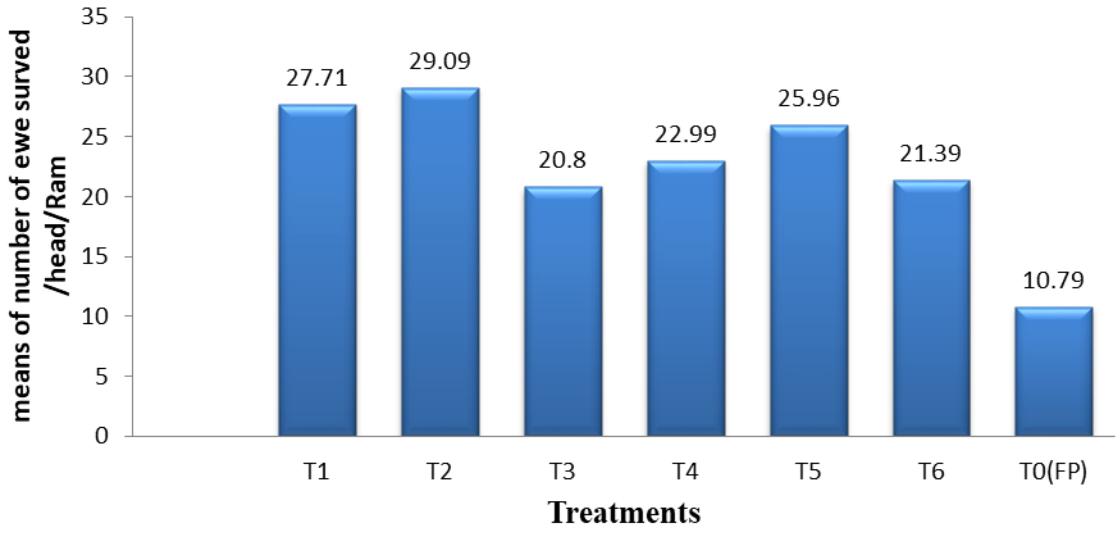

Figure 2 the potential of Menz rams to serve ewes fed on grazing natural pastures and supplemented with different levels of energy and protein feeds

T0/FP= farmers practice, T1 =FP+9.93(50%) ME +8.4%CP, T2 = FP+7.01(30%) ME +8.01%CP, T3 = FP+9.89(50%) ME +12.77%CP, T4 = FP+7.03(30%) ME +12.1%CP, T5=FP+8.08(40%) ME +8.1%CP, T6=FP+8.2(40%) ME +12.2%CP

4.7. Correlation among body condition score, scrotal circumference, and semen characteristics of Menz rams

The correlations between body condition score and the semen characteristics of Menz rams are presented in Table 12. The BC was positively correlated with SC, VOL, ($P<0.0001$) and significantly correlated with BW, PRSE, and MOT ($P<0.05$). Also, the SC positively and significantly correlated with VOL ($P < 0.0001$), PRSE, and VIS ($P<0.05$). The concentration of spermatozoa also positively and significantly correlated ($P<0.0001$) with volume, color, PPRSE, and viscosity. The observed correlation among reproductive parameters in the present study was in agreement with the result reported by Moghaddam *et al*. (2012) on ArkharMerino× Moghani (AM×MG) and Baluchi×Moghani (BL×MG) rams in Iran. The correlation between SC and semen volume was related to spermatogenesis and testosterone secretion (William H. Wolker, 2011; Maksimovic *et al*., 2016, Shenkutie *et al*., 2018). The data analyzed using Pearson correlation obtained a correlation coefficient of 0.89, between SC and semen volume which shows a positive and very strong correlation. This is consistent with Sugiyono (2007) that the correlation coefficient of 0.001 to 0.200 means correlation very weak, correlation coefficient 0.201 to 0.400 means weak correlation, correlation coefficient 0.401 to 0.600 means correlation strong enough, correlation coefficient 0.601-0.800 means strong correlation, correlation coefficient 0.801 to 1.000 means very strong correlation. The study further suggests that the concentration of the semen and volume are highly correlated, it may be related to the amount of seminal fluid secreted from the accessory male sex organs (Niehanus, 2004; Preston *et al*., 2012).

The result of this study agrees with the study of Bintara *et al.* (2017) which stated that SC and semen volume has correlation coefficient of 0.852, which suggests that the SC has a positive and very strong correlation level with semen volume. The result of this study is also in agreement with the study of Hastono and Arifin (2006) which stated that one of the attempts to know the volume of testes is by measuring the circumference of the scrotum, means that the greater the volume of the scrotum or testes the greater the circumference of the scrotum. The observed correlation between scrotum circumference and volume of semen per ejaculation is similar to the report of (Daramola *et al*., 2007 and T.Ahemen *et al*., 2011).

Similarly, this study demonstrated a significant correlation (P<0.001, r = 0.89) between SC and semen volume, which is a direct function of testicular size (AX et al., 2000).

The correlation between the SC, semen volume, and viscosity could be attributed to the fact that the wider SC is expected to have an optimum size of the testis which is expected to secrete a higher amount of testosterone (William H. Walker, 2011; Maksimovic *et al*., 2016, Shenkutie Goshimie *et al*., 2018). The semen color of the Menz rams was correlated with motility, rams semen that is creamy in color has higher motility when compared to those which are of different colors or are watery (Rodriguez-Martinz, 2013; Kridli *et al*., 2007; Olah *et al*., 2013). Semen color different other than creamy color is due to the presence of live/dead sperms or semen which is infected with diseases or pus cells have off colors and hence less motile (Mohammed et al., 2006). The result also suggests that body condition and SC could be used to estimate semen quality in rams.

Table 12 Correlations between semen characteristics of breeding Menz rams fed on grazing natural pastures and supplemented with different levels of energy and protein feeds

	BC	BW	SC	LIB	CON	VOL	COL	PPRSE	VIS	MOT	NOR	ABN
BC	1	0.44*	0.57**	0.01	0.18	0.54**	0.18	0.44*	0.21	0.29*	0.22	-0.22
BW		1	0.3*	-0.08	0.07	0.13	0.16	0.1	0.14	0.02	-0.07	0.07
SC			1	-0.02	0.16	0.89***	0.24	0.4*	0.2*	0.28	0.21	-0.21
LIB				1	-0.04	-0.022	-0.07	-0.02	-0.08	0.01	-0.18	0.18
CON					1	0.49**	0.53**	0.7***	0.6***	0.14	0.15	-0.15
VOL						1	0.45*	0.9***	0.5**	0.2*	0.22	-0.22
COL							1	0.5**	0.8***	0.35*	0.13	-0.13
PRSE								1	0.56**	0.27	0.21	-0.21
VIS									1	0.3*	0.12	-0.12
MOT										1	0.01	-0.01
NOR											1	-0.68**
ABN												1

*=p<0.05, **=p<0.01, and *** =p<0.001

BC = Body Condition, BW = Body Weight, SC = Scrotum Circumference, LIB = Libido, CON = Concentration, VOL = Volume, COL = Color, PRSE = potential of rams to serve ewe, VIS = Viscosity, MOT = Motility, NOR = Normal, ABNO = Abnormal

4.8. Cost of the supplementary feeds

The supplementary feed cost was computed and described for each treatment (Table 11). Except feed cost all other costs were similar to all treatment groups. The results showed that T6 and T3 had the lowest supplementary feed cost as compared to other supplemented groups.

Table 11 Cost of the supplementary feeds

Items	Price (ETB)/kg	Treatment					
		1	2	3	4	5	6
Oats grain	25	350			210		
Wheat bran	20			280			224
Maize bran	25		350			210	
Broken lentil screening	25				105	105	
Salt	15	4.2	4.2	4.2	4.2	4.2	4.2
Total supplemental feed cost (ETB) for flushing		354.2	354.2	284.2	319.2	319.2	228.2

ETB = Ethiopian Birr

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The current study was conducted to evaluate the effect of flushing Menz breeding rams with varying energy and protein level above farmer practice on reproductive characteristics and semen quality. It could be concluded that short-term flushing of Menz breeding rams with energy and protein above farmer practice improved body condition, scrotal circumference, semen volume, concentration spermatozoa, and prediction potential of rams to serve ewe. However, libido, semen color, semen viscosity, and sperm morphology were not affected by short term flushing with energy and protein. Generally, the present experiment result showed that flushing of Menz breeding rams for four weeks before breeding can improve the reproductive performance, semen quality and potential of Menz rams to serve ewes.

5.2. Recommendation

- Based on the cost of supplementary feed T6 and T3 were recommended to improve the reproductive performance, semen quality and potential of rams to serve ewes of breeding Menz sheep rams.
- Since the experiment was conducted in one season (spring or harvesting season), more studies are needed to examine the effect of energy and protein supplementation at different times of the year in order to provide comprehensive recommendation and develop a complete nutritional package for different seasons of the year.
- Wider scaling up

CHAPTER SIX: REFERENCES

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CHAPTER SEVEN: APPENDIXES

Appendix 7.1 ANOVA Tables

ANOVA for Menz ram Initial Body condition

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.83673469	0.30612245	1.46	0.2141
Error	42	8.78571429	0.20918367		
Corrected Total	48	10.62244898			

R-Square	Coeff Var	Root MSE	initial BCs Mean
0.172911	15.50930	0.457366	2.948980

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.83673469	0.30612245	1.46	0.2141

ANOVA for Menz ram Final Body Condition

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.80277778	0.30046296	2.36	0.0492
Error	38	4.84166667	0.12741228		
Corrected Total	44	6.64444444			

R-Square	Coeff Var	Root MSE	Final BCs Mean
0.271321	9.676317	0.356949	3.688889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.80277778	0.30046296	2.36	0.0492

ANOVA for Menz ram Initial Body weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	100.9640816	16.8273469	1.48	0.2078
Error	42	476.9257143	11.3553741		
Corrected Total	48	577.8897959			

R-Square	Coeff Var	Root MSE	initial BWt Mean
0.174712	13.11300	3.369774	25.69796

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	100.9640816	16.8273469	1.48	0.2078

ANOVA for Menz ram Final BWt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	64.8308571	10.8051429	0.87	0.5239
Error	38	470.4891429	12.3812932		
Corrected Total	44	535.3200000			

R-Square	Coeff Var	Root MSE	Final BWt Mean
0.121107	12.16143	3.518706	28.93333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	64.83085714	10.80514286	0.87	0.5239

ANOVA for Menz ram ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	15092.9300	2515.4883	0.83	0.5526
Error	38	114866.2536	3022.7961		
Corrected Total	44	129959.1837			

R-Square	Coeff Var	Root MSE	ADG Mean
0.116136	54.84937	54.97996	100.2381

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	15092.93003	2515.48834	0.83	0.5526

ANOVA for Menz ram initial SC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	13.9081633	2.3180272	0.69	0.6554
Error	42	140.2142857	3.3384354		
Corrected Total	48	154.1224490			

R-Square	Coeff Var	Root MSE	initial SC Mean
0.090241	7.293669	1.827139	25.05102

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	13.90816327	2.31802721	0.69	0.6554

ANOVA for Menz ram Final SC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	44.0404762	7.3400794	2.77	0.0248
Error	38	100.7595238	2.6515664		
Corrected Total	44	144.8000000			

R-Square	Coeff Var	Root MSE	Final SC Mean
0.304147	6.083548	1.628363	26.76667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	44.04047619	7.34007937	2.77	0.0248

ANOVA for Libido Characteristics of Menz ram

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.08571429	0.18095238	1.85	0.1150
Error	38	3.71428571	0.09774436		
Corrected Total	44	4.80000000			

R-Square	Coeff Var	Root MSE	libido Mean
0.226190	6.337316	0.312641	4.933333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.08571429	0.18095238	1.85	0.1150

ANOVA for semen volume of Menz ram

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.84125397	0.14020899	5.57	0.0003
Error	38	0.95652381	0.02517168		
Corrected Total	44	1.79777778			

R-Square	Coeff Var	Root MSE	Volume Mean
0.467941	23.40824	0.158656	0.677778

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	0.84125397	0.14020899	5.57	0.0003

ANOVA for semen color of Menz ram

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.60476190	0.26746032	0.88	0.5212
Error	38	11.59523810	0.30513784		
Corrected Total	44	13.20000000			

R-Square	Coeff Var	Root MSE	color Mean
0.121573	14.53665	0.552393	3.800000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.60476190	0.26746032	0.88	0.5212

ANOVA for Menz ram spermatozoa concentration

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.04023703	0.17337284	2.07	0.0399
Error	38	3.18363617	0.08377990		
Corrected Total	44	4.22387320			

R-Square	Coeff Var	Root MSE	conc Mean
0.246276	20.99678	0.289448	1.378533

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.04023703	0.17337284	2.07	0.0399

ANOVA for Menz ram semen Viscosity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.17301587	0.19550265	1.57	0.1832
Error	38	4.73809524	0.12468672		
Corrected Total	44	5.91111111			

R-Square	Coeff Var	Root MSE	viscosity Mean
0.198443	12.41403	0.353110	2.844444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1.17301587	0.19550265	1.57	0.1832

ANOVA for Menz ram Motility of spermatozoa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3.30634921	0.55105820	1.09	0.3879
Error	38	19.27142857	0.50714286		
Corrected Total	44	22.57777778			

R-Square	Coeff Var	Root MSE	motility Mean
0.146443	16.26715	0.712140	4.377778

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	3.30634921	0.55105820	1.09	0.3879

ANOVA for Menz ram Normal morphology of spermatozoa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	113.749206	18.958201	0.47	0.8269
Error	38	1535.895238	40.418296		
Corrected Total	44	1649.644444			

R-Square	Coeff Var	Root MSE	Normal Mean
0.068954	6.933816	6.357538	91.68889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	113.7492063	18.9582011	0.47	0.8269

ANOVA for Menz ram abnormal morphology of spermatozoa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	72.4158730	12.0693122	0.74	0.6172
Error	38	615.8952381	16.2077694		
Corrected Total	44	688.3111111			

R-Square	Coeff Var	Root MSE	Abnormal Mean
0.105208	52.66422	4.025887	7.644444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	72.41587302	12.06931217	0.74	0.6172

ANOVA for Menz ram Midpiece abnormality morphology of spermatozoa

Source	DF	Sum Squares	Mean Square	F Value	Pr > F
Model	6	6.23492063	1.03915344	0.83	0.5555
Error	38	47.67619048	1.25463659		
Corrected Total	44	53.91111111			

R-Square	Coeff Var	Root MSE	midpiece Mean
0.115652	132.6441	1.120106	0.844444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	6.23492063	1.03915344	0.83	0.5555

ANOVA for Menz ram tail abnormality morphology of spermatozoa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	72.4920635	12.0820106	0.75	0.6107
Error	38	609.4190476	16.0373434		
Corrected Total	44	681.9111111			

R-Square	Coeff Var	Root MSE	tail Mean
0.106307	58.50972	4.004665	6.844444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	72.49206349	12.08201058	0.75	0.6107

ANOVA for Prediction potential of Menz rams to serve Ewe

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1526.016605	254.336101	1.85	0.01162
Error	36	4941.096321	137.252676		
Corrected Total	42	6467.112926			

R-Square	Coeff Var	Root MSE	PPRSW Mean
0.235966	51.94567	11.71549	22.55335

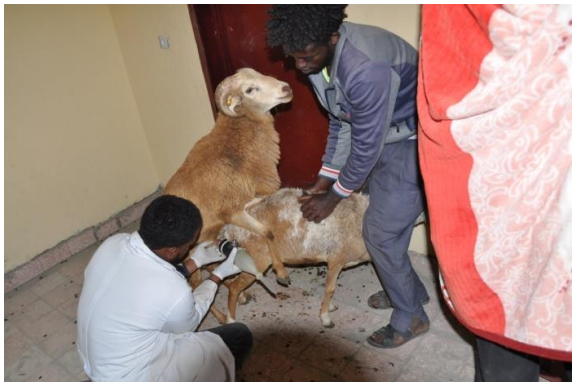
Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	6	1526.016605	254.336101	1.85	0.0019

Appendix 7.3 Appendix figures

Scrotum circumference measurement

a. b. Body Condition measurement

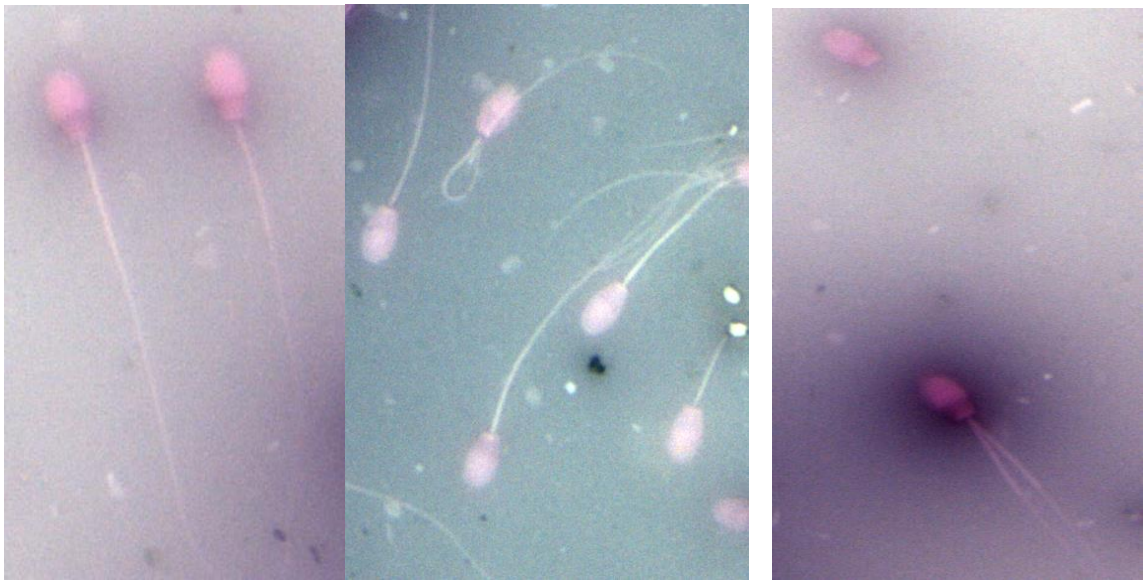
C. Body weight measurement



D. Semen collection using AV

E. Mini lab with equipment

F. microscopic evaluation



Morphology of spermatozoa (Normal, coiled tail, double tail and tailless from left to right)

BIOGRAPHICAL SKETCH

The author was born on May 13, 1994, in Gishe Rabel, North Shewa Zone of Amhara Regional State, Ethiopia. He attended his primary education at Tenamba Primary School and Secondary and preparatory education at Rabel Secondary and preparatory high School, Rabel. After completing his high school education, he joined Wollo University in 2013 and was awarded a BSc Degree in Animal Sciences in 2015 with a very high distinction award. After graduation in 2016, he joined the feed and nutrition team of the Amhara Region Agricultural Research Institute (ARARI), Debre Birhan Agricultural Research Center Livestock Research Directorate. He completed short-term training at ARARI and the Ethiopia Institute of Agricultural Research (EIAR) on research planning, implementation and reporting and received a certificate of completion. He also received an International Certificate in Entrepreneurship Skill Development Training from ICARDA. He has conducted various research projects related to feeds and nutrition in collaboration with other researchers. The author also conducts research with national and international research institutes (ILRI, ICARDA, AFRICA RISING, FEED the FUTURE Ethiopia. The author was enrolled in November 2020 to pursue his Master of Science study at Bahir Dar University, majoring in Feeds and Animal Nutrition.