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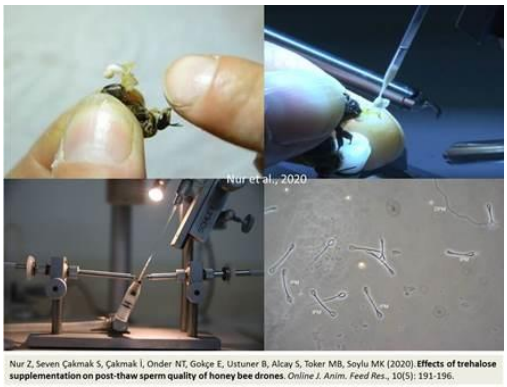
Volume 10 (5); September 25, 2020

Research Paper



Nur Z, Seven Çakmak S, Çakmak İ, Onder NT, Gokçe E, Ustuner B, Alcay S, Toker MB, Soylu MK (2020). **Effects of trehalose supplementation on post-thaw sperm quality of honey bee drones**. *Online J. Anim. Feed Res.*, 10(5): 191-196.

Effects of trehalose supplementation on post-thaw sperm quality of honey bee drones.

Nur Z, Seven Çakmak S, Çakmak İ, Onder NT, Gokçe E, Ustuner B, Alcay S, Toker MB, Soylu MK

Online J. Anim. Feed Res., 10(5): 191-196, 2020; pii: S222877012000027-10

Abstract: Sperm cryopreservation has led to an increase in widespread use and has it made it more practical to use artificial insemination not only for domestic animals but also for non-mammalian species and humans. Dimethyl Sulfoxide (DMSO) was the most frequently used cryoprotectant by protecting honey bee drone semen when freezing it. The objective of this study was to determine the effects of Trehalose (0.05M, 0.1M or none at all) on extending the viability of semen with 12% DMSO that was based on sperm motility and plasma membrane functional integrity of frozen drone semen. Three different freezing extender solutions were designated as follows; the 0.05M Trehalose, 0.1M Trehalose and Trehalose free (control group). Semen motility and plasma membrane functional integrity were evaluated under phase-contrast microscopy (400X). We found that in control group, DMSO is a critical substance in freezing extender and supports post-thaw sperm motility (53%) and plasma membrane functional integrity (79%) to some extent. Addition of 0.05M Trehalose to the extender leads to a small recovery of post-thaw motility (55%) and plasma membrane integrity (89%), but when Trehalose is added at 0.1M concentration, this led to significantly better post-thaw motility (62%) and plasma membrane integrity (91%). In conclusion, the freeze-thaw process is detrimental to post-thaw drone semen viability. The addition of 0.1 or 0.05M Trehalose to the freezing media containing 12% DMSO has been seen better post-thaw cell motility and plasma membrane integrity of spermatozoa.
Keywords: Honey bee, Drone, Semen, Cryoprotectant, DMSO, Trehalose

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Research Paper

Implementation of community based breeding program to improve growth rate and milk production performance of abergelle goat.

Amare B, Gobeze M and Wondim B.

Online J. Anim. Feed Res., 10(5): 197-202, 2020; pii: S222877012000028-10

Abstract: The study aimed to identify farmers breeding objective and trait preference criteria, devising and implementing a pilot selective breeding scheme and establishing a model goat development scheme that can be applied under village conditions. A total of 1372 goats from 32 households were monitored for selection and data collection on growth performance, milk production, reproductive performance and other important characteristics. Best bucks were selected at yearly basis based on their indexed estimated breeding value and unselected bucks were culled from the population through castration and sale. Birth type, year of birth and parity had significant effect on pre-weaning growth performances. In this intervened pilot village with three round selections mean birth and yearling weight of kids had increased from 1.6±0.05 Kg and 15.4±0.4 Kg to 2.3±0.06 Kg and 16.8±0.45 Kg, respectively. Total lactation milk yield was significantly affected by birth season and year while lactation length was affected by parity of doe's in addition to birth season and year. Average daily milk yield of doe's was 422.3±120.3 ml and it was significantly differed between years. Higher body weight of the adult male (birth weight through yearling weight) with its color and strong feeding behavior were the most preferable traits, on the other hand milk production and good mothering ability were set for the doe's. In general, the breeding program was feasible, simple and compatible strategy to the smallholder farmers breeding practices as it avoids negative selection by allowing elite selected bucks for next generation. Genetic improvement in a single pilot-village will not bring an overall population improvement of the breed, and hence there is a need of scaling out of the breeding program to all the neighboring communities. Reproductive technologies (estrus synchronization with timed artificial insemination) need to be used. Since larger number of nine month to yearling male goats are leaving the village in one or other reasons, selection of bucks starting from nine months of age should be emphasized in the future. All doe's are not productive in terms of higher litter size and milk production at every stage of their reproductive age. So, keeping of very high parity dams will not provide profitable income to the households. Forage development strategies should be designed to improve data quality, increase production capacity of the breed and sustainability of the breeding program.
Keywords: Abergelle goat, Birth weight, Selection, Traditional breeding system, Yearling weight

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Research Paper

Prevalence of ectoparasites in small ruminants (case: afar region of Ethiopia).

Feki E, Gebre S, Shumet A, Gobena Y, Mohammed H, Gebregergious A.

Online J. Anim. Feed Res., 10(5): 203-209, 2020; pii: S222877012000029-10

Abstract: A study was conducted from August 2016 to November 2017 to investigate the prevalance of major ectoparasites of small ruminant and associated risk factors in fourteen districts found in the three Zones of Afar Regional State of Ethiopia. A total of 5376 small ruminants were examined to determine the prevalence of ecto-parasites, includes 3696 goats and 1680 sheep. 1443 (39.00%) goats and 839 (49.90%) sheep were found infested with different ectoparasites. The result of the study showed that statically significant difference was found between species of sheep and goats in related to ectoparasite infestation. The overall prevalence of ectoparasite indiocates that 2282 (42.45%) small ruminants were infested by ectoparasites. The most common ectoparasites encountered in order of their predominance were 1968 (36.6%), 155 (2.88%), 105 (1.95%) and 54 (0.56 %) ticks, mange mites, lice and flea infestation, respectively. In the present study, five genera of ticks (*Rhipicephalus, Hyalomma, Amblyomma and Boophilus*), *two genera of lice (Linognathus and Damalina) three genera of mites* (Sarcoptes, Demodex and Psoroptes), and one genera of flea ctenocephalides were identified on the study animals. *Rhipicephalus, Hylomma, Boophilus, Ambylomma* was identified as the predominant genera. The prevalence of ectoparasite infestation for different age groups, sex and body condition score were found to vary significantly. In conclusion, among ectoparasites species recorded in the area ticks was found to be highly prevalent in sheep and goats. Tick was predominant followed by mite, lice and flea. The present study revealed an overall ectoparasite prevalence of 2282 (42.45%) in both small ruminant species. Of this, 839 (49.9%) and 1443 (39%) was in sheep and goats, respectively. It also revealed that ticks, mites, lice and fleas are common ectoparasites in the study area. to reduce high prevalence of ectoparasites and their impact on the productivity in small ruminants requires immediate attention, control interventions.
Keywords: Ectoparasite, Ethiopia, Prevalence, Ruminant, Ticks.

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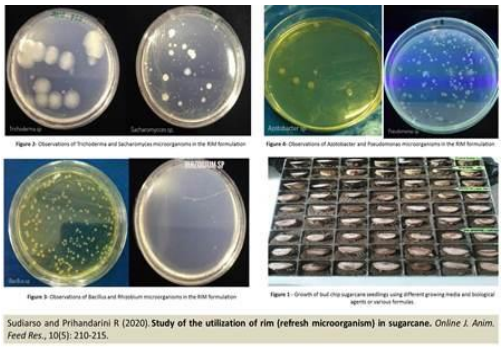
Research Paper



Study of the utilization of RIM (refresh microorganism) in sugarcane.

Sudiarso and Prihandarini R.

Online J. Anim. Feed Res., 10(5): 210-215, 2020; pii: S222877012000030-10

Abstract: Sugarcane is used by its stems as the main raw material for sugar. In addition to improving soil structure, soil organic matter also supplies soil nutrients, especially Nitrogen, Phosphor and Sulfur, increases soil aggregate stability, increases groundwater holding capacity and increases soil recoverability. To overcome this problem, the use of RIM microorganism (refresh microorganism) technology in sugarcane fields needs to be done to reduce the use of chemical fertilizers which will ultimately lead to the realization of the application of organic farming systems in sugarcane cultivation. The purpose of this study was to study the effect of different dosage applications of RIM and various sources of organic matter on the growth and yield of sugarcane. This research consisted of two stages, namely in the greenhouse and in the field, namely (1) testing the growth of bud chip sugarcane seedlings using different growth media and microorganism technology (2) testing the effect of RIM applications and organic matter on the growth and yield of sugarcane. The use of RIM's Biological Agents by using Tricho derma, Sacharomyces, Bacillus, Rhizobium, Azotobacter and Pseudomonas bacteria was able to increase the growth of sugarcane buds. The combination of using RIM and manure can increase the number of leaves and the percentage of growing sugarcane.
Keywords: Beneficial microorganism, Organic Material. RIM's Biological Agent, Sugarcane.

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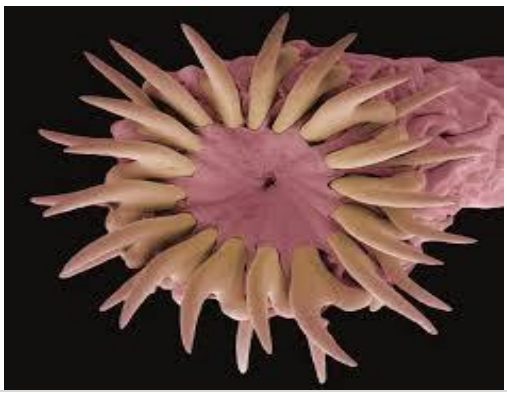
Review



Systematic review on gastrointestinal helminths of domestic ruminants in Ethiopia.

Fentahun T.

Online J. Anim. Feed Res., 10(5): 216-230, 2020; pii: S222877012000031-10

Abstract: This systemic review was conducted to identify, critically assess, and bring together available data from primary researches conducted so far on gastrointestinal (GI) helminthes of domestic ruminants in Ethiopia. In the country, GI helminths of domestic ruminants have been identified; examined and informative statistics has

been extracted since a few decades ago. For this review, relevant articles were retrieved from English databases: PubMed, Google Scholar, Science Direct, Web of Science and Scientific Information Database (SID). Additional studies were recognized by scanning the African Journal Online (AJOL) that includes the Ethiopian Veterinary Journal and Bulletin of Animal Health and Production. Out of retrieved (n=154) articles, thirty three (n=37) articles which fulfilled the eligibility criteria were selected. Accordingly, twenty three GI helminthes species which belong to the three classes of helminthes have been found to occur in domestic ruminants in the country. The main genera reported so far are *Haemonchus*, Strongyloides, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum*, *Fasciola*, *Monezia* and *Paramphistomum* whereas, *Haemochus contortus*, *Moneizia expansa* and *Fasciolahepatica* are the most frequently reported species from Nematode, Cestode and Trematode classes respectively. The overall GI helminths prevalence ranged from 2.3% to 100% were reported. Simple flotation, sedimentation, modified McMaster technique and faecal culture are the most common and routine diagnostic methods which have been used in the country. Management aspects like husbandry practices, climate and host influences are found to be the principal contributing factors that affect GI helminths infections. So far, the control of GI parasites in the country is mainly focusing on the use of anthelmintics. Consequently, due to the lack of effective control strategies, antihelmintics are exclusively used which result in antihelmintics resistance. Generally, occurrence, epidemiological features, realistic control strategies, common diagnostic procedures and frequently encountered species are reviewed. Finally, the relevance of epidemiological knowledge and the development of efficient, sustainable and conventional control measures which cover wider ago-climatic zones of the country are suggested for controlling GI helminths infections and should be assessed timely.
Keywords: Anthelmintics, Domestic ruminant, Ethiopia, Gastrointestinal helminthes.

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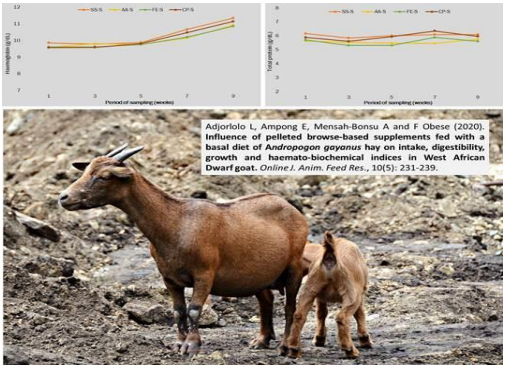
Research Paper

Influence of pelleted browse-based supplements fed with a basal diet of *Andropogon gayanus* hay on intake, digestibility, growth and haemato-biochemical indices in West African Dwarf goat.



Adjorlolo L, Ampong E, Mensah-Bonsu A and F Obese.

Online J. Anim. Feed Res., 10(5): 231-239, 2020; pii: S222877012000032-10

Abstract: This study evaluated the nutritional value of pelleted supplements based on four major feed resources fed to small ruminants by smallholder farmers in the Accra Plains. Leaves of *Samanea saman, Acacia auriculiformis* and *Ficus exasperata,* and cassava peels were dried, milled, mixed with other ingredients and pelleted to form *Samanea saman* (SS-S)*, Acacia auriculiformis* (AA-S)*, Ficus exasperata* (FE-S) and cassava peel (CP-S) based supplements. The supplements were fed to twenty West African Dwarf goats on a basal diet of *Andropogon gayanus* (Gamba grass) hay in a completely randomised design experiment. Voluntary intake of dry matter did not differ (P>0.05) by the type of supplement. However, crude protein intake was higher (P<0.05) in goats fed FE-S than those fed CP-S. Acid detergent fibre intake was higher (P<0.05) for SS-S, AA-S and FE-S than CP-S. Digestibility of dry matter, organic matter, crude protein, acid detergent fibre and neutral detergent fibre were lowest (P<0.05) for goats on CP-S. Average daily weight gain and Feed conversion ratio were not affected (P>0.05) by dietary treatments. Also, dietary treatment did not affect (P>0.05) the concentrations haematological and blood biochemical constituents determined except urea which was higher (P<0.05) in goats fed SS-S than the other treatments. In conclusion, the above results suggest that browse-based and cassava peel-based supplements could be fed to confined goats on roughage diets especially in the dry season without any deleterious effects on intake, growth and physiology of goats.
Keywords: Accra plains**,** Performance**,** Shrub Leaves, Smallholder Farmers, Supplementation

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Research Paper

Phenotypic characterization of genetic resources of indigenous cattle in West Gondar zone of Ethiopia.

Emru E, Abegaz S, Getu A, Mengistu A, Fantahun T.

Online J. Anim. Feed Res., 10(5): 240-250, 2020; pii: S222877012000033-10

Abstract: This study was conducted to identify and phenotypic characterization of the existing indigenous cattle genetic resources in the three districts of West Gondar zone of Amhara Region based on their descriptive morphological characteristics. A total of six sampling sites were purposively selected based on the distribution of unique cattle types. Key informant and focus group discussions were used to identify the new genetic resources. A total of 180 adult male and 720 female cattle were selected for 20 morphological and 9 biometrical traits. The focus group discussions revealed that, diversified indigenous cattle were found (Fellata in Quara, Rutana in Metema, Miramir in West Armachiho and Qocherie from all Districts). The overall mean linear body measurements of male cattle for horn length, body length, heart girth, canon bone length and cannon bone circumferences were 23.92 cm, 127.97 cm, 161.15cm, 20.37 cm and 19.81 respectively. Females had horn length (25.86 cm), body length (122.85 cm), pelvic width (37.47 cm) and canon bone length (19.26 cm), canon bone circumferences

(18.36 cm) and mouth circumferences (36 cm). In a discriminate analysis, all sampled populations were classified with the overall hit rates of 75% for males and 60% for females. The shortest and longest mehalanobis distances were between Abrajira with Dubaba (0.762) and Gendawuha Birshign with Mirt Gelegu (37.88) for male and Gendawuha Birshign with Dubaba (0.358) and Gendawuha Birshign with Mirt Gelegu (29.37) for females, respectively. In stepwise discriminate analysis the top traits which were highly discriminated male cattle populations were cannon bone length, horn length and cannon bone circumferences. This was similar in females except the replacement of cannon bone circumferences with mouth circumferences. Three clusters were formed (Fellata from Mirt Gelegu in cluster one and Qocherie from Gendawuha Birshign, Dubaba and Abrajira in cluster two and Rutta from Lominat Forgena and Miramir from Gilal Wuha in cluster three. Three indigenous cattle types were identified with their phenotypic features and further molecular characterization should be done to confirm their genetic distinctiveness.
Keywords: Cattle, Characterization, Genetic Resources, Phenotype, West Gondar Region.

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EFFECTS OF TREHALOSE SUPPLEMENTATION ON POST-THAW SPERM QUALITY OF HONEY BEE DRONES

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Supporting Information (This research had been funded by TÜBITAK (Project number: 2050586)

ABSTRACT: Sperm cryopreservation has led to an increase in widespread use and has it made it more practical to use artificial insemination not only for domestic animals but also for non-mammalian species and humans. Dimethyl Sulfoxide (DMSO) was the most frequently used cryoprotectant by protecting honey bee drone semen when freezing it. The objective of this study was to determine the effects of Trehalose (0.05M, 0.1M or none at all) on extending the viability of semen with 12% DMSO that was based on sperm motility and plasma membrane functional integrity of frozen drone semen. Three different freezing extender solutions were designated as follows; the 0.05M Trehalose, 0.1M Trehalose and Trehalose free (control group). Semen motility and plasma membrane functional integrity were evaluated under phase-contrast microscopy (400X). We found that in control group, DMSO is a critical substance in freezing extender and supports post-thaw sperm motility (53%) and plasma membrane functional integrity (79%) to some extent. Addition of 0.05M Trehalose to the extender leads to a small recovery of post-thaw motility (55%) and plasma membrane integrity (89%), but when Trehalose is added at 0.1M concentration, this led to significantly better post-thaw motility (62%) and plasma membrane integrity (91%). In conclusion, the freeze-thaw process is detrimental to post-thaw drone semen viability. The addition of 0.1 or 0.05M Trehalose to the freezing media containing 12% DMSO has been seen better post-thaw cell motility and plasma membrane integrity of spermatozoa.

Keywords: Honey bee, Drone, Semen, Cryoprotectant, DMSO, Trehalose

RESEARCH ARTICLE

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INTRODUCTION

Honey bee contribution in pollination of crops (*Apis mellifera* L.) and end products (honey, royal jelly, wax, pollen, and propolis) are crucial for agriculture and beekeepers. Recently the variety of subspecies and populations of honey bees have declined dramatically by virtue of man-made (habitat destruction, usage of pesticide, transport stress, management failures and poor queen health) and natural factors (starvation, parasites, winter colony losses) (Çakmak and Çakmak, 2016; Moritz and Erler, 2016). The losses of honey bees vary from region to region, but in general recently there has been large scale colony losses (35% to 75%) observed (Neumann and Norman, 2010). Therefore, the ability to preserve honey bee genetic material and to artificially inseminate for breeding different honey bee genetic lines are regarded as a critical tool for improving bee health and producing the beekeeping products mentioned above. Maintenance of genetic diversity, protection of desirable genetic lineages, prevention of colony loss, and increasing the productivity of bee colonies are possible if honey bee drone sperm preservation and artificial insemination of queen bees are probable with frozen thawed sperm (Collins, 2000; Cobey et al., 2013; Pallard et al., 2017).

Semen extender solution composition has a pivotal effect when overcoming the deleterious effects of ultra-low temperatures and sustaining the fertilization viability of cryopreserved semen (Gul et al., 2017; Pallard et al., 2017; Alcay et al., 2019a,b). Cryoprotectants are known as one of the essential ingredient to extend the shell-life of semen and are used for preventing the detrimental effects of freezing, intracellular ice crystallization, and the effects of freeze-thaw cycles (Watson and Fuller, 2001; Domingo et al., 2019). These molecules are classified as internal or penetrating and external or non-permeating cryoprotectants (Salamon and Maxwell, 2000). Internal cryoprotectants increase the plasma membrane fluidity, enhance the dehydration of spermatozoon, and reduce the intracellular ice formation, thereby improving the freezing ability and survivability of spermatozoa (Holt, 2000).

On the other hand, external cryoprotectants act as additional solute and decrease the freezing temperature of extender solution, reduce extracellular ice formation, and protect spermatozoon against cryopreservation as well (Amann, 1999). Out of all of these cryopotectants, dimethyl sulfoxide (DMSO) has attracted the most attention because to date it provides the best post-thaw motility and viability results for honey bee spermatozoa cryopreservation (Taylor et al., 2009; Hopkins ad Herr, 2010; Wegener et al., 2014; Alcay et al., 2016, Alcay at al., 2019a, b); Yaniz et al., 2019). However, there are other cryoprotective agents (CPAs), which are commonly used to protect survival of spermatozoa at low

Citation: Nur Z, Seven Çakmak S, Çakmak İ, Onder NT, Gokçe E, Ustuner B, Alcay S, Toker MB, Soylu MK (2020). Effects of trehalose supplementation on post-thaw sperm quality of honey bee drones. *Online J. Anim. Feed Res*., 10(5): 191-196.

temperatures. Due to toxicity concerns of the permeable CPAs, disaccharides (trehalose, maltose and sucrose), amino acids (EDTA, Proline, Glycine, Glutamic acid, Aminobutyric acid, Glutaric acid, Ammonium acetate), and proteins (Bovine serum albumin, gelatin, peptones and sericin) have been recently considered instead (Hubalek, 2003; Igbal et al., 2016; Mosca et al., 2016). Considering how other insect species have successfully used Trehalose for, sperm preservation, Threhalose was selected as a good candidate to test to see if it can increase preservation of honey bee sperm as well. The objective of the ongoing experiments was to evaluate the effects of various concentrations of Trehalose (0.05M, 0.1M or none at all) as viable freezing extenders that are also supplemented with 12% DMSO. The effectiveness of treatments on extending the shelf-life of honey bee spermatozoa was evaluated based on post-thaw drone semen motility and plasma membrane functional integrity.

MATERIALS AND METHODS

Animals

Apis mellifera anatoliaca drones were collected from the Beekeeping Development-Application and Research Center (AGAM) colonies and semen freezing was carried out at the Laboratory of Andrology within the Faculty of Veterinary Medicine, located in Bursa Uludag University, Turkey between May and July 2017. Mature drones that were 16 days age or older chosen from 5 different colonies that were established with drone wax foundations. The colonies used in this experiment were managed using standard beekeeping practices and were only treated for Varroa mite infestations using amitraz strips.

Chemicals

Catalase, DMSO (D2650), Trehalose and Amoxicillin were bought from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO, USA). The other chemicals (Na citrate, $NaHCO_3$, KCl) used were purchased from Merck (Merck & Co., Inc. Germany).

Semen collection and dilution

Semen was collected by squeezing drone abdomens as described by Collins and Donoghue (1999) using a Schley syringe tip 1.10 (Schley Instrumental Insemination equipment, Lich, Germany) under a stereo microscope (Collins and Donoghue, 1999). A total of 0.8 μL of saline solution was drawn into a capillary, followed by 3μL of semen (approximately 3-4 mature drones) for each freezing group.

Sperm motility

The diluted and thawed sperm motility was assessed under a phase-contrast microscope, at 400 X magnification. Observed sperm motility was scored on a scale of 0 to 5 corresponding to 0%, 20%, 40%, 60%, 80% and ≥80% amount of motility respectively (Nur et al., 2012).

Plasma membrane integrity

Sperm plasma membrane functional integrity was evaluated by the Water test (Nur et al., 2012). The 1.0 μL of diluted semen was added to 250 μL of distilled water and rested for 5 min at room temperature. One drop of incubated semen was placed on a microscope slide and the percentage of coiled tail spermatozoa was determined under a phase-contrast microscope (400X) (Nur et al., 2012). Sperm plasma membrane integrity was also assessed at the post-thaw stage as well.

Semen dilution and freezing

As suggested by Taylor et al. (2009) extender solutions IV (Na Citrate 2.43g, $NaHCO_3$ 0.21g, KCl 0.04g, Amoxicillin 0.03g, Catalase 200μL in a volume 100ml) were used. The extender's Ph was fixed to 8.1 and split into three groups I: Trehalose free (control group), II: 0.05 M Trehalose and 0.1M Trehalose. Obtained semen (3μL) was diluted (1 part semen to10 parts extender solutions) with one of the freezing extender group solutions at room temperature and loaded into a 0.25ml straw (Alcay et al., 2015). The semen filled straws were cooled to 5°C within 1 h in a cold cabinet and then were equilibrated for 2 h. Equilibrated straws were then frozen in liquid nitrogen vapor (5cm above the level of LN2, −80°C-120) for 10 min and then dipped into liquid nitrogen at roughly -196°C. From each group two straws were thawed at 37°C for 30 s in a water bath which then followed with a post-thaw semen motility and plasma membrane integrity evaluation. The procedure was repeated 5 times for each group (5x2=10straws).

Statistical analyses

The obtained data were subjected to the Kruskal Wallis –Mann Whitney U test for the motility and plasma membrane integrity tests, comparing across the different extender solutions for cryopreservation of spermatozoa. The SPSS statistical package (SPSS 10.0 for Windows, SPSS, Chicago, IL, U.S.A), was used for all statistical tests.

RESULTS

The semen motility and plasma membrane integrity as a function of Trehalose content at the two different stages were presented in Figures 1 and 2. There were no differences among diluted semen motility between control and the treatment

Citation: Nur Z, Seven Çakmak S, Çakmak İ, Onder NT, Gokçe E, Ustuner B, Alcay S, Toker MB, Soylu MK (2020). Effects of trehalose supplementation on post-thaw sperm quality of honey bee drones. *Online J. Anim. Feed Res*., 10(5): 191-196.

groups. No differences were observed across the diluted semen plasma membrane functional integrity of used groups (Figure 1).

Although freeze-thaw cycle affect the post-thaw sperm motility and plasma membrane integrity for used groups, spermatozoa had greater post-thaw motility and plasma membrane integrity when frozen in trehalose supplemented extender in comparison when frozen in Trehalose free extender. The freeze-thawing process was less harmful on drone semen plasma functional membrane integrity, compared to sperm motility especially in trehalose-supplemented group. Post-thaw sperm motility of Trehalose supplemented groups were better than the Trehalose free group (P>0.05). Also post-thaw plasma membrane integrity of the 0.1M Trehalose supplemented group (91%) was better than the 0.05 Trehalose (89%) (P>0.05), and Trehalose free group (79%) (P<0.05) (Figure 2).

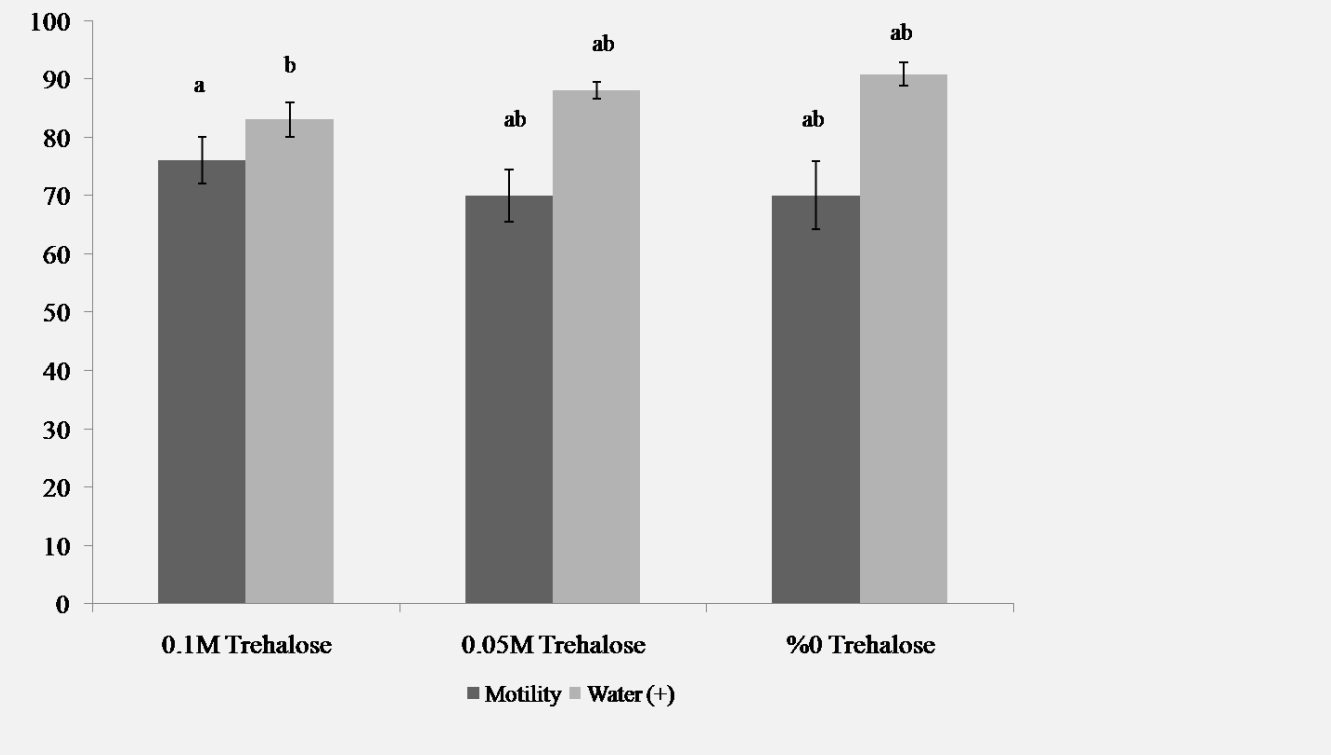

Figure 1 - Diluted semen motility (%), and plasma membrane functional integrity (%). [a,b:] Groups with different superscripts for same parameters are significantly different (P<0.05).

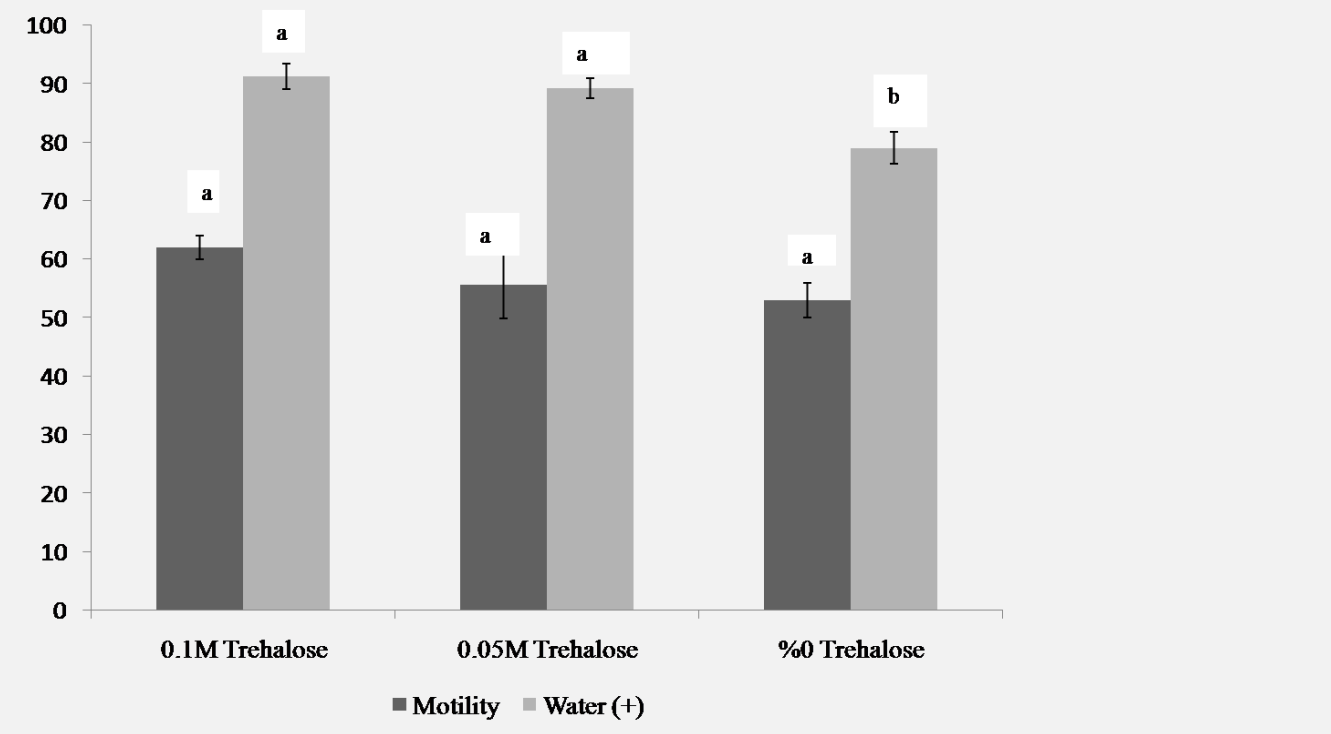

Figure 2 - Post-thaw semen motility (%), and plasma membrane functional integrity (%). [a,b:] Groups with different superscripts for same parameters are significantly different (P<0.05).

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DISCUSSION

Semen preservation requires a decrease or interruption of spermatozoa metabolism which is realized with cryopreservation. This technique includes diluting, cooling, freezing, and storing the sperm at -196°C in liquid nitrogen, followed by the thawing of the stored sperm. However, the processes of cryopreservation can affect sperm motility, viability and fertility; permanently or temporarily. Irreversible reduction of spermatozoa metabolism originates from the dilution effect, cold shock treatment, the formation of intracellular ice crystals, osmotic stress, and lipid peroxidation that can occur throughout the cryopreservation process (Isachenko, 2003; Domingo et al., 2019).

The extenders used includes, carbohydrates (trehalose), salts (Na Citrate, $NaHCO_3$, KCl), enzyme (catalase), cryoprotectant (DMSO, trehalose) and antibiotics. These chemicals interact with different part of spermatozoa and lead to osmotic and toxic stresses, and induce biochemical changes in cell metabolism immediately after dilution (Ak et al., 2010; Domingo et al., 2019; Yániz et al., 2019). There were no differences among diluted sperm motility and plasma membrane functional integrity within studied groups generally. The extender ingredients, dilution time, rate, temperatures, cooling to 5°C and freezing curve and presence of cryoprotectant also affect post-thaw semen quality (Ak at al., 2010; Nur at al., 2012; Alcay et al., 2015; Alcay et al., 2019a,b). The cryoprotectants that were used for preventing the detrimental effects of cooling, intracellular ice crystallization throughout the freezing and thawing processes are known as one of the essential ingredients of semen extenders (Watson and Fuller, 2001; Domingo et al., 2019). Post-thaw sperm motility (53%) and plasma membrane integrity (79%) of control group were lower than diluted semen motility (70%; $P<0.01$) and plasma membrane integrity (90%; $P<0.01$). The transitional population of immotile sperm with intact plasma membranes reveals that these sperms lost mobility characteristics, but still had functional membranes. These findings indicate that using extender solutions have an ability to preserve post-thaw drone sperm plasma membrane integrity to some degree. Therefore, it appears that post-thaw drone plasma membrane integrity can withstand stress more than the motility ability of the sperm when subjected to freezing related damages (Alcay et al., 2015). Casper et al. (1996) indicated that less than 50% of sperms have intact membranes in samples with complete astenozoospermia.

Cryoprotective agents including the widely used compound DMSO that was used in the freezing media have significant cytotoxic effects on spermatozoa, both in short term and long term storage. They can induce some factors from mitochondria to nucleus and poly-(ADPribose)-polymerase (PARP) activation that cause translocation of apoptosis (Hanslick et al., 2009). In addition, DMSO affects cell plasma membrane structures and induces pore formation (Notman et al., 2006). As a cryoprotective agent DMSO yields better post-thaw sperm quality in general and for this reason it has become one of the main extender ingredients in many studies to date (Paillard et al., 2017; Gül et al., 2017; Alcay et al., 2019a,b).

The present experiments were designed to improve post-thaw drone semen quality by adding different concentrations of Trehalose (0.05M, 0.1M or none at all) in freezing extender with 12% DMSO. The addition of 0.05M Trehalose to the extender with 12% DMSO leads to a small increase in recovery of post-thaw motility ($P>0.05$) and plasma membrane integrity ($P<0.05$), but when added at a 0.1M concentration this led to even better post-thaw motility ($P>0.05$) and plasma membrane integrity ($P<0.05$). Trehalose has specific interactions with sperm membrane phospholipids and minimizes the degree of cell damage during the freeze-thaw cycle by increasing the extender solution osmolarity (Storey et al., 1998; Solocinski et al., 2017). For example, supplementation of 0.2-0.6M Trehalose to media containing 10% DMSO improves post-thaw cell viability in several mammalian cell types (Beattie et al., 1997, Buchanan et al., 2004). However, the exact mechanism(s) by which Trehalose cryoprotects the cellular structures at ultra-low temperatures remains unknown. We observed that the addition of 0.05 and 0.1M Trehalose to the freezing extender solution containing 12% DMSO, increased post-thaw drone sperm plasma membrane integrity as well (Figure 2).

CONCLUSION

In conclusion, the freeze-thaw cycle is detrimental to post-thaw drone semen viability. The addition of 0.1 or 0.05M Trehalose to freezing media containing 12% Dimethyl Sulfoxide has been demonstrated to improve both post-thaw cell motility and plasma membrane integrity. More research is needed, to improve post-thaw sperm quality with other possible candidate substances in the future for honey bee artificial insemination applications.

DECLERATIONS

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Authors' contributions

Prof. Dr. Z. Nur performed watering, freezing, thawing, evaluation, statistical analysis and writing manuscript, PhD student S. Çakmak collected semen from drones, Prof. Dr. İ. Çakmak reared, collected drones, interpreted data, contributed to manuscript writing and revisions, E. Gokçe performed watering, freezing, thawing and evaluation, Assoc. Prof. Dr. B. Ustuner and S. Alçay performed thawing, evaluation, statistical analysis, and manuscript writing, Research assistants N. Onder and M. Toker performed freezing of semen, Prof. Dr. M Soylu contributed to manuscript writing.

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Competing Interests
Authors declare no conflict of interest.

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IMPLEMENTATION OF COMMUNITY BASED BREEDING PROGRAM TO IMPROVE GROWTH RATE AND MILK PRODUCTION PERFORMANCE OF ABERGELLE GOAT

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The study aimed to identify farmers breeding objective and trait preference criteria, devising and implementing a pilot selective breeding scheme and establishing a model goat development scheme that can be applied under village conditions. A total of 1372 goats from 32 households were monitored for selection and data collection on growth performance, milk production, reproductive performance and other important characteristics. Best bucks were selected at yearly basis based on their indexed estimated breeding value and unselected bucks were culled from the population through castration and sale. Birth type, year of birth and parity had significant effect on pre-weaning growth performances. In this intervened pilot village with three round selections mean birth and yearling weight of kids had increased from 1.6±0.05 Kg and 15.4±0.4 Kg to 2.3±0.06 Kg and 16.8±0.45 Kg, respectively. Total lactation milk yield was significantly affected by birth season and year while lactation length was affected by parity of doe's in addition to birth season and year. Average daily milk yield of doe's was 422.3±120.3 ml and it was significantly differed between years. Higher body weight of the adult male (birth weight through yearling weight) with its color and strong feeding behavior were the most preferable traits, on the other hand milk production and good mothering ability were set for the doe's. In general, the breeding program was feasible, simple and compatible strategy to the smallholder farmers breeding practices as it avoids negative selection by allowing elite selected bucks for next generation. Genetic improvement in a single pilot-village will not bring an overall population improvement of the breed, and hence there is a need of scaling out of the breeding program to all the neighboring communities. Reproductive technologies (estrus synchronization with timed artificial insemination) need to be used. Since larger number of nine month to yearling male goats are leaving the village in one or other reasons, selection of bucks starting from nine months of age should be emphasized in the future. All doe's are not productive in terms of higher litter size and milk production at every stage of their reproductive age. So, keeping of very high parity dams will not provide profitable income to the households. Forage development strategies should be designed to improve data quality, increase production capacity of the breed and sustainability of the breeding program.

Key words: Abergelle goat, Birth weight, Selection, Traditional breeding system, Yearling weight

INTRODUCTION

Smallholder farmers in are obliged to search options to cope up under the existing conditions and pushed to livestock production in general, and small ruminant husbandry especially goats in particular, which become the major component of the farming system in the area (Yeheyis et al., 2012; Belay, 2013; Alubel, 2015). Abergelle goat, one of the most important goat breeds in Ethiopia have a population of 300,000 (Farm Africa, 1996) distributing in all agro-ecological zones of Waghimra contributes about 70% of liquid cash incomes of the household in lowland areas (FAO, 2019). This breed is characterized by its lower body weight, lower production potential, longer kidding interval and lower litter size with better meat quality and temperament in comparison to other indigenous goat breeds (Belay and Taye, 2013; Solomon, 2014; Alubel, 2015; Vorobyov et al., 2019). In addition, the growing demand of meat at the domestic as well as at the international markets increases the importance of goat in the national economy of the developing country (Solomon et al., 2014). Despite of the large population and multidirectional roles of goats in the area (Ethiopia), their productivity and contribution to the household as well as to the national economy is far below to its potential. Many of biological, environmental and socioeconomic constraints critically affected the sector. Among the constraints aforementioned here, biological factors especially poor genetic performance of indigenous breeds takes the lion share of overall lower productivity of livestock sector. Systematic breeding programs that can improve the genetic performance of indigenous breeds are not in place in our country. Little is known about the goat production system, breeding structure, genetic potential, breeding objective and their constraints and opportunities. Many small ruminant cross breeding activities in tropical countries are not effective due to incompatibility of genotypes to farmers breeding objectives, management method and prevailing environment of low input smallholding production system (Ayalew et al., 2003; Kosgey et al., 2006; Solomon, 2014). Thus, selective pure breeding of the adapted

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indigenous breeds is the best possible option of genetic improvement in the tropical countries (Marshall et al., 2019). Pure breeding applying community based breeding program is more appropriate breeding program for such a production systems (Solomon et al., 2009; Solomon et al., 2014). It is a new approach of genetic improvement program proposed for the low input traditional smallholder farming system as it considers the indigenous knowledge of the communities on breeding practices and breeding objectives (Aynalem et al., 2011; Wurzinger et al., 2011; Solomon, 2013; Solomon, 2014). A holistic community based selective breeding with growth only or very few traits considering the existing production system was a recommended approach for genetic improvement of Abergelle goat breed (Solomon et al., 2014). Therefore, the study was designed with the objectives of: defining farmers' goat selective breeding objectives, devising and implementing a pilot breeding scheme that can be applied under village conditions and finally establishing a model goat development village for technology testing, demonstration and promotion.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Abergelle district, located at 12º56´02´´N and 38º57´22´´E which is 15 and 45 Km far from the district Niruak and Sekota towns, respectively. The district has an area of 176,664.56 ha. Abergelle district has rugged topography characterized by mountains, steep escarpments and deeply incised valleys. The production system of Abergelle district is mixed crop-livestock system with high priority of Abergelle goat production. The traditional agro-ecological classification of the district comprises of Kola and dry-Woyna-Dega/dry-sub-moist highland with a respective share of 85 and 15 percent (Solomon, 2014). The rainfall of the district is very short with an erratic distribution. The mean annual average rainfall of the area was 250-750 mm. with mean annual temperature of 23-37ºC. the main rainy season in the area starts from late June and to mid-July and ends during late August.

Selection Criteria of the village

The district was selected based on goat production potential, separate herding practice of their flock in the village, accessibility of roa and feed resources under irrigation and willingness of the farmers to participate in a community based goat improvement program. Initially, a total of 23 goat keepers were selected and had a continuous discussion on the principle and implication of community based goat improvement program. At the end of the discussions, 17 volunteer farmers were selected for final implementation of the activity and further baseline survey study. General household characteristics, purpose of keeping goats, livestock ownership, flock structure, trait selection criteria's of farmers, performance of goats, management and breeding practices and goat production constraints had been assessed. General information and farming systems of the study area were obtained from survey report by Solomon (2014) and Sekota Dry land Agricultural Research Center survey report as the district is one of the center's research mandate area. As well, reports of district office of agriculture and rural development were considered as secondary information during selection of the project village.

Recording and flock management

Two participating farmers from the project village were recruited and trained as an enumerator. Their roles were to coordinate the breeding program and collect pedigree and performance data from the participating village flocks. All animals in the villages were uniquely identified using plastic ear tags. In addition, local names were provided for each individual that helped us during the loss of an ear tag and to simplify the data recording processes. Data collection commenced prior to the start of the first round of selection. Baseline information collected includes parity of the doe's using farmer recall method, age of the doe's based on their dentition and farmers information, total flock number with each age class category of the households; and date of birth, dam identity, milk yield of the dam, birth weight and subsequent weights of kids sired by non-selected village bucks at the start of the breeding program. The performance of kids sired by unselected village sires served as a baseline or contrasts against which the genetic progress resulting from selection was assessed. After the selection activity started, data on mating, kidding and body weights were recorded. The enumerators made rounds of visits to the villages every morning to record kids born identify them by ear tags, milk data measurement and weigh the newborn. The kids were also weighed at three, six, nine and twelve months of age. Milk yield data were recorded up to twelve weeks at a week interval.

Selection and mating

A one-tier breeding structure was adopted; i.e. selection was implemented in the whole household goat population. All first birth kids of the population born from unselected previous village sires were evaluated and selected as first round breeding population, after all, the growth performance of individuals and milk production performance of their respective dams were recorded. Index selection method were implemented for the first two round selections (on yearling weight and dam ADMY traits) and for the recent two rounds selection through estimated breeding value of bucks were undertaken. All 10 to 12 month old bucks from all flocks in the project villages were evaluated together as cohorts. Selection of the best young buck was planned to be selected based on their yearling weight corrected for non-genetic factors (sex, birth type, season of birth, year of birth and parity of the dam) and to subject these criteria further to farmers' selection criteria which have been defined earlier (Solomon, 2014). Farmers, however, put heavier weights on their own subjective morpho-metrics type of criteria (i.e. color, body size and conformation) to select the bucks. The selected bucks were assigned to buck groups following an own flock mating plan due to larger flock sizes at each household by considering the previous mating history of bucks to avoid inbreeding (Solomon et al., 2009). Unselected bucks were culled through castration and sale as soon as the selection was done. In cases of small flock number faced during the selection process, group mating system was used. Bucks were rotated among the

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individual members based on mapped rotation modality by considering the grazing management of flocks, settlement and previous mating history. After completion of a single breeding season bucks were rotated to other farmers by considering the pre-set conditions and finally at the end of service period, they fattened at the cooperative for income generation.

Data analysis

Descriptive statistics were used to perform simple population characteristics. Own flock ranking and group ranking methods described by Solomon (2014) was used to identify the breeding objectives. Production and reproduction performance (quantitative) data were analyzed using the GLM procedures of SAS software. Birth type, sex, season of birth, parity and year were fitted as fixed factors for body weight variables whereas season of birth, parity and year were fitted for milk production traits. Least square mean with respective standard error was separated using Tukey-Kramer test. The following models for body weight and milk yield variables with the fixed effects were fitted.

Yijkl =μ + Bti + Sj +Sbk +P l + Ym+eijklm

Where:

Yijlm = the observed growth performance of goat by weight mainly at six month to yearling weight

μ = overall mean

Bti= is the effect of ith birth type (Single and twin)

Sj = is the effect of jth sex (male and female)

Sbk = is the effect of kth birth season (wet and dry)

Pl= is the effect of lth parity (first to ninth)

Ym= is the effect of the mth year (2014-2017)

eijklm= is random residual error

Yijk= μ + Sbi + Pj+Yk + eijk

Where:

Yijkl = the observed milk yield

μ = overall mean

Sbi = is the effect of ith birth season (wet and dry)

Pj = is the effect of jth parity (1-9))

Yk = is the effect of kth season (2014-2017)

eijk = is random residual error

RESULTS AND DISCUSSION

Breeding objectives and trait selection criteria of farmers

The primary objective of keeping goats in the study area was to use them as an immediate source of cash income and diverse livestock products that sustain the livelihoods of the community due to frequent crop production failure. The most preferred trait for doe's in the community were milk yield, good mothering ability and strong feeding behavior (drought resistance) in the existing extreme eroded and undulating areas. And for males, farmers had given more priority for color, body conformation and body size trait. The result is illustrated in Table 1.

Population structure, flock size and off take rates

The preliminary survey result of this study revealed that the flock size of goats per house hold (mean ± SD) was 47.75±29. Based on sex 27% of the populations are males and 73% are females. Reproductive doe's constitute the larger number (48%) in the flock because of farmers demand on increased number of offspring's. The results of this study is slightly lower than the reports of Belay (2008) and Solomon (2014) who reported 56.6% and 51.8%, respectively. Lower number in this study was due to smaller number of household data used in comparison to them. Death, sale, share and slaughtering (Figure 1) were the major off take reasons in the monitored flock and the overall off take rate was higher at yearling weight and around. This is because during this stage more goats were brought to the market, consumed at household level during festivities and shared to herders. Population structure (mean ± SD) with in each age class and percentage contribution is presented in Table 2.

Milk Production

Milk is the most important product consumed by all households. Milk production performance during the selection period presented in (Table 3) was significantly different ($p<0.0001$) between years. It was significantly varied during each year in a decreasing fashion. The mean average daily milk yield (±SD) was 338.8±210 ml. Doe's that have given birth during wet seasons were produced higher amount of milk and longer lactation lengths because of the availability of feed was good in this season. The reasons for milk production decline in each year were related to; first, there was higher variability of lactation lengths in each year due to scarcity of feed and longer dry season that could not supported milk production in the recent two years. Second, flock mobility and dynamics were the most critical problems during the peak periods of lactations since farmers obliged to migrate their flocks to distant areas in search of feed for their animals in the long dry seasons and this resulted in improper implementation and data recording during these periods.

Growth performance

Growth performance was the most single important trait that farmers experienced to select their next parent stock conventionally. All growth traits (birth weight, weaning weight, six month weight, nine month weight and yearling weight) described in Table 3 and Table 4 was significantly affected by year during the course of selection period. The overall mean of weight at birth, three months, six months nine months and twelve months were 2.0±0.04 kg, 7.2±0.17 kg, 10.1±0.25 kg, 13.0±0.24 kg and 15.9±0.4 kg, respectively. Sex was not significantly affected by pre weaning and post weaning weights except at six months of age. Birth type had a significant effect up on the weaning ages and then after it was not affected. This could be due to the dependence of kids on their dams during their earlier ages and after their weaning the

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kids graze on the same grazing land and forages. Season of birth only had a significant effect at birth weight and it didn't affect all other growth performance traits. This is mainly because of the performance of grazing lands in the study area were similar that could not make the difference. Males, single born kids and those born during wet seasons had significantly higher birth weight than females, twin kids and dry season born ones, respectively. This could be due to higher physiological performance of males, under nourishment of the embryo in twins and the availability of forage during wet seasons that support increased follicular growth in does, respectively. In addition, the increase in weight at different age during the respective years of selection reflected the effectiveness of the breeding program that is implemented with a good management practices. The result of this study was higher than the reports of Belay and Taye (2013) which was 1.91, 6.84, 9.13 and 14.25 Kg for birth weight, weaning weight, six month weight and yearling weight, respectively. Growth performance traits were not significantly affected by parity of the doe but doe's with earlier and higher parity had a potential of giving lower litter. This could be due to underdeveloped state of the reproductive features in first parity dams compared with older doe's that have reached physiological maturity. It was in agreement with some other studies and the results of Belay and Taye (2013). Kidding interval, litter size and age at first kidding of the flock was almost the same with the results of Solomon (2014), 12.4 months, 1.04 and 15.5 months and lower than the reports of Belay (2008). Twining was only 3.4% in the intervened population of goats. This could be due to the fact that in this study growth performance records were involved in the selection process as it was a recommended breeding design for the breed and recording of multiple traits was not feasible in the community.

Table 1 - List of preferred traits by farmers (own flock ranking method for females and group animal ranking for males), adopted from Solomon (2014).

Sex	Traits	Percentage (%)
Female	Milk Yield	20.47
	Drought resistance	14.96
	Body size	14.17
	Kid growth	11.81
	Twining	10.24
	Kidding Interval	9.45
	Other cumulative traits	18.89
Male	Body size	21.03
	Color	23.86
	Body conformation	10.12
	Height	9.09
	Fast growth	7.95
	Other cumulative traits	25

Table 2- Population structure, percentage contribution and off take amounts by different reasons at each households (N=764).

Age group	Mean	SD	Percentage (%)
Male kid	1.6	1.6	3.1
Female kid	0.6	1.8	1.2
Weaned male	12.1	4	13.1
Weaned female	22	9.1	23.8
Doe	44.2	13.6	48
Buck	7.4	3	8
Castrated	2.6	1.8	2.7

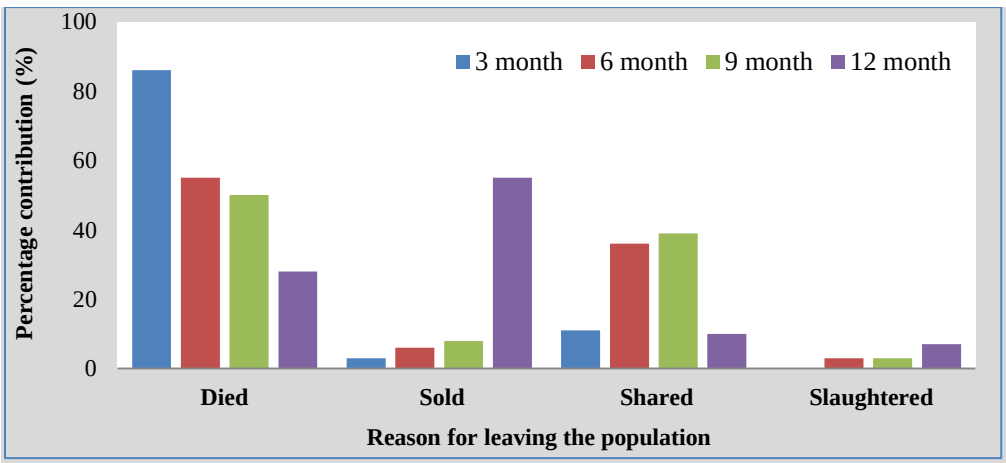

Figure 1 - Percentage of individuals leaving the population by different reason

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Table 3 - Least square means of daily milk yield and lactation length of goats under village condition

Variables	ADMY (ml)		LL (weeks)	
	N	LSM ± SE	N	LSM ± SE
Over all	1033	338.8	1033	8.9
CV (%)	-	39.2	-	35.2
Season of birth (p)	-	0.0001	-	0.0002
Dry	423	226.4±13.8^{b}	423	6.2±0.3^{b}
Wet	610	368.9±13.3^{a}	610	8.7±0.3
Year (p)		0.0001		0.0002
2006	366	440±14.4^{a}	366	9.7±0.4^{a}
2007	324	409.6±14.1^{b}	324	7.1±0.4^{b}
2008	344	375.1±13.4^{c}	344	5.5±0.4
Parity	-	0.0002	-	0.0003
1	159	224.3±13.5^{b}	159	7.6±0.4^{b}
2	167	269.2±13.3^{a}	167	8.4±0.4^{a}
3	166	281.5±13.5^{a}	166	9.0±0.4^{a}
4	201	292.5±13.3^{a}	201	8.8±0.3^{a}
5	145	257.3±13.3^{a}	145	8.5±0.3^{a}
6	99	275.7±14.9^{a}	99	7.7±0.4^{a}
7	48	280.7±18.1^{a}	48	7.4±0.5^{b}
8	19	275.7±25.6^{a}	19	7.6±0.8^{b}

ADMY = Average daily milk yield, LL= Lactation length

Table 4 - Least square means (± SE) of live body weights at different age (Kg) of Abergelle goats under village condition

Variables	BWT		TMWT		SMWT		NMWT		YWT	
	N	LSM ± SE	N	LSM ± SE	N	LSM ± SE	N	LSM ± SE	N	LSM ± SE
Over all	1372	2	942	7.2	836	10.09	632	13	371	15.9
CV%	26.5	-	20.4	-	21.13	-	12.8	-	15.6	-
Sex (p)	-	0.06	-	0.06	-	0.04	-	0.07	-	0.07
Male	707	2.02±0.04	498	7.4±0.17	452	10.1±0.25^{a}	333	13.36±0.24	181	16.6±0.4
Female	665	1.97±0.04	444	7.3±0.17	384	9.79±0.26^{b}	299	13.14±0.25	190	16.6±0.4
Birth type (p)		0.0001		0.001		0.051		0.06		0.06
Single	1271	2.07±0.04^{a}	881	7.7±0.23^{a}	783	9.95±0.34	603	13.48±0.35	360	17.00±0.77
Twin	51	1.93±0.06^{b}	31	7.03±0.15^{b}	27	9.92±0.22	15	13.02±0.22	11	16.13±0.24
Season of birth (p)	-	0.0001	-	0.06	-	0.06	-	0.062	-	0.064
Dry	533	1.96±0.05^{b}	407	7.4±0.17	335	10.1±0.26	223	13.38±0.26	118	16.9±0.44
Wet	839	2.05±0.04^{a}	535	7.34±0.17	501	9.8±0.25	409	13.12±0.24	253	16.23±0.4
Year (p)	-	0.0001		0.0001	0.0001	0.0001	0.0001	0.0001		0.0001
2014	395	1.7±0.05^{d}	319	7.01±0.18^{b}	263	9.34±0.27^{b}	220	12.76±0.26^{b}	156	15.4±0.4^{b}
2015	393	1.9±0.05^{c}	294	.07±0.18^{b}	278	9.21±0.26^{b}	214	13.06±0.25^{b}	92	17.48±0.45^{a}
2016	373	2.1±0.05^{b}	329	8.02±0.17^{a}	295	11.26±0.26^{a}	198	13.94±0.26^{a}	123	16.8±0.45^{ab}
2017	210	2.4±0.06^{a}	-	-	-	-	-	-	-	-
Parity (p)	-	0.05	-	0.052	-	0.06	-	0.06	0.06	0.06
1	217	1.87±0.05	147	7.11±0.16	127	9.87±0.24	93	13.3±0.24	64	16.8±0.52
2	215	1.95±0.05	154	7.68±0.15	138	10.23±0.23	99	13.35±0.24	56	16.3±0.53
3	204	2.06±0.05	150	7.54±0.15	130	9.85±0.24	112	13.23±0.23	73	16.34±0.51
4	226	2.03±0.05	179	7.59±0.14	160	10.1±0.22	124	13.320.22	82	16.78±0.5
5	193	1.99±0.04	143	7.67±0.15	127	10.28±0.23	96	13.42±0.23	49	16.52±0.5
6	133	2.04±0.05	86	7.71±0.17	81	10.7±0.26	52	13.32±0.27	31	17.21±0.57
7	75	2.06±0.06	40	7.01±0.24	32	9.95±0.4	24	12.5±0.35	11	16.74±0.8
8	34	2.05±0.09	17	7.29±0.36	15	9.81±0.56	10	13.02±0.53	4	15.8±1.25

BWT=birth weight, TMWT=three month weight, SMWT=six month weight, NMWT=nine month weight, YWT= yearling weight, N= total population, LSM= least square means and SE= standard error,

CONCLUSION AND RECOMMENDATIONS

Community based breeding program is compatible and simple strategy that goes in line with farmers breeding practice as it can avoid negative selection due to the elite sires were able to stay and mate at the breeding population. Higher body weight of the adult male (birth weight through yearling weight) with its color and strong feeding behavior were the most preferable traits, on the other hand milk production and good mothering ability were set for the doe's. Mean birth weight of kids has increased from 1.7 Kg to 2.4 Kg in the intervened pilot village. Off take rates were high during nine month to yearling periods that the farmers either sale the animals or share them to the herder to cover their wider demand. Based on the above strong justification the following recommendations are forwarded.

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- Genetic improvement in a single pilot-village will not bring an overall population improvement of the breed, and hence there is a need of scaling out of the breeding program to all the neighboring communities.
- Selection of sire before nine month should be recommended in order to reduce negative selection.
- Reproductive technologies (estrus synchronization with timed artificial insemination) need to be used in the community based villages to provide wider improved genotypes within a single breeding season.
- All doe's are not productive in terms of higher litter size and milk production at every stage of their reproductive age. So, keeping of very high parity dams will not provide profitable income to the households.
- Since larger number of nine month to yearling male goats are leaving the village in one or other reasons, selection of bucks starting from nine months of age should be emphasized in the future.
- Forage development strategies should be designed to improve data quality, improve production capacity of the breed and sustainability of the breeding program.

DECLARATIONS

The authors declare that we have not conflict of interest. Bewketu Amare, Mulat Gobeze and Bekahagn Wondem have declared and agree the rule of the journal and put the signatures on the declaration form. We all are first Authors/corresponding Authors of the papers. The contributions of the Authors are from initiation of the paper until final write up.

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PREVALENCE OF ECTOPARASITES IN SMALL RUMINANTS (CASE: AFAR REGION OF ETHIOPIA)

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: A study was conducted from August 2016 to November 2017 to investigate the prevalance of major ectoparasites of small ruminant and associated risk factors in fourteen districts found in the three Zones of Afar Regional State of Ethiopia. A total of 5376 small ruminants were examined to determine the prevalence of ecto-parasites, includes 3696 goats and 1680 sheep. 1443 (39.00%) goats and 839 (49.90%) sheep were found infested with different ectoparasites. The result of the study showed that statically significant difference was found between species of sheep and goats in related to ectoparasite infestation. The overall prevalence of ectoparasite indiocates that 2282 (42.45%) small ruminants were infested by ectoparasites. The most common ectoparasites encountered in order of their predominance were 1968 (36.6%), 155 (2.88%), 105 (1.95%) and 54 (0.56 %) ticks, mange mites, lice and flea infestation, respectively. In the present study, five genera of ticks (*Rhipicephalus, Hyalomma, Amblyomma and Boophilus*), *two genera of lice (Linognathus and Damalina) three genera of mites* (Sarcoptes, Demodex and Psoroptes), and one genera of flea ctenocephalides were identified on the study animals. *Rhipicephalus, Hylomma, Boophilus, Ambylomma* was identified as the predominant genera. The prevalence of ectoparasite infestation for different age groups, sex and body condition score were found to vary significantly. In conclusion, among ectoparasites species recorded in the area ticks was found to be highly prevalent in sheep and goats. Tick was predominant followed by mite, lice and flea. The present study revealed an overall ectoparasite prevalence of 2282 (42.45%) in both small ruminant species. Of this, 839 (49.9%) and 1443 (39%) was in sheep and goats, respectively. It also revealed that ticks, mites, lice and fleas are common ectoparasites in the study area. to reduce high prevalence of ectoparasites and their impact on the productivity in small ruminants requires immediate attention, control interventions.

Keywords: Ectoparasite, Ethiopia, Prevalence, Ruminant, Ticks.

INTRODUCTION

In Ethiopia, sheep and goats contribute a substantial proportion of the nation's meat supply and milk. The total numbers of sheep and goats in Ethiopia are estimated about 25.5 and 23.4 million respectively (CSA, 2003). Sheep and goats constitute about 30% of the total livestock population of the country. Gryscels and Anderson (1993) and are among important contributors to food production in Ethiopia, providing 35% of meat consumption and 14% of milk consumption (Asfaw et al., 1998).

Small ruminants are source of income for agricultural community and are also one of Ethiopia's major sources of foreign currency through exportation of live animals, meat and skin (Dessie et al, 2010; Mekuria et al., 2018). However in Ethiopia, contribution of sheep and goats to food production, rural and export income are far below the expected potential. This is because small ruminant production in Ethiopia is constrained by the compound effects of diseases, poor feeding and poor management (Kassa, 2005 and Ayele et al, 2003). Ecto-parasites including ticks, lice, mites etc. play an important role in the transmission of certain diseases (Mohd Zain et al., 2015). Infested animals scratch, rub and bite the affected areas and this end up with skin damage (Seyoum et al., 2015). Moreover, infected ruminants are the most important vectors of protozoan, bacterial, viral and rickettsial diseases (Radostits et al, 2007; Rhabari et al, 2009 and Stuchin et al., 2016).

The increasing severity and periodic rapid spread of the ecto-parasites and skin diseases in the Afar Region demanded that the different institutes to conduct a survey to characterize the true status of ecto-parasites and skin diseases problem and recommend possible control measures: Therefore, the objective of this study was:

- To identify different types of ecto-parasites and associated risk factors in small ruminants.
- To determine the prevalence ecto-parasites in sheep and goats.
- To recommend possible control and prevention measures of ecto-parasites in the region (Radostits et al, 2007; Rhabari et al., 2009 and Stuchin et al., 2016).

So, aim of present study was investigating the prevalance of major ectoparasites of small ruminant and associated risk factors in fourteen districts found in the three Zones of Afar Regional State of Ethiopia.

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MATERIALS AND METHODS

Study areas

The study was carried out in fourteen districts selected from zone one, four and five of Afar regional state, namely; Five districts of Zone 1 (AwsiRasu) namely (Afambo, Chifra, Ayssaita, Dubti, Mile), four districts of Zone 5 (HariRasu), (Telalak, Dewe, Hadalella & Dalifage) and Five districts of Zone 4 (FantenaRasu), (Golina, Awra, Ewa, Yalo & Teru) were selected for this study. The livestock population in the Afar region estimated at 3.6 million cattle, 2 million sheep, 3 million goats, 0.9 million camels and 0.2 million equines (ERIPAE, 2000). The Afar National Regional State is characterized by an arid and semi-arid climate with low and erratic rainfall. The altitude of the region ranges from 120m below sea level to 1500m above sea level. Temperatures vary from 20^{0}C in higher elevations to 48^{0}C in lower elevations. Rainfall is bi-modal throughout the region with a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The production system of the Afar region is dominated by pastoralist (90%) from which agro-pastoralist (10%) is now emerging following some permanent and temporary rivers on which small scale irrigation is developed. The season are traditionally classified as kerma (July-September), which is the long rainy season; sugum (March-April), which is the short rainy season; hagai (May-June) which is the hot dry spell; and gilal (October-February) which is the cool season. Gilal is sometimes interrupted by rains in January and February.

Animals and sampling

A cross-sectional study was used to investigate the occurrence of ecto-parasites in sheep, goats and associated risk factors. The study was conducted from August-2016 to November-2017, from the three zonal administrations 14 /fourteen districts/were selected for this study. The study districts selected based on the inclusion criteria on the high sheep and goats population, extent of parasite infection and infestation, accessibility of their Peasant associations and willingness of the pastoralists to participate in the survey. The studied animals were randomly selected using a systematic sampling technique from traditionally managed sheep and goats populations in the respective districts. The studied population was indigenous breed of sheep and goats kept under pastoral type of production which allows free grazing, usually mixed with other animals.

Sample size determination

Sample size was determined as described by Thrusfield (2005). Accordingly, 50% expected prevalence of ectoparasites infestations in each study agro-ecology, 5% acceptable error and 95% confidence level was applied to determine the sample size of study sheep and goats in each study agro-ecology.

$$n = \frac{1.96^2 pexp\ (1-pexp)}{d^2}$$

Where n = sample size, d = desired absolute precision (0.05), P_{ex} = expected prevalence (50%), thus the desired sample size for P_{ex} = 0.05 is n = 384. Sampling was 384 from each district. Proportionally distributed based on the total large ruminant population in the study districts and PAs.

Data collection

Data was collected such as species type, age, sex, and body condition score. The ages of the animals were estimated using the definition described by Aiello and Mays (1998). When lambs and kids were less than 6 month old, they were considered as “young animals” whereas when small ruminants were more than 6 month old they were included in the “adult” group. And also the age determine by using dental formula. Body condition scores will be determined by modifying the system of Gatenby (2002) animals either poor or good body condition.

Sample collection and handling procedure

During clinical examination the skin will be palpated across all parts of the animal for the presence of ectoparasites, and gross lesions suggestive of a clinical form of parasitic infestations. Animals found infested will be considered positive. From sheep and goats ticks, lice and fleas were collected with forceps from their predilection sites of attachment. The ticks were removed from the host skins whilst retaining their mouth parts for identification using forceps. Coat brushing techniques was used for collection of lice. They will be placed in labeled universal bottles containing 70% ethanol and identified under a stereoscopic microscope according to the descriptions of ticks (Walker et al., 2003). When skin lesions were evidenced skin scraping from suspected cases of mange were collected and preserved in 10% formalin. Mite identification was made according to Wall and Shearer (2001) and Taylor et al. (2007).

Ethical Regulation

Handling, sampling and all of animal related cantacts were in according to ethical regulation standart of Pastoral and Agro-pastoral Research Institute of Ethiopia.

Statistical analysis

The collected data were entered in Microsoft excel. An intercooled Stata 7 software (Stata Corporation, 2001) statistical program was employed for the data analysis. The prevalence of tick was determined by dividing the number of

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positive samples by the total sample size, and was expressed as percentage. Chi-square ($\chi 2$) test was used to assess if there was a statistically significant difference in tick infestation with in different groups. For this analysis P-value less than 0.05 was considered significant.

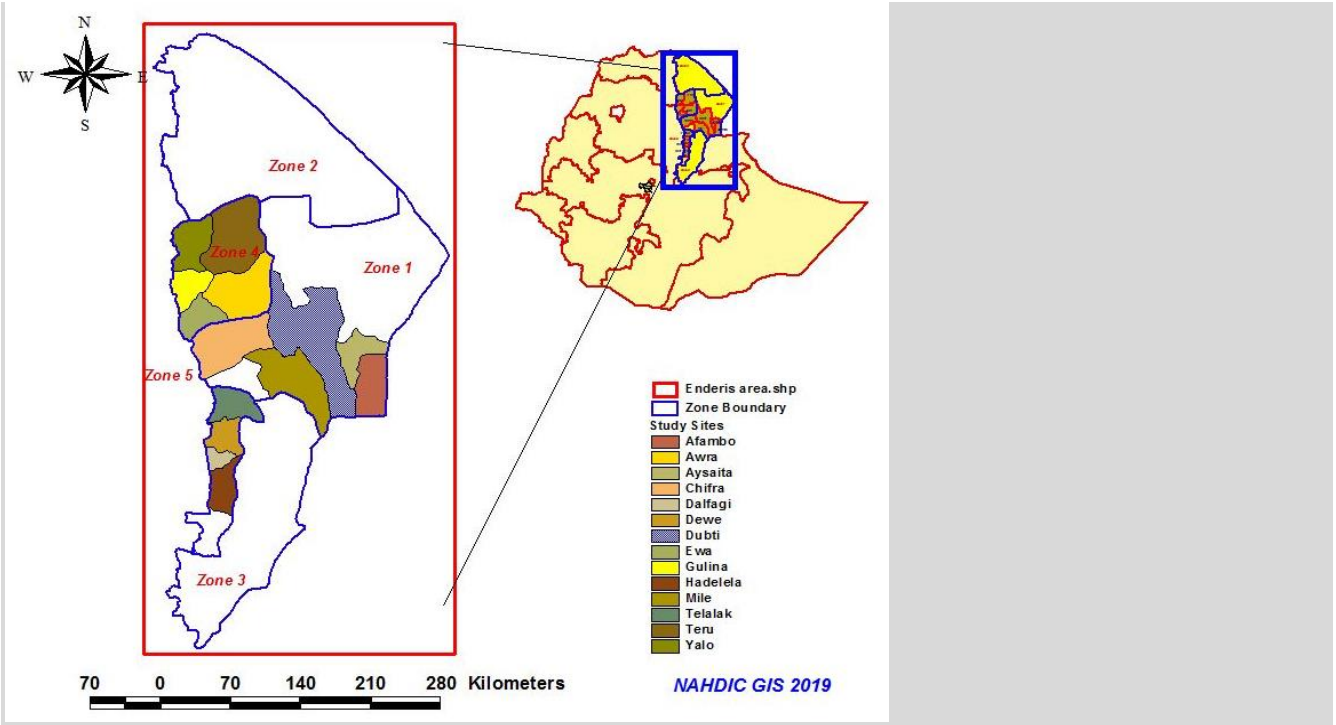

Figure 1 - Map of study districts in the region. ** National Animal Health Diagnostic and Investigation Centre (NADIC) GIS, 2019*

RESULTS and DISCUSSION

Prevalence of ecto-parasites disease

A total of 5376 small ruminants were examined to determine the prevalence of ecto-parasites infestation in 14 districts of Afar region. Of these, 2282 (42.45%) were infested by ecto-parasites identified. The overall prevalence of ectoparasites was higher in sheep than goats (Table 1). Ticks were identified as the predominant ectoparasites in small ruminants followed by mite, lice and Flea infestations (Table 2). The total number of ectoparasites collected were 2282 (42.45 %). Of these 1968 (36.6%), 155 (2.88%), 105 (1.95 %) and 54 (0.56 %) accounted for tick, mange mites, lice and flea infestation, respectively (Table 2).

Table 1 - Prevalence of ectoparasites in small ruminants of Afar region, Ethiopia

Characteristics		Parasites states of the area		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Positive	Negative				
Animals	Goats	1443(39%)	2253(61%)	1	0.0001	1	0.001
	Sheep	839(49.9%)	841(50.1%)	1.558 (1.387, 1.750)		0.642(0.572, 0.721)	

*statically significant P<0.05. COR: Crude odd ratios and AOR: Adjusted odds ratios. CI: confidence interval.

Table 2 - Percentage of ectoparasites prevalence found in small ruminants in the Afar region, Ethiopia

Ectoparasites	No. of positive shoats	%
Tick	1968	36.61
Mange	155	2.88
Lice	105	1.95
Flea	54	0.56
Total ecoparasites count	2282	42.41

Prevalence of ectoparasites by Genus level

In the present study four genera of ticks (*Rhipicephalus, Hyalomma, Boophilus and Amblyomma, three genera of mites* (sarcoptes, Demodex and Psoroptes), two genera *lice (Linognathus and Damalina)* and one genera of flea Ctenocephalides were identified. Of the total of 3696 goats and 1680 sheep examined for the infestation of ticks, mite, lice and flea; 1968 (36.61%), 155 (2.88%), 105 (1.95%), 54(0.56%) of shoats were infested with these ectoparasites respectively (Table 3).

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Prevalence of ecto-parasites at species level

Nine species of ticks which belong to the five genera were identified. *Rhipicephaluspulchellus,* 599 (11.14%) *Rhipicephalus evertsi,* 495 (9.21%) *and Rhipicephalus Pravus,* 191 (3.55%) were the dominant ones. Mange mites genera identified were; Sarcoptes 77 (1.43%), Psoroptes 48 (0.89%) and Demodex 30 (0.56%). The most prevalent lice species found on animals were Damalina *ovis,* 33 (1.96%), *Linognathus ovis,* 20 (1.19%) on sheep and *Linognathus caprae,* 28 (0.76%) and *Damalina caprae,* 24 (0.65%) on goats. The overall prevalence of Ctenocephalidia was 22 (0.48%) in sheep and goats (Table 4).

Table 3 - Prevalence of ectoparasites at the genus level

Types	Genus	Goat 3696	Sheep1680	Shoat 5376
		Prevalence	Prevalence	Prevalence
Ticks	*Rhipicephalus*	918 (23.94%)	528 (31.43%)	1446 (26.90%)
	Hyalomma	126 (3.41 %)	72 (3.92%)	198 (3.68%)
	Boophilus	109 (2.95%)	56 (3.33%)	165 (3.07%)
	Amblyomma	97 (2.62%)	62 (3.69%)	159 (2.96%)
	Sub total	1250 (33.82%)	718 (42.74%)	1968 (36.61%)
Mite	*Sarcoptes*	67 (1.81%)	10 (0.60%)	77 (1.43%)
	Psoroptes	16(0.43%)	32 (1.90%)	48 (0.89%)
	Demodex	26 (0.70%)	4 (0.24%)	30 (0.56%)
	Sub total	109 (2.95%)	46 (2.74%)	155 (2.88%)
Lice	*Damalinia*	24 (0.65%)	33 (1.96%)	57 (1.06%)
	Linognathus	28 (0.76%)	20 (1.19%)	48 (0.89%)
	Sub total	52 (1.41%)	53 (3.15%)	105 (1.95%)
Flea	*Ctenocephalides*	32 (0.60%)	22 (0.48%)	54 (0.56%)
	Sub total	32 (0.60%)	22 (0.48%)	54 (0.56%)
	Total	1443 (39.00%)	839 (49.90%)	2282 (42.45%)

Table 4- Prevalence of tick species in small ruminants of Afar region, Ethipia

Genera and species of ecto-parasites		Goat 3697	Sheep 1679	Shoats 5376
		Prevalence	Prevalence	Prevalence
Rhipicephalus	*Pulchellus*	386 (10.44%)	213 (12.68 %)	599 (11.14%)
	Evertsi evertsi	297 (8.04%)	198 (11.79%)	495 (9.21%)
	Pravus	131 (3.54%)	60 (3.57%)	191 (3.55%)
	Praetexatus	104 (2.81%)	57 (3.39%)	161 (2.99%)
	Over all	918 (24.84%)	528 (31.43%)	1446 (26.90%)
Hyalomma	*Truncatum*	72 (1.95%)	42 (2.50%)	114(2.12%)
	Dromedarii	39 (1.06%)	17 (1.01%)	56 (1.04%)
	Anatolicum anatolicum	15 (0.41%)	13 (0.77%)	28 (0.52%)
	Over all	126 (3.41%)	72 (3.92%)	198 (3.68%)
Boophilus	*Decoloratus*	109 (2.95%)	56 (3.33%)	165 (3.07%)
	Over all	109 (2.95%)	56 (3.33%)	165 (3.07%)
Amblyomma	*Gemma*	80 (2.16%)	54 (3.21%)	134 (2.49%)
	Variegatum	17 (0.46%)	8 (0.48%)	25 (0.47%)
	Over all	97 (2.62%)	62 (3.69%)	159 (2.96%)

Prevalence of ectoparasites in sheep and goats by different host related factors

An effort was made to determine the prevalence of ectoparasites in relation to different host factors such as sex, age, and body condition scores. The result is presented in table 5. In regard to sex, male small ruminants were more infested (64%) than females (23.6%) and this was found to be statistically significant (Table 5). The result shows statically different ($P<0.05$). The prevalence of infestation with different ectoparasites age and body condition score groups. The study revealed a higher prevalence in male, adult age groups and poor body condition score animals higher than good body condition (Table 5).

Table 5 - Prevalence of ectoparasites in sheep and goats by different host related factors.

Characteristics		Status of parasites		COR (95%CI)	p-value
		Positive	Negative		
Sex	Female	678 (23.6%)	2191 (76.4%)	1	< 0.001 [a]
	Male	1604 (64%)	903 (36%)	5.740 (5.098, 6.463)	
Age	Young	500 (30%)	1168 (70%)	1	< 0.001 [a]
	Old	1782 (48.1%)	1926 (51.9%)	2.161 (1.911, 2.444)	
BCS	Poor	2244 (90.9%)	225 (9.1%)	1	< 0.001 [a]
	Good	38 (1.3%)	2869 (98.7%)	0.001 (0.001, 0.002)	

*With in different row a common superscript statically significant (P<0.05).

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A total of 3697 goats and 1679 sheep were examined for the infestation of ectoparasites. The overall prevalence of ectoparasite infestation in the present study was found to be 2282 (42.45%). The present finding of ectoparasites in the study area was lower than the prevalence study reported from Zone four of Afar region by Fikre et al. (2015), with the prevalence of 94.62% and 91.86% in sheep and goats respectively. Bekele et al. (2011) reported the prevalence of 99.38% and 96.92% in sheep and goats respectively in Wolmera districts of Oromia region central Ethiopia; Tewodros et al (2012) stated prevalence of 80.97% in sheep and 72.07% in goats around Gonder town, Ethiopia. Our findings showed higher than the result explained by Teshome (2002) that stated the prevalence of 23.8% in sheep and 16% in goats and 15.41% and Yacob et al. (2008). In addition the present study revealed that there is a significant variation in the prevalence of ectoparasites among the animal species, sex of animals, age groups and body conditions of the animals (Table 1, 5 and 6). The rates of infection in this survey were relatively more in sheep (49.9%) than goats (39.0%) which agree with a study conducted by Yacob et al. (2008) that in Wolaita Soddo region reported that the rate of infestation by ectoparasites higher in sheep than goats 68.7% and 28.4%, respectively. Similarly also, the host differences reported in present region by Fikre et al. (2015), that is (94.62%) sheep and (91.86%) goats were found infested with ectoparasites. But, Due to this goats appeared to be significantly more resistant than sheep. The result is in goat lower because of their self-grooming, licking, scratching, rubbing and grazing behaviors, which would contribute to rapid ectoparasite elimination, management practice and in the environmental conditions.

These study shows that ticks, mite, lice, flea; 1968 (36.61%), 155 (2.88%), 105 (1.95%), 54(0.56%) of shoats were infested with above-mentioned of these ectoparasites, respectively. Ticks were found to be the most prevalent ectoparasites in both sheep and goats throughout the study areas. The present result indicates that infestation still need more efforts to achieve expected control result and reduction of impact excreted by ticks but on the other hand others ectoparasite species like mite, lice and flea infestation result is reduced on the present study result. A relative low prevalence of tick infestation (31.8%) in sheep and (18.6%) in goats were reported by Teshome (1994) and were as low result were reported by Zelalem (1994), 23.8% in sheep 16% in goats from the Sidama Zone in Southern Ethiopia.

Different study indicated that ectoparasites affected both sexes. In this study, a higher prevalence of ectoparasite infection was in male sex group (64%) than female sex group (23.6%) and this was significantly ($P<0.05$) different (Table 5). Similarly, Tewodros (2012) reported higher ectoparasite prevalence was observed in male than female and also the result collaborated with those reported by Abebe et al. (2011) and Sertse and Wossene A. (2007). However, dissimilar to the previous reports of Yacob et al. (2008), was also noticed that females were significantly more frequently affected than males (75.45% vs. 61.54%): The variation compare with our findings is due to areas of people use one male for many flocks of sheep and goats in the areas, due to this the males has opportunity to frequent contact with infested goats and sheep.

In present study the prevalence of ectoparasites of adult age group (48.1%) was higher than young age group (30%) (Table 5). The result was significant association ($P<0.05$). This finding was similar with Tefera (2004) and Fikre et al. (2015), with the infection rate of 51.05% and 54.2% in young and adult age, and also the prevalence of ectoparasite was higher in adult (96.91% in sheep ,93.83% in goat) than young, and (88.52% in sheep, 86.25% in goat) respectively. The result dissimilar with the result of Yakob et al. (2008), that reported the prevalence of 53% and 15% for adult and young age group small ruminants in Wolaita Sodo, respectively. This is may be due to differences to infection as a result of variation in also the management system where animals are allowed to graze together in communal fields.

Concerning the prevalence of different ectoparasites high infested in poor body conditions than that of good body condition of small ruminants. Our findings showed that the prevalence of poor body condition (90.9%) and good body condition (1.3%; Table 5). Present finding is similar with results reported by Madeira et al. (2000), Sertse and Wessene (2007), Mulugata et al. (2010) and Tewodros (2012).

Table 6 - Prevalence of ectoparasites in different districts

Zone	Districts	Positive	Negative	COR (95%CI)	p-value
Zone 4 (Fantena Rasu)	Ewa	188 (49.0%)	196 (51%)	1.099 (0.828, 1.458)	0.516[a]
	Awra	150 (39.1%)	234 (60.9)	0.734 (0.551, 0.978)	0.035 [a]
	Gulina	147 (38.3%)	237(61.7%)	0.710 (0.533, 0.947)	0.020 [a]
	Yallo	108 (28.1%)	276 (71.9%)	0.448 (0.332, 0.605)	0.001[b]
	Teru	88 (22.9%)	296 (77.1%)	0.340 (0.249, 0.465)	0.001[c]
Zone 1 (Awsi Rasu)	Afambo	155 (40.4%)	229 (59.6%)	0.775 (0.582, 1.032)	0.005[b]
	Asayta	179 (46.6%)	205 (53.4%)	1	0.000[d]
	Dubti	161 (41.9%)	223 (58.1%)	0.827 (0.622, 1.100)	0.191[a]
	Mille	138 (35.9%)	246 (64.1%)	0.642 (0.481, 0.858)	0.003[e]
	Chifra	144 (37.5%)	240 (62.5%)	0.687 (0.515, 0.916)	0.011[a]
Zone 5 (Hari Rasu),	Telalak	188 (49.0%)	196 (51.0%)	1.099 (0.828, 1.458)	0.516[a]
	Dewe	250 (65.1%)	134 (34.9%)	2.137 (1.598, 2.856)	0.000[b]
	Dalifage	197 (51.3%)	187 (48.7%)	1.206 (0.909, 1.602)	0.194[a]
	Hadelela	189 (49.2%)	195 (50.8%)	1.110 (0.836, 1.473)	0.470[a]

Different superscripts within subgroup represent statically significance of difference prevalence $P<0.05$. COR: Crude odd ratios. CI: confidence interval.

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CONCLUSION

Its concluded that, among ectoparasites species recorded in the area *ticks* was found to be highly prevalent in sheep and goats tick was predominant followed by mite, *lice* and *flea.* The present study revealed an overall ectoparasite prevalence of 2282 (42.45%) in both small ruminant species. Of this, 839 (49.9%) and 1443 (39%) was in sheep and goats, respectively. It also revealed that ticks, mites, lice and fleas are common ectoparasites in the study area.

It is suggested that, due to this higher economic losses occur through animal deaths and damages of the skin. Hence, the following recommendations are forwarded:

- Treatment campaign needs to cover of all affected areas and population of small ruminants because of free livestock movement in the region for grazing and watering.
- Sustainable veterinary services and improved management practice and well-coordinated control interventions and Strong monitoring and evaluation measures during the control campaign is very essential.
- It is valuable to implement effective Extension services and training programs aiming at awareness creation about the importance and control of ecto-parasites for livestock owners is very important.
- Moreover further epidemiological investigation is needed in the study area.

DECLARATIONS

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Authors' contribution

All authors contributed equally to this research work. All authors read and approved the final manuscript.

Availability of data and materials

Data will be made available up on request of the primary author

Consent to publish

Not applicable.

Competing interests

The data can be available to the journal upon request.

Conflict of interest

The authors declare they have no competing of interests.

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STUDY OF THE UTILIZATION OF RIM (REFRESH MICROORGANISM) IN SUGARCANE

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Sugarcane is used by its stems as the main raw material for sugar. In addition to improving soil structure, soil organic matter also supplies soil nutrients, especially Nitrogen, Phosphor and Sulfur, increases soil aggregate stability, increases groundwater holding capacity and increases soil recoverability. To overcome this problem, the use of RIM microorganism (refresh microorganism) technology in sugarcane fields needs to be done to reduce the use of chemical fertilizers which will ultimately lead to the realization of the application of organic farming systems in sugarcane cultivation. The purpose of this study was to study the effect of different dosage applications of RIM and various sources of organic matter on the growth and yield of sugarcane. This research consisted of two stages, namely in the greenhouse and in the field, namely (1) testing the growth of bud chip sugarcane seedlings using different growth media and microorganism technology (2) testing the effect of RIM applications and organic matter on the growth and yield of sugarcane. The use of RIM's Biological Agents by using Tricho derma, Sacharomyces, Bacillus, Rhizobium, Azotobacter and Pseudomonas bacteria was able to increase the growth of sugarcane buds. The combination of using RIM and manure can increase the number of leaves and the percentage of growing sugarcane.

Keywords: Beneficial microorganism, Organic Material. RIM's Biological Agent, Sugarcane.

INTRODUCTION

Sugarcane (*Sacharum officinarum* L.) is a plant in the Graminae family which is used by its stems as the main raw material for sugar, as well as animal feed. Along with the development of the population, Indonesia has not been able to meet the national sugar needs. This is evidenced by the decline in sugarcane productivity in 2017 which only reached 5.4 tonnes/ha compared to the 2016 projection of 7.75 tonnes/ha (Agricultural Statistics at a Glance 2018). The low productivity of sugarcane, followed by an increase in sugar consumption, makes Indonesia have to import sugar from abroad. Domestic sugar needs are mostly met from imported sugar in the form of raw sugar and crystal sugar, even in 2018 it was reported that National sugar production only reached 2.1 million tons per year, while the need for consumption and refined sugar reached 6.8 million tons.

The low sugarcane production can be seen from the on-farm side, including the low soil fertility caused by the low content of organic matter in the soil. Soil organic matter has biological, physical and chemical functions of soil which are mutually bound to one another. Soil organic matter in addition to providing nutrients for soil microbial activity, nutrients used by microorganisms are useful for accelerating their activity in order to increase the rate of decomposition of organic matter and release of nutrients (Sutanto, 2016). It was stated that that drought on the soil will result in reduced chlorophyll a,b content, the ratio of chlorophyll a,b decreases physiological activity and will reduce sugarcane plant growth and sugar productivity (Ferreira et al., 2017). Further, it was determined(that about 60% of rice fields in Java show less than 1% organic matter, meanwhile for the agricultural system to be sustainable, it must have organic matter greater than 2%. Rhizhobacterium was able to increase the growth and photosynthesis of sugarcane plants (Rampazzo et al., 2018).

Dependence on chemical fertilizers causes chemical fertilizer subsidies to increase every year. The environmental burden caused by the continuous use of chemical fertilizers in high doses is increasingly closing the soil pores, so that the absorption of the soil to rain water decreases and results in frequent flooding and drought (Thierfelder et al., 2018). In addition, the continuous use of chemical fertilizers causes the soil to become hard so that crop production, especially sugar cane, is getting smaller with a low yield of sugar. To overcome this problem, the use of microorganisms in sugarcane fields needs to be done to reduce the use of chemical fertilizers which will eventually lead to the realization of the application of organic farming systems in sugarcane cultivation. Microorganism technology engineering is expected to improve soil fertility so that it will increase the production and yield of sugarcane.

The current research purposes were 1) study the effect of different application of RIM dosages and various sources of organic matter on soil fertility and sugarcane crops; 2) Studying the effect of RIM and the dosage of organic matter on the growth and yield of sugarcane. The research outputs to be achieved are: (a) publication in national or international seminar proceedings, and (b) technology package for organic sugarcane cultivation.

Citation: Sudiarso and Prihandarini R (2020). Study of the utilization of rim (refresh microorganism) in sugarcane. *Online J. Anim. Feed Res.*, 10(5): 210-215.

MATERIALS AND METHODS

This study consisted of two stages, namely in the greenhouse and in the field, namely, 1) the growth test of bud chip sugarcane seedlings using different growing media and hygienic agents, 2) testing the effect of RIM application and organic matter on the growth and yield of sugarcane (Figures 1-4).

The first stage of research was the growth test of bud chip sugarcane seedlings using different growing media combined with different microorganism formulas. The objective of this research is to obtain an optimal growing environment for the growth of bud chip sugarcane. The experimental design used was a completely randomized design; the study was conducted in a greenhouse. Synergy test between a consortium of microorganisms between photosynthetic bacteria Sacharomyces (S), Azospirillum sp (A), Azotobacter (Z), Rhizobium (R), Pseudomonas (P), Bacillus (B) and Trichoderma sp. (T) with a comparison, the formula is F1, F2, F3, F4 and F5. Plant shoot variables observed were the percentage of shoots that grew on the pottray and time of shoot growth.

The second stage of research was a continuation of stage 1, the growth of shoots was followed by the influence of the growth of sugarcane shoots with RIM treatment combined with various kinds of organic matter and the concentration of biological agents on the growth and yield of sugarcane. The experimental design used was a randomized block design with 7 treatments on various planting media samples:

S0: control, S1: RIM 5 gr, S2: RIM 10 gr S3: RIM 20 gr, S4: manure + RIM 5 gr, S5: manure + RIM 10 gr, S6: manure + RIM 20 gr and S7: fertilizer cage. So there are 8 treatments, each treatment is repeated 3 times. There are 24 research treatments. Plant variables that are observed are plant height and number of leaves. Observation of soil fertility includes laboratory analysis of soil nutrient content before and after treatment, and soil microorganism population. The data obtained were analyzed using analysis of variance and significant difference test.

Figure 1 - Growth of bud chip sugarcane seedlings using different growing media and biological agents or various formulas.

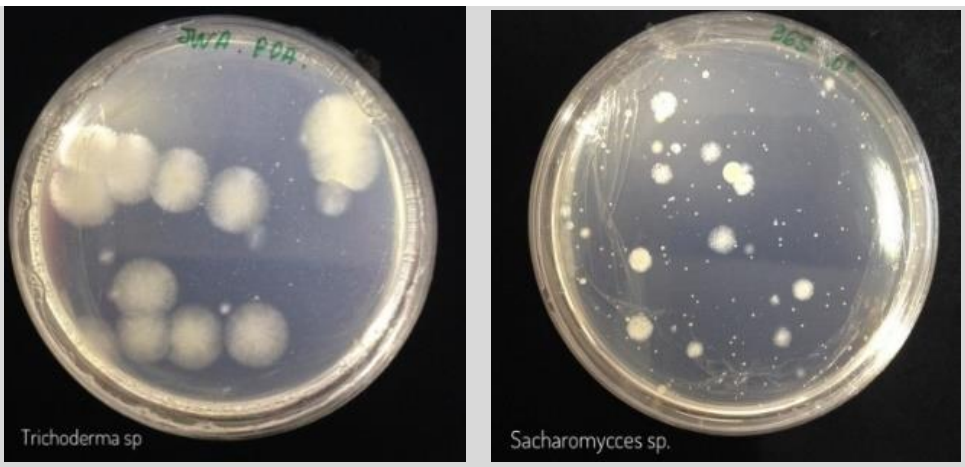

Figure 2 - Observations of Trichoderma and Sacharomyces microorganisms in the RIM formulation.

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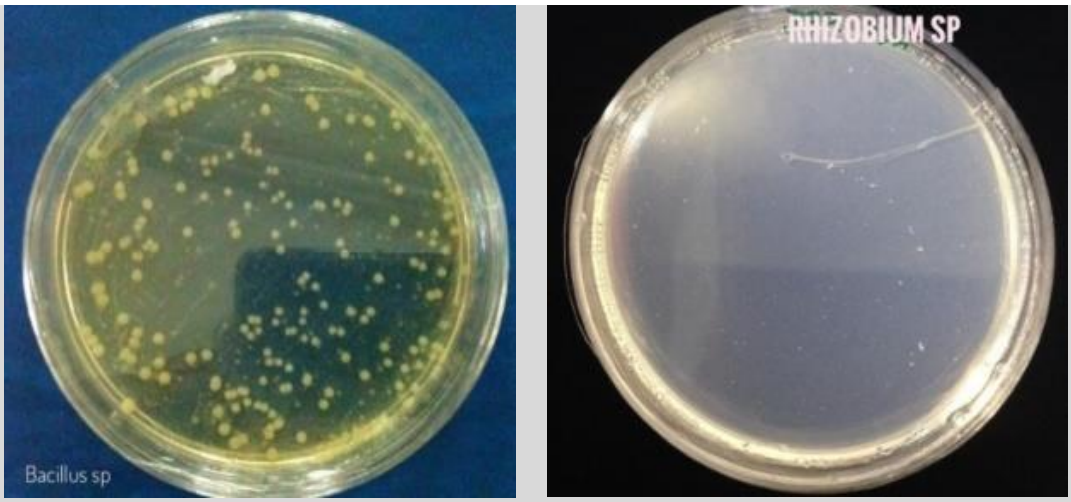

Figure 3 - Observations of Bacillus and Rhizobium microorganisms in the RIM formulation.

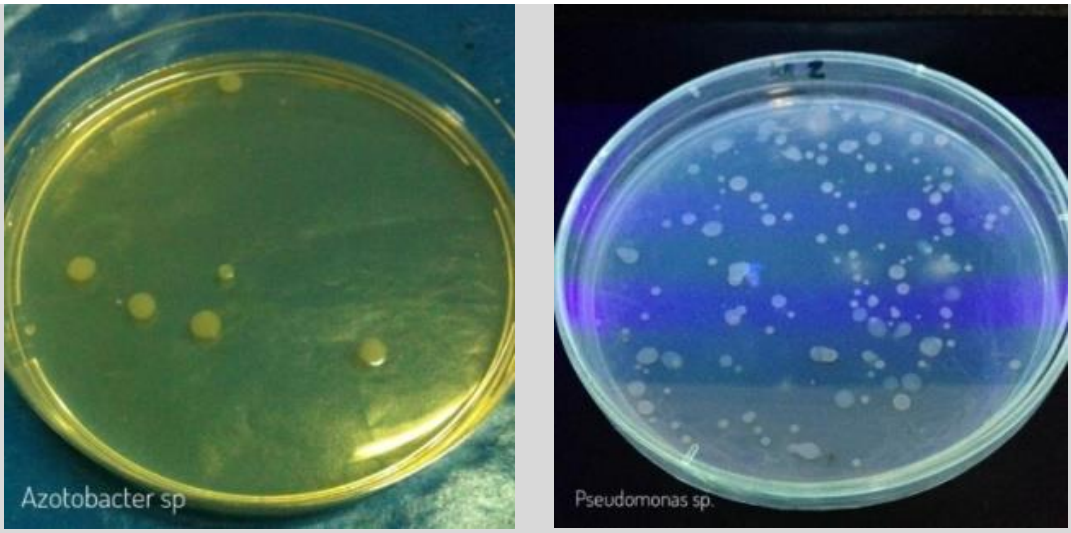

Figure 4 - Observations of Azotobacter and Pseudomonas microorganisms in the RIM formulation.

RESULTS

Bud chip cane seed growth test using different growing media and biological agents

Based on the synergy test between the microorganism consortium between the photosynthetic bacteria Sacharomyces (S), Azospirillum sp (A), Azotobacter (Z), Rhizobium (R), Pseudomonas (P), Bacillus (B) and Trichoderma sp (T) with a comparison, the formula is obtained F1, F2, F3, F4 and F5 that are used can synergize with one another. From the results of the incubation for 24 hours, each formula has a predominance and can be mixed and F3 is the best formula and is used in further research. Based on this test and formulation, a formula is obtained that can increase the speed of growth of sugarcane seeds and is expected to be used as biological fertilizer for sugarcane plants. However, it still needs to be studied in more detail whether the formula is correct in a soil biology laboratory then it is followed by a test of the content of the best Microorganism Technology formula, which is named RIM (Refresh Microorganism). The results of this RIM formulation were then tested in sugarcane bud chip nurseries in the Laboratory and sugarcane nurseries at the Green House. The process of sugarcane bud chip seeding was continued at the Green House with a combination treatment of RIM with manure. The results of the analysis of variance showed that the application of RIM, manure and control on sugarcane bud chips had no significant effect on shoot growth time (Table 1) but had a significant effect on the percentage of shoot growth (Table 2). Observation of the percentage of shoots growing stated that the addition of RIM resulted in a diversity in the percentage of bud chip that grew where the treatments of S2, S3, S4, S5, S6 and S7 were significantly different from the percentage of shoots growing in the S0 treatment (Control).

Plant height

The results of the analysis of variance showed that giving RIM had a significant effect on the height of the sugarcane bud chip. At the age of 50 DAP, the height of the bud chip plants in S0 (control) treatment was significantly different from the S1 treatment and S2, S3, S4, S5, S6 and S7 treatments. However, at the age of 30 and 40 DAP there was no significant difference between these treatments. The provision of manure and RIM showed a significant difference compared to control. At the age of 70 HST, giving RIM alone or giving manure alone was not significantly different from Control. The combination of RIM and manure contributed significantly to the increase in plant height (Table 3).

Citation: Sudiarso and Prihandarini R (2020). Study of the utilization of rim (refresh microorganism) in sugarcane. *Online J. Anim. Feed Res*., 10(5): 210-215.

Number of leaves

The results of the analysis of variety showed that the application of RIM and manure had no significant effect on the number of sugarcane bud chip leaves at the age of 30 and 40 DAP, but at the age of 50, 60 and 70 DAP there was a significant difference. Based on the results of observations made at the age of 50 to 70 days afterwards, the number of bud chip leaves in each treatment had a significantly different value (Table 4). The effect of giving RIM alone or simultaneously with manure can increase the number of leaves.

Plant nutrient elements

Content of Nitrogen and Potassium. The content of N and K elements in this study increased the provision of RIM and manure (S1, S2, S3, S4, S5, S5, S6 and S7, analysis of various K content had a significant effect compared to control (S0) between these treatments. increase the content of Nitrogen and Potassium in sugarcane seeds at the end of this research 70 days after planting. Potassium different from the control this difference can be seen in Figure 5.

Contents of P, calcium, magnesium and pH. The elemental content of phosphorus, calcium, magnesium increases with the provision of RIM and manure. This can be seen from the increase in elements of P, Ca, Mg S1, S2, S3, S4, S5, S6 and S7 compared to S0 treatment (control). The highest P content was obtained in the S3 treatment (giving RIM 20 gr/lt), but it was not significantly different from the S2, S5 and S6 treatments (giving RIM and manure). Calcium content in S3 treatment (giving RIM 20 gr/lt) was not significantly different from the treatment with RIM and manure (S4 and S5). The difference in the treatment of giving RIM 10 gr/ltd of manure alone does not provide a significant difference in the calcium content, which can be seen in Figure 6. Meanwhile, the phosphorus content of sugarcane seeds up to 70 days after planting was relatively different in the treatment given by RIM, a combination of RIM and manure and manure alone compared to the control. However, the pH was not different in all treatments, there was no significant difference.

Table 1 - The effect of giving RIM various doses and combinations of manure application on sugarcane bud chip growing time.

Treatment	Shoots growth time (days)
S0 (Control)	6.75
S1 (RIM 5 g)	7.50
S2 (RIM 10 g)	8.50
S3 (RIM 20 g)	8.75
S4 (RIM 5 g + manure)	7.25
S5 (RIM 10 g + manure)	8.50
S6 (RIM 20 g + manure)	8.90
S7 (manure)	7.80
BNT5%	NR (No Response)

Table 2 - Effect of RIM on various doses and combinations of manure application on the percentage of buds growing sugar cane chips.

Treatment	Percentage of shoots (%)
S0 (control)	53.75 [a]
S1 (RIM 5 g)	67.50 [b]
S2 (RIM 10 g)	71.50 [bc]
S3 (RIM 20 g)	76.75 [bc]
S4 (RIM 5 g + manure)	77.25 [c]
S5 (RIM 10 g + manure)	78.50 [c]
S6 (RIM 20 g + manure)	86.90 [d]
S7 (manure)	68.70 [b]

*Note: Figures accompanied by the same letter are not significantly different based on the LSD test at the 5% level

Table 3 - Effect of RIM on various doses and combinations of manure application on sugarcane bud chip plant height.

Plant Height (cm) / Treatment	Shoots Growth Time (days)				
	30	40	50	60	70
S0 (control)	9.36	10.56	11.79 [a]	13.25 [a]	15.33 [a]
S1 (RIM 5 g)	9.58	11.32	13.55 [b]	14.88 [b]	15.88 [a]
S2 (RIM 10 g)	9.63	11.39	13.89 [b]	14.78 [b]	16.22 [b]
S3 (RIM 20 g)	9.78	11.45	14.55 [bc]	15.36 [bc]	16.59 [b]
S4 (RIM 5 g + manure)	9.65	11.36	13.78 [bc]	14.99 [bc]	16.55 [b]
S5 (RIM 10 g + manure)	9.77	11.79	15.44 [cd]	15.89 [c]	16.52 [b]
S6 (RIM 20 g + manure)	9.82	11.89	15.88 [d]	15.99 [c]	17.22 [c]
S7 (manure)	9.52	11.22	13.77	14.33[b]	15.89 [a]
BNT 5%	Nr	Nr	1.5	0.8	0.7

*Note: Figures accompanied by the same letter are not significantly different based on the LSD test at the 5% level

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Table 4 - Effect of RIM on various doses and combinations of manure application on the number of leaves of sugarcane bud chips

Treatment \ Number of Leaves	Shoots Growth Time (days)				
	30	40	50	60	70
S0 (control)	1.36	1.56	3.79 a	3.95 a	5.33 a
S1 (RIM 5 g)	1.58	1.82	3.55 b	4.88 b	5.88 a
S2 (RIM 10 g)	1.63	1.39	3.89 b	4.78 b	6.22 b
S3 (RIM 20 g)	1.78	1.45	4.55 bc	5.86 bc	6.59 b
S4 (RIM 5 g + manure)	1.65	1.36	3.78 bc	5.99 bc	6.55 b
S5 (RIM 10 g + manure)	1.77	1.79	5.44 cd	5.89 c	6.52 b
S6 (RIM 20 g + manure)	1.82	1.89	5.88 d	5.99 c	6.22 b
S7 (manure)	1.52	1.22	3.77 bc	4.33 b	5.89 a
BNT 5%	nr	nr	1.5	0.5	0.7

*Note: Figures accompanied by the same letter are not significantly different based on the LSD test at the 5% level

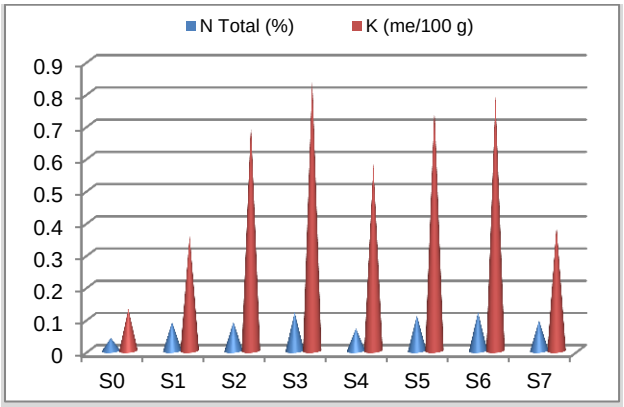

Figure 5 - Nitrogen and potassium nutrient content in sugarcane bud chip seeding research.

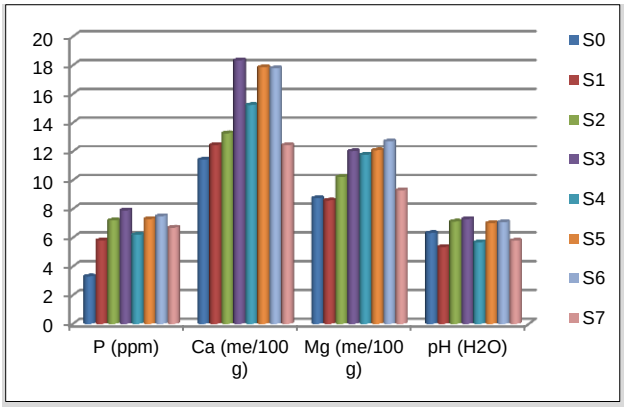

Figure 6 - Nutrient content of phosphorus, calcium, magnesium and pH in sugarcane bud chip breeding research.

DISCUSSION

The results showed that from the time of growth the treatments given by RIM and the provision of manure and the combination (S0, S1, S2, S3, S4, S5, S6 and S7) were not significantly different from the control (S0). However, the parameters of the proportion of plant proportions, plant height and number of plants treated with S0 (control) were significantly different from other treatments and the lowest treatment was at certain observation ages.

This is because in the S0 treatment (control) there was no colony of RIM bacteria which was able to increase plant growth from hormones and some organic compounds produced by these bacteria (Hindersah et al., 2004; McMillan, 2007). In addition to hormones and organic compounds, RIM can increase resistance to pathogen attack, for example *Colletotrichum falcatum* (Rahni, 2012), one way for RIM bacteria to increase plant resistance to pathogenic attack is by producing secondary metabolites such as siderophore. antibiotics, hydrogen cyanide, extracellular enzymes and induce plant resistance and are able to synthesize pathogenic cell wall degradation enzymes such as chitination, 1,3-glucanase, 1,4-glucanase, cellulase, lipase and protease and produce 1-aminocyclopropane, ACC deaminase (Baharun et al., 2003; Haung et al., 2004; Sutariati, 2006).

The results showed that the growth of bud chips with S0 treatment (control) was lower than that of other treatments (S1, S2, S3, S4, S5, S6 and S7) for certain parameters and age of observation. Giving RIM and manure could increase the percentage of shoot growth and plant height compared to the control. Observation data from several parameters that represent the growth of bud chip treatment S0 (control) is the observation data of bud chip plant height parameters where the S0 treatment (control) at the age of 70 DAS is almost equivalent to all treatments given RIM at the age of 60 DAP. This means that the growth of bud chip with S0 treatment (control) is slower than other treatments. This is in accordance with the results of research that the use of bacteria can increase growth compared to control treatment (Syamsuddin and Ulim, 2013). According to Suwahyono et al. (2011), the microbes contained in biological fertilizers when applied to plants can bind nitrogen from the air, dissolve phosphates that are bound in the soil and break down complex organic compounds into simpler compounds and stimulate plant growth.

Trichoderma, Sacharomyces, Bacillus, Rhizobium, Azotobacter and Pseudomonas found in the RIM formulation can be consortium and work together to increase the acceleration of growth of shoots from clear sugarcane. Especially, if it is mixed with manure which is food for these bacteria, Suwahyono et al. (2011) stated that the combination treatment of N, P and K fertilizers as well as biological fertilizers produced a larger stem diameter compared to the single biological fertilizer treatment.

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CONCLUSION

Its concluded that the use of RIM's biological agents (such as refresh microorganisms) using Tricho derma, Sacharomyces, Bacillus, Rhizobium, Azotobacter and Pseudomonas bacteria can increase the growth of sugarcane buds. The combination of using RIM and manure can increase the number of leaves and the percentage of growth of sugarcane bud chips. It is necessary to continue research in the field in order to know the production and yield.

DECLARATIONS

Consent to publish
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Authors' Contribution
All the three authors reviewed the paper and contributed in developing the content.

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SYSTEMATIC REVIEW ON GASTROINTESTINAL HELMINTHS OF DOMESTIC RUMINANTS IN ETHIOPIA

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Supporting Information

REVIEW

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ABSTRACT: This systemic review was conducted to identify, critically assess, and bring together available data from primary researches conducted so far on gastrointestinal (GI) helminthes of domestic ruminants in Ethiopia. In the country, GI helminths of domestic ruminants have been identified; examined and informative statistics has been extracted since a few decades ago. For this review, relevant articles were retrieved from English databases: PubMed, Google Scholar, Science Direct, Web of Science and Scientific Information Database (SID). Additional studies were recognized by scanning the African Journal Online (AJOL) that includes the Ethiopian Veterinary Journal and Bulletin of Animal Health and Production. Out of retrieved (n=154) articles, thirty three (n=37) articles which fulfilled the eligibility criteria were selected. Accordingly, twenty three GI helminthes species which belong to the three classes of helminthes have been found to occur in domestic ruminants in the country. The main genera reported so far are *Haemonchus*, Strongyloides, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum*, *Fasciola*, *Monezia* and *Paramphistomum* whereas, *Haemochus contortus*, *Moneizia expansa* and *Fasciolahepatica* are the most frequently reported species from Nematode, Cestode and Trematode classes respectively. The overall GI helminths prevalence ranged from 2.3% to 100% were reported. Simple flotation, sedimentation, modified McMaster technique and faecal culture are the most common and routine diagnostic methods which have been used in the country. Management aspects like husbandry practices, climate and host influences are found to be the principal contributing factors that affect GI helminths infections. So far, the control of GI parasites in the country is mainly focusing on the use of anthelmintics. Consequently, due to the lack of effective control strategies, antihelmintics are exclusively used which result in antihelmintics resistance. Generally, occurrence, epidemiological features, realistic control strategies, common diagnostic procedures and frequently encountered species are reviewed. Finally, the relevance of epidemiological knowledge and the development of efficient, sustainable and conventional control measures which cover wider ago-climatic zones of the country are suggested for controlling GI helminths infections and should be assessed timely.

Keywords: Anthelmintics, Domestic ruminant, Ethiopia, Gastrointestinal helminthes.

INTRODUCTION

As a result of having different agro-ecological zones and favorable environmental situations in Ethiopia, the country is believed to be endowed numerous livestock species and suitable for livestock production. It has the largest livestock population in Africa (Tilahun and Schmidt, 2012; CSA, 2013). According to CSA (2013) report, an estimated statistics showed that about 54 million cattle, 25.5 million sheep and 24.06 million goats are found in the country. Of the total cattle population, 98.95% are local breeds and the remaining are hybrid and exotic breeds. Furthermore, 99.8% of the sheep and nearly all goat population of the country are local breeds (CSA, 2013).

However, diseases have numerous negative impacts on production and productivity. Among diverse animal diseases encountered in the country, helminthes infections remain one of the most important limiting factors and a bottlenecking production and productivity these days (Elsa et al., 2012). By chance, the gastrointestinal tract of ruminants harbors variety of parasites particularly helminthes which can cause both clinical and subclinical parasitism. As stated by Lebbie et al. (1994), GI helminthes infections are of a global concern for livestock industry, which have devastating impact in Sub-Saharan Africa in general and in Ethiopia in particular as a result of wider range of agro-ecological factors which are fitting for diversified hosts and parasite species. Hence, gastrointestinal (GI) helminthiasis has become among the most important diseases encountered by livestock sector of Ethiopia and has been considered to be one of the major constraints in the development of the sector (Regassa et al., 2006). In Ethiopia, helminthiasis is responsible for 25% mortality and 3.8% weight loss in highland sheep (Bekele et al., 1992).

Citation: Fentahun T (2020). Systematic review on gastrointestinal helminths of domestic ruminants in Ethiopia. *Online J. Anim. Feed Res.*, 10(5): 216-230.

According to Zahid et al. (2005), the helminthes infections of ruminants are mostly caused by nematodes like Strongyliodes spp., *Ostertagia* Spp., *Bunostomum* and Trichuris spp.; Cestodes such as *Moniezia* spp., *Taenia* spp. and Trematodes such as *Paramphistomum* spp., *Fasciola* spp. and *Shistosoma* spp. Perry et al. (2002) reported that GI nematodes have been ranked highest on globalindex with *Haemonchus contortus* on top. Moreover, Trichostrongyloidea that include genera such as *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Nematodirus*, and the Strongyloidea and Ancylostomatoidea with *Oesophagostomum* and *Bunostomum*, are the economically most important and widely prevalent GI nematodes (Takele et al., 2013; Winter et al., 2018). In addition, such a pervasive occurrence of the metacestodes; *Cysticercus ovis* and *Cysticercus tenuicollis*; Hydatid cyst (*E.granulosus*); *Fasciola hepatic* and *Fasciola gigantic* are most prevalent in the country and considered to be of great economic importance (Lemma et al., 1985; Regassa et al., 2009; Nigatu et al., 2009; Kebede et al., 2009; Feyesa et al., 2010; Nigatu, 2010; Endale et al., 2013; Abebe et al., 2015; Beyene and Hiko, 2019).

There are many associated risk factors influencing the occurrence and epidemiology of GI helminthes including age, sex, weather condition and husbandry or management practices (Khan et al., 2009). Factors such as host age, physiological status, breed, parasite species involved, and the epidemiological patterns (husbandry practices and climate variables) determine the degree of infection (Tembely and Hansen, 1996; Menkir et al., 2007). In Ethiopia, several studies have been conducted on ruminant helminthiasis in various regions reporting a prevalence ranged from 2.3–100% (Fikru et al., 2006; Abebe and Esayas, 2001; Yirsaw and Zewdu, 2015).

A number of published research reports have been found on GI helminthes of domestic ruminants in Ethiopia. However, these reports are found in a separate and unorganized way. So, comprehensive and well organized documentation about GI helminthes of domestic ruminant in the country is essential to support researchers, professionals and policy-makers to develop further actions on the control and prevention strategies. Therefore, the aim of this systematic review was to identify, assess critically, and bring together available data from primary research conducted so far on gastrointestinal (GI) helminthes of domestic ruminants in Ethiopia.

MATERIALS and METHODS

Source, selection strategies and protocol

This systematic review was carried out on GI helminthes of Ethiopian ruminant using available electronic and non-electronic databases. The electronic search was used as the primary search method. The main electronic databases used were PubMed, Google Scholar, Science Direct, Web of Science, Scientific Information Database (SID) were accessed from University of Gondar, Ethiopia. Relevant studies have been identified from English databases in Pub Med, Google Scholar, Science Direct, Web of Science, Scientific Information Database (SID). Additional studies were recognized by scanning the African Journal Online (AJOL) that includes the Ethiopian Veterinary Journal and Bulletin of Animal Health and Production. Moreover, complete and congress articles like original descriptive studies (designated as cross-sectional study) in sheep, goats and cattle were also considered. Epidemiological parameters such as prevalence of GI helminthes infection among sheep, goat and cattle, and main contributing factors like age, sex, and geographical sub-regions of Ethiopia were considered thoroughly. An intensive data searching was made to collect available information (Figure 3). The searching strategies used were combining the phrases close to GI helminths in large and small ruminants in Ethiopia as indicated in Figure 1. Searches were restricted to peer-reviewed articles published nationally or internationally in English language.

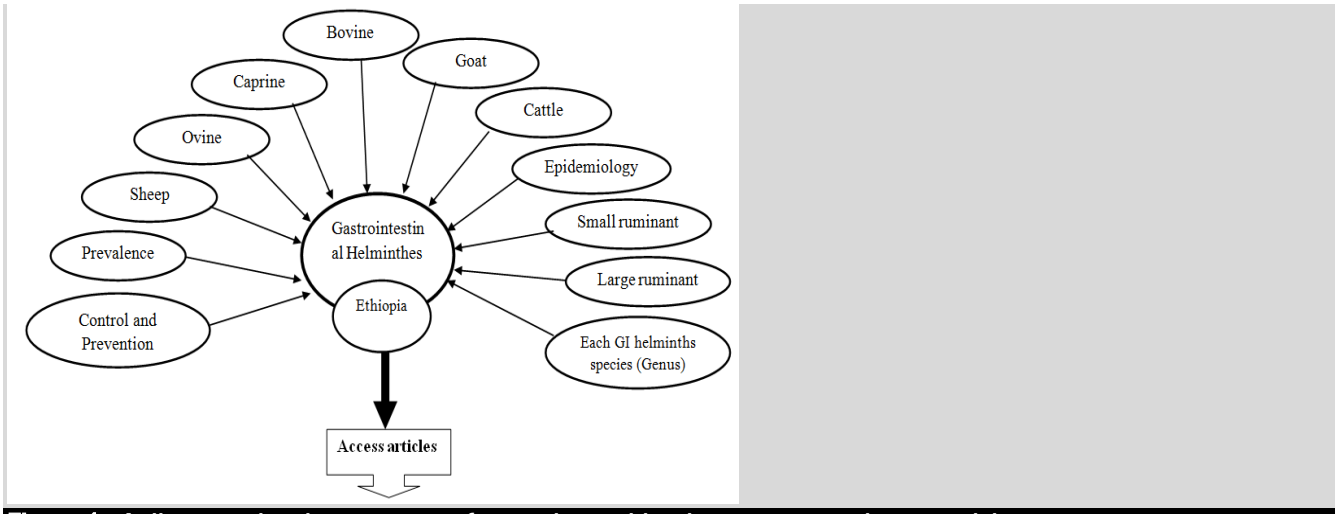

Figure 1 - A diagram showing a strategy for words combination to access relevant articles.

Citation: Fentahun T (2020). Systematic review on gastrointestinal helminths of domestic ruminants in Ethiopia. *Online J. Anim. Feed Res.*, 10(5): 216-230.

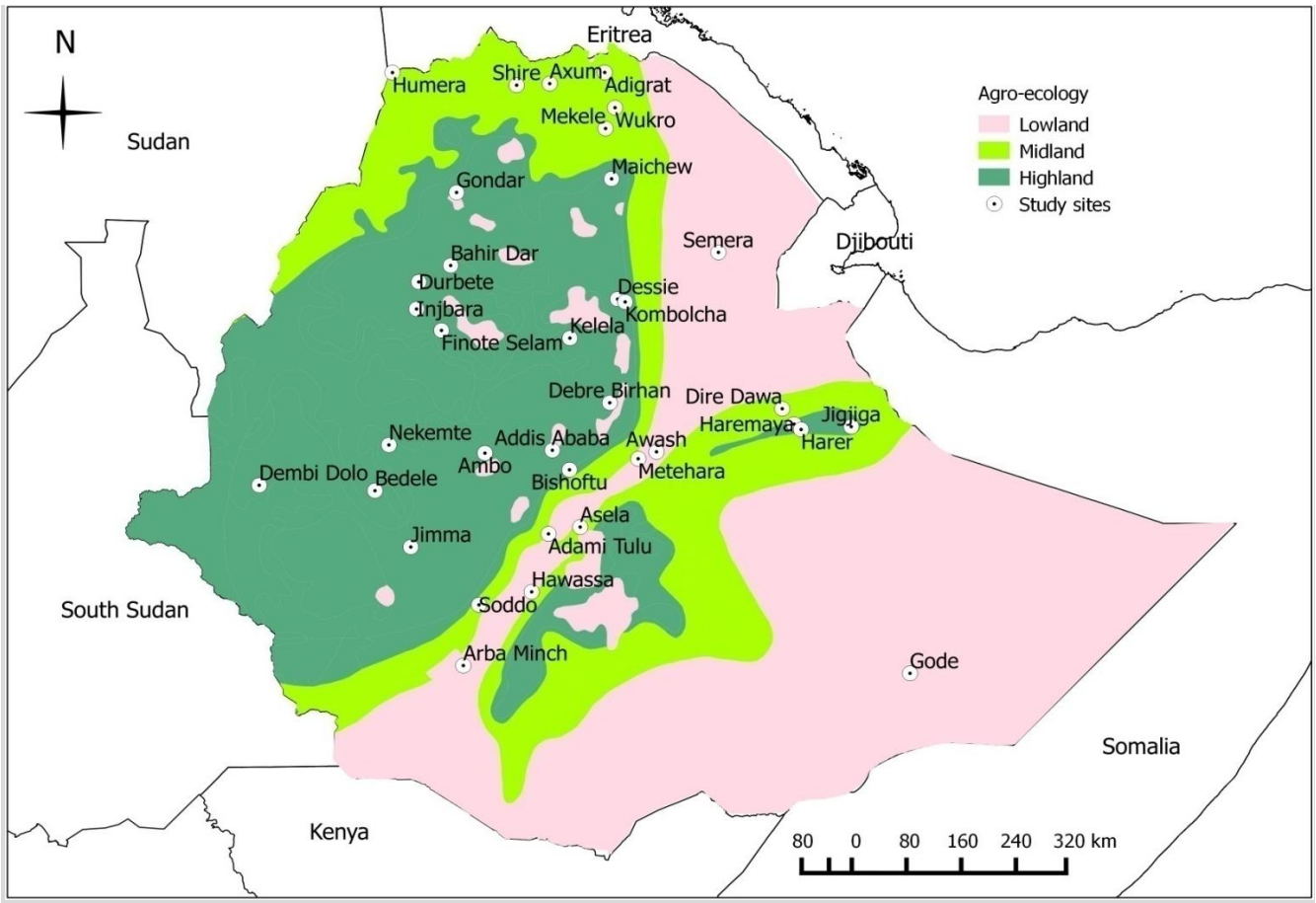

Figure 2- Map of Ethiopia showing study sites of GI helminthes of domestic ruminants.

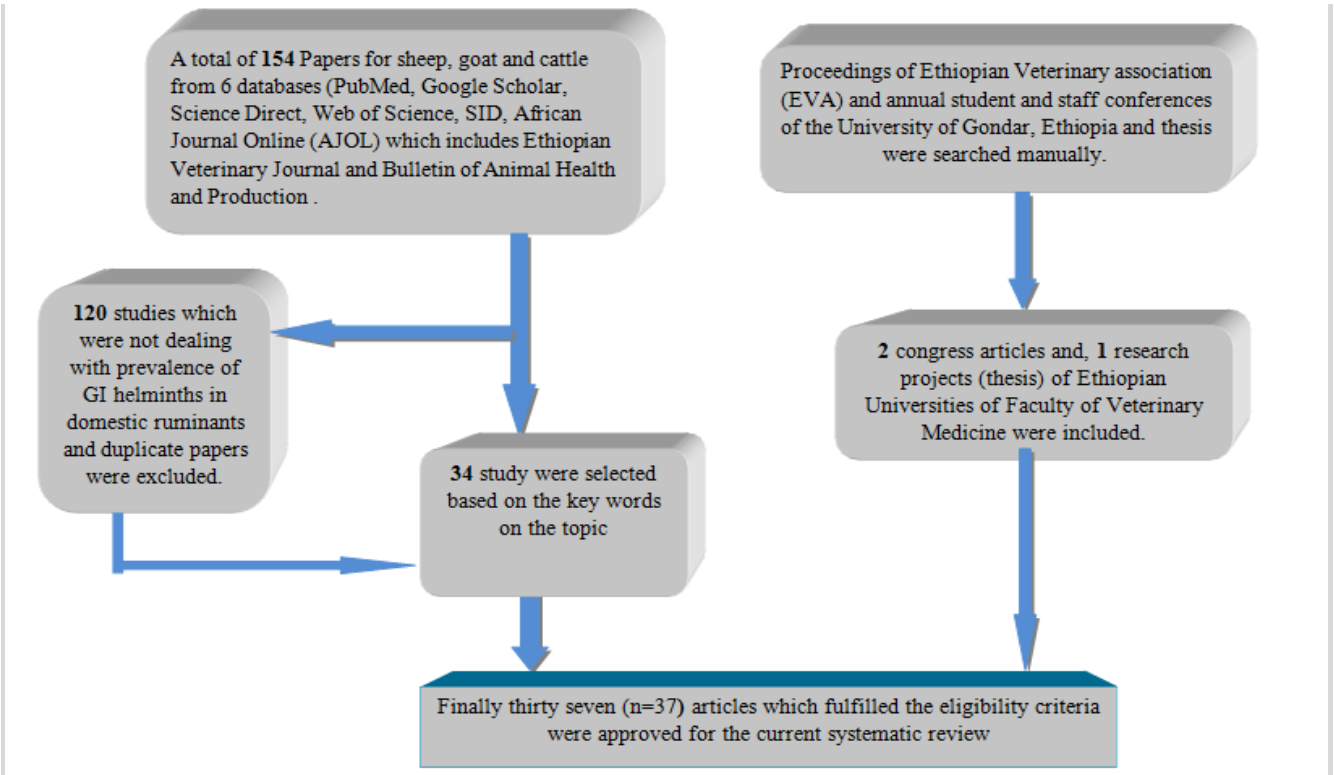

Figure 3 - Flow diagram describing the study design process and literature search on GI helminthes in domestic ruminants.

Data type and collecting methods

Published papers were scanned by quick reading to select relevant articles. Important articles were defined and included in the current review as one that contained information on GI helminthes of cattle, sheep and goat in Ethiopia. References of all relevant articles were searched to identify articles that were missed by the electronic search. Any

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identified article was subjected to the same inclusion process as a data type. Following that all relevant articles were reviewed, extracted and compiled in a searchable database (Microsoft Access software, ver. 2007). Extracted information included authors name, study sites, year of the study commenced and ended, year of publication, study design, species of animals, sampling procedure, number of animals selected, body condition, sex, altitude of the study site, laboratory procedure to detect the parasite, testing methodology and prevalence of GI helminthes reported including their associated risk factors.

Reports collected on GI helminths in Ethiopia

The initial electronic searches yielded a total of (n=154) studies. After scrutinizing these and eliminating duplicates, thirty three (n=37) were considered (Figure 4) including thesis containing relevant information concerning GI helminths of ruminants in Ethiopia. This review work documented that of the relevant articles about 8% (3) were not retrieved by any of the search engines, 19% (7) were retrieved by a single search engine, 27% (10) by two, 16% (6) by three and 30% (11) by all four databases. Providing a balanced and an impartial summary of the topic using these representative studies was at the core of this review. No restrictions other than identifying ovine, caprine and bovine GI helminthes in Ethiopia were imposed on the inclusion criteria. This minimized literature selection bias and provided an exhaustive list of GI helminths. However, for factors associated with domestic ruminant GI helminthes in Ethiopia, strict inclusion criteria were applied in order to only select the critical studies. In order for the current review to be critically appraised, repeatability was crucial. This was achieved by documenting every step of the review process.

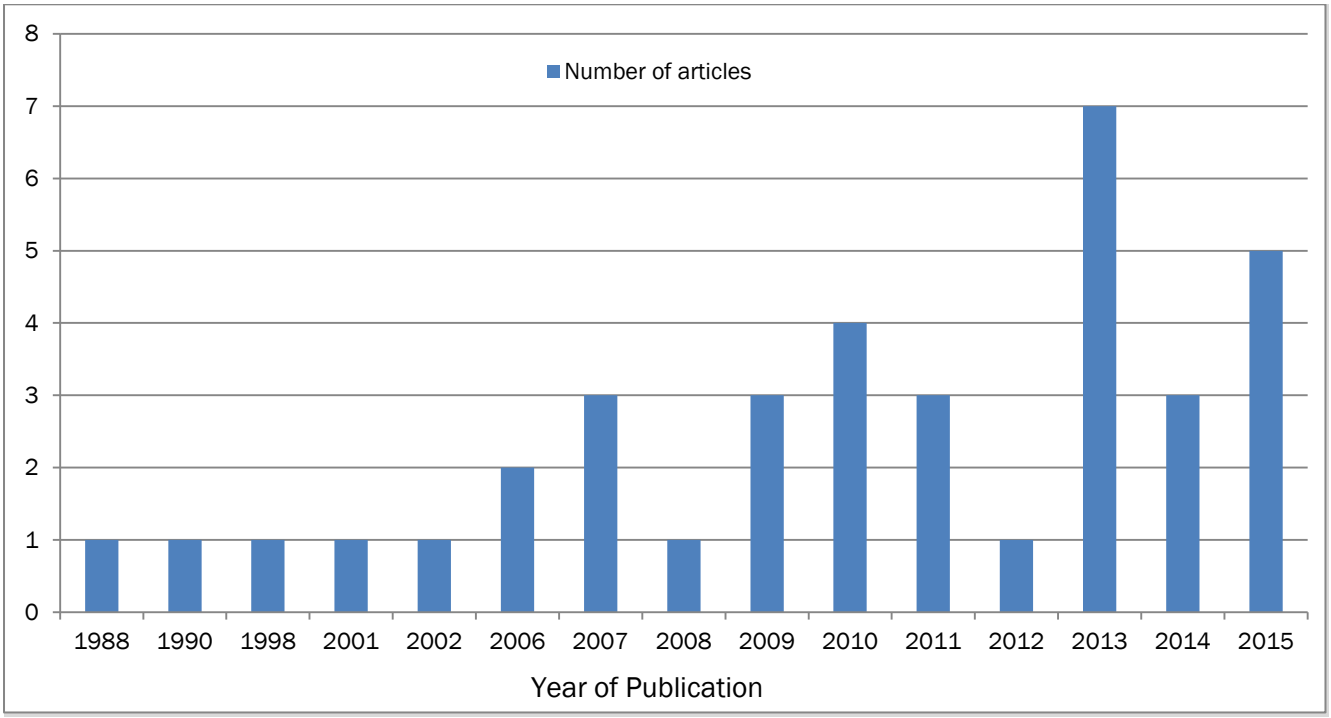

Figure 4 - Frequency of articles relevant to the systematic review on GI helminths of domestic ruminants in Ethiopia.

Occurrence and spatial distribution

The present review work revealed that the occurrences of GI helminthes infections were reported in different parts of the country (Figure 2). The prevalence of GI helminthes is summarized in table 1. The majority of the prevalence studies were specific on some helminthes genera like Strongyles, *Fasciola*, *Shistosoma* and some families like GI nematodes, Trematodes and Cestodes. However, adequate sources were not accessed compared to the number of studies done on specific species and families of the GI helminthes of domestic ruminants. In addition, most studies are based on the coprological examinations and few studies were conducted on abattoir surveys in the country.

According to the result obtained from the review, the overall prevalence of GI helminthes is ranged from 13.2% in Awash River Basin (Afar region) to 81.5% in Debre Zeit/Bishoftu (Bersissa et al., 2011; Moti et al., 2013). Furthermore, the review underlined that the majority of the identified species are found from nematodes and the lowest from Cestode. On the other hand, *Haemochuscontortus*, *Moneiziaexpansa* and *Fasciolahepatica* are the predominant species from Nematode, Cestode and Trematode classes respectively. Furthermore, *Nematodirus* spp., *Toxocaravitulorum* and *Schistosoma* spp. are less prevalent (Table 2). In Ethiopia the majority of the studies were focusing only on small ruminants' GI helminthes than large ruminants. Therefore, the highest prevalence of the parasite was reported from sheep (91.2%) in Gode (Ogaden), Southern Ethiopia than cattle (21.9%) in Nekemete in Western part of Ethiopia (Table 1). On the other hand, most Abattoir survey reports have also shown a status of GI helminths of cattle than small ruminants.

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Table 1 - List of selected studies on GI helminthes of domestic ruminants in Ethiopia

Study site(s)	Study animals and Sample size				Study period	ST	Diagnostic technique	Prevalence (%)				Sample	Author(year)
	Sheep	Goat	Cattle	Total				Sheep	Goat	Cattle	Total		
Adama	92	208	852	1152	Nov2007-Apr2008	CS	PME	29.3	6.7	46.8	-	Liver	Getawa et al. (2010)
Adami Tulu	-	499	-	499	Jul1997-Jun1998	CS	MM	-	45.5	-	45.5	F	Etana (2002)
Addis Ababa	560	-		560	Sep 1985 - Jun 1986	CS	PME	19.6	-	-	19.6	Liver	Bekele et al.(1988)
Ambo	90	30	-	120	Feb-May 2013	CS	DS,FL,SD	47.8	53.3	-	-	F	Temesgen and Walanso (2015)
Arba Minch	241+20	357+25	-	598 + 45	Jan2010-Aug 2011	CS	SD,FL, MM,PME	79.7; 85	63.6; 68	-	70.1;	F, GIT	Nejib et al. (2014)
Asella	-	-	384	384	Nov 2013-Apr 2014	CS	FL	-	-	49	49	F	Addisu and Berihu (2014)
Asella	408	-	-	408	Nov 2008-Apr 2009	CS	MMM	68.1	-	-	68.1	F	Diriba et al. (2013)
Awash	3,697	-	-	3,697	Jan-Dec 2005	CS	EAC	13.2	-	-	13.2	F	Ahmed et al.(2007)
Bahir Dar	340	-	420		May 2005 - Dec2006			10.6	-	34.05	-	Liver	Nigatu et al. (2009)
Bahir Dar	384	-	384	768	Nov2010-March 2011	CS	SD, PME	10.9; 16.9	- -	31.5; 45.3	-	F, Liver	Ayalew and Endalkachew (2013)
Bahir Dar	-	-	384	4134	Nov2008-March2009	CS	SD, PME	-	-	39.95	39.95	F, Liver	Fikirtemariam et al. (2013)
Bahir Dar	-	-	384	384	Nov2013-April 2014	CS	SD	32.3	-	-	32.3	F	Yitayal et al. (2015)
Bedele	-	-	500	500	Oct 2011-March 2012	CS	SD, FL, MM	-	-	64.2	64.2	F	Moti et al. (2013)
Bishoftu (Debre Zeit)	1152	1536	-	2688	Dec2005 - Jun 2006	CS	PME	58.5	43.8	-	-	Liver	Jibat et al. (2008)
Bishoftu (Debre Zeit)	157	65	-	222	Nov2007-Apr2008	CS	FL,SD, CC	81.0	83.0	-	81.5	F	Bersissa et al. (2011)
Bishoftu (Debre Zeit)	-	-	326	326	Nov2011-Apr 2012	CS	FL, SD,	-	-	61	61	F	Cheru et al. (2014)
Debre Berhan	2500	-	-	2500	1987	CS	SD,FL	18	-	-	18	F, PME	Njau et al.(1990)
Dembi Dolo	255	245	257	757	2003-2004	CS	DS, SD,FL, MM	75.3	84.1	50.2	69.6	F	Fikru et al.(2006)
Dessie	510	420	-	930	Nov2011-Mar 2012	CS	PME	21.04	27.61	-	-	OM, PE, Liver,MS	Abebe et al.(2015)
Dire Dawa	425	420	-	845	Nov2011-April 2012	CS	PME	22.8	26.4	-	24.6	Liver, PE OM	Endale et al. (2013)
Durbete	202-	-	330	532	Oct 2014- Apr 2015	CS	SD	2.3	-	24.6	26.9	F	Yirsaw and Zewdu (2015)
Gondar	458	100	-	558	Nov-Jan 2008	CS	SD, FL	46.07	55	-	47.67	F	Shimelis et al. (2011)
Gondar, Finote Selam, Injibara	-	-	22,755	22,755	Sept 2002-2007	CS	PME	-	-	79.5	79.5	Liver	Nigatu (2010)
Haremaya, Harar, Dire Dawa, Jijiga	655	632	-	1,287	May 2003-Apr 2005	CS	PME				-	GIT	Menkir et al. (2007)
Hawassa	284	226	-	510	Nov2008-Feb 2009	CS	MMM	47.2	38.9	-	-	F	Abebe et al. (2010)
Hawassa	-	-	632	632	Dec2008 – Mar 2009	CS	PME	-	-	52.6	52.6	Liver	Feyesa et al. (2010)
Hawassa	180	132	-	312	Jan-Jun 2006	CS	PME	91.1	87.1	-	-	A	Thomas et al. (2007)
Jimma	-	-	210	210	Nov2008-Apr 2009	CS	FL,SD, MMM	-	-	77.6	77.6	F	Hailu et al. (2011)
Kelala	230	-	154	384	Sept1997-Apr1998	CS	FL, SD	53	-	38	-	F	Tesfaye (1998)
Kombolcha	-	-	400	400	2011	CS	PME	-	-	17	17	Liver	Fufa et al. (2012)
Mekelle	-	-	1023	1023	Nov2007 - Feb2008	CS	PME	-	-	7. 23	7. 23	Liver	Getachew and Ashwani (2013)
Mekelle, Adigrat, Axum, Humera, Maichew, Shire	-	-	5,194	5,194	2007/8	CS	PME	-	-	22.1	22.1	Liver	Kebede et al. (2009)
Metehara, Semera, Jigjiga	92	91	-	183	Nov1998-Apr 1999	CS	MMM, PME	97.03	100	-	-	F , GIT	Abebe and Esayas(2001)
Nekemete	-	-	384	384	Nov2011-March 2012	CS	PME	-	-	21.9	21.9	GIT	Alula et al. (2013)
Ogaden-Gode	114	82	-	196	Aug2003-March 2004	CS	PME	91.2	82.9	-	-	A	Kumsa and Wossene (2006)
Wolaita Soddo	-	-	415	415	Nov2007 - Apr2008	CS	PME	-	-	11.3	-	Liver	Regassa et al. (2009)
Wukro	384	-	-	384	Nov 2013-Apr 2014	CS	PME	40.9	-	-	40.9	A	Lidya and Berihun (2015)

-: No information available; ST=Study design; PME=Postmortem examination; CS=Cross sectional; SD=Sedimentation, FL=Flotation, MM=Mc Master; DS=Direct Smear; MMM=Modified Mc Master; F= Faeces; A=Abomasum; GIT=Gastrointestinal content; EAC= Ethyl-acetate Centrifugation; PE=peritoneum; OM=Omentum; MS=Mesentry; RT= Retrospective

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Table 2 - GI helminthes species/genus composition; collected from each selected studies in Ethiopia.

Species of parasite identified (%)												Location	Altitude of study area	Author (year)
Nematode	Species of animal			Cestode	Species of animal			Trematode	Species of animal					
	Sheep	Goat	Cattle		Sheep	Goat	Cattle		Sheep	Goat	Cattle			
-	-	-	852	Hydatid cyst	40.0	55.6	37.1	-	-	-	-	Adama	1770	Getaw et al. (2010)
H.contortus	-	30.3	-											
Strongyloidesspp.	-	16.4	-											
Trichostrongylus spp.	-	13.8	-	*M. expansa*	-	14.6	-	-	-	-	-			
Trichuris spp.	-	2.2	-									Adami Tulu	900	Etana (2002)
Nematodirus spp	-	2.8	-	*M. benedeni*	-	4.0	-	-	-	-	-			
Chabertia ovina	-	5.0	-											
Bunostomum spp.	-	5.4	-											
-	-	-	-	*T.hydatigena and Hydatid cyst*	18.6	-	-	-	-	-		Addis Ababa	2,326	Bekele et al. (1988)
Strongyles spp.	47.8	53.3	-	-	-	-	-	*Fasciola* spp.	4.4	6.7	-	Ambo	2185	Temesgen and Walanso (2015)
H.contortus	A*	A*	-	-										
Trichostrongylus spp.	A*	A*	-	-										
Teladorsagia spp.	A*	A*	-	-								Arba Minch	1400	Nejib et al. (2016)
T.colubriformis	A*	A*	-	-										
Haemonchus spp.	A*	A*	-	-										
*Ostertagia*spp	-	-	1.8											
*Oesophagostomum*sp	-	-	1.3											
Strongloid spp.	-	-	2.6											
Trychostrongylusspp	-	-	3.6									Assela	1500-2300	Addisu and Berihu (2014)
Hemonchusspp.	-	-	11.7											
Bunostomum spp.	-	-	4.4											
Strongylesspp	64.0	-	-											
Strongyloidesspp	7.4	-	-									Asella	2500-3000	Diriba et al. (2013)
Trichurisspp	3.7	-	-											
-	-	-	-	-	-	-	-	*Fasciola* spp.	13.2%	-	-	Awash	550-850	Ahmed et al. (2007)
-	-	-	-	*E. granulosus*	25.0	-	16.1	-	-	-	-	Bahir Dar	1784	Nigatu et al. (2009)
-	-	-	-	-	-	-	-	*F. hepatica* and *F.gigantica*	16.9	-	45.31	Bahir Dar	1784	Ayalew and Endalkachew (2013)
-	-	-	-	-	-	-	-	*F.hepatica*	-	-	89.7			
								F.gigantic	-	-	3.63	Bahir Dar	1784	Fikirtemariam et al. (2013)
								Mixed	-	-	6.67			
-	-	-	-	-	-	-	-	*F. hepatica*	-	-	32.3	Bahir Dar	1784	Yitayal et al. (2015)
Trichuris spp.	-	-	6 .8	-	-	-	-	*Fasciola* spp	-	-	51.4	Bedele	2060	Moti et al. (2013)
Trichostrongyles spp.	-	-	11.0	-	-	-	-	Paramphistomu spp,	-	-	18.7			
-	-	-	-	*St.hepatica*	9.5	12.1	-	*Fasciola* spp.	6.9	3.6	-	Bishoftu	2155	Jibat et al. (2008)
				C.tenuicollis	5.2	8.3	-							
Strongyle type	56.60	61.0	-	M.expansa	13	29.2	-	*Fasciola*spp.	0.63	0	-	Bishoftu	2155	Bersissa et al. (2011)
Strongyloides spp.	8.2	15.4	-											

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Trichuris spp.	5.0	0.0	-											
Strongyle spp. Trichuris spp. *Toxocara* spp. Trichuris spp.	- - - -	- - - -	41.4 41.4 5.2 5.2	*Monezia* spp.	-	-	2.8	*Fasciola* spp. *Paramphistomum* spp.	- -	- -	36.5 18.4	Bishoftu	2155	Cheru et al. (2014)
Trichuris ovis *T, axei* *H. contortus* *Ostertagia* spp.	25.4 19.4 2.9 1.5	- - - -	- - - -	*C. ovis* *Moniezia* spp.	11.9 7.5	- -	- -	*Fasciola hepatica*	4.5%	-	-	Debre Berhan	2500-300	Njau et al. (1990)
Ascaris spp. Strongyle spp. Dictyocaulus spp. Trichuris spp.	1.6 70.2 4.3 4.5	2.5 78.4 1.2 9.8	2.8 47 0.4 1.6	-				*Fasciola* spp.	0	0	0.4	Dembi Dolo	1500-2000	Fikru et al. (2006)
-	-	-	-	*C.tenuicollis, C.ovis* Hydatid cysts	45.69, 8.43 9.02	72.38, 8.57 1.90	- - -	-	-	-	-	Dessie	2,400	Abebe et al. (2015)
-	-	-	-	*C.tenuicollis*	22.8	26.4	-	-	-	-	-	Dire Dawa	1204	Endale et al. (2013)
Shistisoma spp.	2.3	-	24.6	-	-	-	-	-	-	-	-	Durbete	1600	Yirsaw and Zewdu (2015)
Strongyle spp. Trichuris spp.	34.06 4.8	54 3	- -	*Monezia* Spp.	3.93	0	-	*F. hepatica* *Paramphistomum spp.*	8.52 0.87	6.99 0	- -	Gondar	1500-2000	Shimelis et al. (2011)
-	-	-	-	Hydatid cysts	-	-	38.8	-	-	-	-	Gondar, Finote Selam, Injibara	1917-2560	Nigatu (2010)
H.contortus. *T.axei* *T.vitrinus* *Trichostrongylus* spp. *Nematodirus spathir* *Cooperia curticei* Strongyloides spp. *Bunostomum* spp *Oes.columbianum* *Oesophagostomum* *Chabertia ovina* Trichuris spp	A* A* A* A* A* A* A* A* A* A* A* A* A*	A* A* A* A* A* A* A* A* A* A* A* A* A*	- - - - - - - - - - - - -	*M. expansa* *Av.centripunctata* *St.globipunctata* *St.hepatica* *Cysticercus ovis)* *C.tenuicollis* *E. granulosus* (cysts)	61 20 24 39 26 79 68	53 21 27 36 22 53 65		*P.microbothrium* *F.hepatica* *F. gigantica* *Dicrocoelium dendriticum*	25 26 20 7	21 3 10 2		Haremaya, Harar, Dire Dawa, Jijiga	2000	Menkir et al. (2007)
Haemonchus spp. *Trichostrongylus* spp. *Oesophagostomum* Trichuris spp. *Bunostomum* spp.	51.6 38.7 22.6 27.6 9.7	64.7 41.2 23.5 12.5 11.8										Hawassa	1790	Abebe et al. (2010)
-	-	-	-	Hydatid cysts	-	-	6.9	6.9	-	-		Hawassa	1790	Feyesa et al. (2010)
Haemonchus spp. *T.axei* Teladorsagia spp.	81.1 47.2 19.4	76.5 39.4 20.5	- - -									Hawassa	1790	Thomas et al. (2007)

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Strongyels spp. Strongyloid spp. Trichuris spp. Ascaris spp. Capillaria spp. *Nematodirus* spp.	- - - - - -	- - - - - -	32.4 3.3 1.9 2.4 1.4 0.9	M. expansa	-	-	5.2	*Fasciola* spp. *Paramphistomum*	- -	- -	23.3 48.6	Jimma	1750	Hailu et al. (2011)
Strongyles spp. Trichuris spp. Strongyloides spp. *Nematodirus* spp.	27.58 2.95 3.20 0.49	- - - -	3.6 0.74 0.6 5.3	*Moniezia* sp.	4.18	-	0.4	*F. hepatica*	1.47	-	4.8	Kelala,	1580	Tesfaye (1998)
-	-	-	-	Hydatid cysts	-	-	4.25	-	-	-	-	Kombolcha	500 - 1840	Fufa et al. (2012)
-	-	-	-	*Cysticercus bovis*	-	-	6. 74	-	-	-	-	Mekelle	2000-2200	Getachew and Ashwani (2013)
-	-	-	-	Hydatid cyst (*E.granulosus*)	-	-	8.1	-	-	-	-	Mekelle, Adigrat, Axum Humera, Maiche, Shire	1400 - 2500	Kebede et al. (2009)
Haemonchus spp. *T.axei* *T.colubriformis* *Bunostomum* spp. Strongyloides spp. Oesophagostomm. Trichuris spp.	90.82 55.8 87.15 38.97 38.02 74.8 51.75	96.5 64.3 90 35.2 43.6 70.8 48.18	-	*M.expansa* *C. tenuicolis* *Avitellina spp.* *Stilesia spp.*	27.6 32.8 33.7 31.6	20.6 34.0 35.1 28.8	-	-	-	-	-	Metehara, Semera, Jigjiga	2300	Abebe and Esayas (2001)
-	-	-	-	-	-	-		*Fasciola* spp.	-	-	21.9	Nekemete	2,088	Alula et al.(2013)
Haemonchus spp. *Trichostrongylus* spp.	91.2 37.7	82.9 40.2	- -	- -	- -	- -	- -	- -	- -	- -	- -	Ogaden	1200	Kumsa and Wossene (2006)
-	-	-	-	*C. bovis* Hydatid cyst	- -	- -	0.0 9.9	-	-	-	-	Wolaita Soddo	2500	Regassa et al. (2009)
H.contortus	40.9	-	-	-	-	-	-	-	-	-	-	Wukro	1977	Lidya and Berihun (2015)

- : No information available, A*= no specific prevalence is mentioned

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Species composition and seasonal dynamics

In Ethiopia, the presence of GI helminthes infections in domestic ruminants is reported by many authors (Thomas et al., 2007; Menkir et al., 2007; Abebe et al., 2010; Shimelis et al. 2011; Diriba et al., 2013; Addisu and Berihu, 2014 and Cheru et al. 2014). According to the current review, about twenty three (23) GI helminthes species belonging to Nematode, Cestode and Trematode categories have been reported from infected cattle, sheep and goats (Table 2). Whereas, Menkir et al. (2007), Abebe et al. (2010), Hailu et al. (2011) and Shimelis et al. (2011) who reported that the most prevalent genera of GI helminthes were *Haemonchus*, Trichostronglyus. *Oesophagostomum*, *Nematodirus*, *Cooperia*, *Toxocara* and *Bunostomum* from Nematodes; *Monezia* and *Cyticercus*from Cestodes and *Fasciola*, *Paramphistomum* and *Shistosoma* from Trematode classes (Table 3).

Table 3 - Summary of Species compositions of GI helminthes of domesticated ruminants in Ethiopia.

Major Class	Species	Host	Predilection site(s)
Nematode	*Haemonchus contortus*	Cattle, sheep, goats	Abomasums
	Haemonchus placei	Cattle	Abomasums
	Ostertagia circumcincta	Cattle, Sheep, Goats	Abomasums
	Trichostrongylus axei	Cattle, Sheep, Goats	Small intestine
	Trichostrongylus colubriformis	Cattle, Sheep, Goats	Small intestine
	Cooperia curticei	Cattle, Sheep, Goats	Small intestine
	Strongyloides papillosus	Cattle, Sheep, Goats	Small intestine
	Ostertagia Spp.	Cattle, Sheep, Goats	Abomasum, Large intestine
	Chabertiaovina	Cattle, Sheep, Goats	Large intestine
	Oesophagostomum columbianum	Sheep, Goat	Large intestine
	Strongyloidespapillosus	Cattle, Sheep, Goats	Small intestine
	Trichuris ovis	Cattle, Sheep, Goats	Large intestine
	Nematodirus filicollis	Cattle, Sheep, Goats	Small intestine
	Nematodirus spathiger	Sheep	Small intestine
	Trichostrongylus vitrinus	Sheep, Goat	Small intestine
	Toxocara vitulorum	Cattle (calves)	Small intestine
Cestode	*Monezia expansa*	Sheep , Goat	Small intestine
Trematode	*Cysticercustenuicollis (Taenia hydatigena)*	Sheep , Goat	Omentum Mesenteries Peritoneum, Liver
	Ecchnoccocus granulossus	Sheep , Goat	Omentum Mesenteries Peritoneum, Liver
	Cysticercus ovis (Taenia ovis)	Sheep , Goat	Omentum Mesenteries Peritoneum, Liver
	Avitellina centripunctata (Av.centripunctata)	Sheep , Goat	Small intestine
	Stilesia globipunctata (St.globipunctata)	Sheep , Goat	Small intestine
	Stilesia hepatica (St. hepatica)	Sheep , Goat	Liver, biliary ducts
	Fasciola hepatica	Sheep , Goat Cattle	Liver
	Fasciola gigantica	Sheep , Goat Cattle	Liver
	Paramphistomum cervi	Sheep , Goat Cattle	Rumen
	Paramphistomum microbothrium	Sheep , Goat Cattle	Rumen
	Schistosoma bovis	Sheep , Goat Cattle	Liver, intestine, mesenteric lymph nodes and mesenteric veins

According to this systemic review, GI helminthiasis in domestic ruminants is severe and increasingly become an important focusing area of research in the country situation. A study conducted in western Oromia on GI parasites showed that Strangles were the most prevalent parasites encountered in the area (Moti et al., 2013). Similar study by Shimelis et al. (2011); Cheru et al. (2014), Nejib et al. (2016) and Diriba et al. (2013) reported that Strongyles were the most prevalent parasites encountered in North Gondar, Debre Zeit (East Shoa zone), Arbaminch (GamoGofa zone) and Asella respectively. As it has been reported by Abebe et al. (2015); Endale et al. (2013); Feyesa et al. (2010); Nigatu (2010); Kebede et al. (2009); Nigatu et al. (2009); Regassa et al. (2009); Jibat et al. (2008); Menkir et al. (2007); Bekele et al. (1988), metacestodes (larval cestodes) *Cysticercus* ovis (*Taeniaovis*), *Cysticercus tenuicollis* (*T. hydatigena*) and Hydatid cysts (*Echinococcus granulosus*) are the most prevalent species in Eastern Ethiopia. On the other hand, gastrointestinal infections as a result of adult cestodes such as *Avitellina centripunctata*, *Moniezia expansa* and *Stilesia globipunctata*, and bile duct infections with Stilesia hepatica were frequently reported in different parts of the country (Abebe and Esayas, 2001; Etana, 2002; Menkir et al., 2007; Bersissa et al., 2011). Nonetheless, a higher prevalence of strongyles infection was recorded in the midland and highland than the lowland, and in wet season than the dry season. The mean fecal egg count was found to be significantly higher in the midland area and in wet season (Nejib et al., 2016). However, according to a study by Abebe and Esayas (2001) in the arid and semiarid zones of Eastern Ethiopia revealed that during the dry seasons of the year, a greater prevalence rates of GI helminthes were recorded in sheep and goats, 95.6 % and 100 % respectively. Furthermore, according to an abattoir survey by Menkir et al. (2007) at 4 abattoirs located in the semi-arid zone of Eastern Ethiopia, the mean burdens of adult nematodes were generally moderate in both sheep and goats and showed patterns of seasonal abundance that corresponded with the bi-modal annual rainfall pattern, with

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highest burdens around the middle of the rainy season. There were significant differences in the mean worm burdens and abundance of the different nematode species between the four geographic locations, with worm burdens in the Haramaya and Harar areas greater than those observed in the Dire Dawa and Jijiga locations (Abebe and Esayas, 2001; Menkir et al., 2007; Abebe et al., 2010; Berssisa et al., 2011; Endale et al., 2013). The seasonality of the GI helminthes distribution is associated with the relative humidity and rainfall (Debela, 2002; Menkir, 2007). A number of reports throughout the country indicated that there are remarkable changes in faecal egg counts and prevalence of helminths infection as a result of seasonal variation and seasonal rainfall pattern (Fikru et al., 2006; Menkir et al., 2007; Takele et al., 2013). Furthermore, relatively higher GI Helminths egg counts were found in mid altitude and highland zones than in lowland due to the influence of existing fluctuations in geographic and climatic conditions between each zone (Demelash et al., 2006; Takele et al., 2013).

Epidemiological factors

The epidemiology of the GI helminthiasis relies on factors such as the infection pressure in the environment and the susceptibility of the host, species and pathogen factor (Tilahun, 1995). From epidemiological point of view, the infective stages which eventually become available to the host depend on the independent and interactive influences of several factors in the macro- and micro-environment (Urquhart et al., 1994; Woledemariam, 2005; Regassa et al., 2006; Takele et al., 2013). Parasitic, host and environmental factors are the most frequently reported determinants for the epidemiology of helminths (Etana, 2002; Fikru et al., 2006; Ahmed et al., 2007; Menkir, 2007; Shimelis et al., 2011; Yirsaw and Zewdu, 2015). As stated by Shimelis et al. (2011), the prevalence of helminthiasis at species level was about 46.07% and 55% in sheep and goats, respectively. Almost similar report was documented in and around Ambo town with the proportion of 47.8% and 53.3% in sheep and goats, correspondingly (Temesgen and Walanso, 2015). Among the collected articles, the highest post mortem examination result was reported in Eastern Ethiopia (100%) (Abebe and Esayas, 2001). Strong association between GI helminths and poor body condition was coupled with heavy intensity of infection in the majority of infected animals (Abebe et al., 2010; Diriba et al. 2013; Alula et al., 2013; Temesgen and Walanso, 2015). According to Abebe et al. (2010) and Cheru et al. (2014), the burden of GI parasites and total EPG was significant in different body conditions.

Host factor

Sex, age, breed, nutrition, physiological status and presence or absence of inters- infections aggravate the severity of infection (Demelash et al., 2006; Menkir et al., 2007).Clinical parasitic gastroenteritis has been reported in young animals whilst infections in mature animals are generally subclinical in nature (Thomas et al., 2007). The lower occurrence in adults has been attributed to immunological maturity as the animals grow and the increase in acquired resistance due to repeated exposure (Biffa et al., 2004). While, some local breeds are known to be genetically resistant to GIhelminths infections than others (Tibbo, 2006). In Ethiopia, the local sheep breeds (Washera, Farta, Afar, Menz, Horo); Goat breeds (Begait, Abewrgelie, Keffa,) and Cattle breeds (Boran , Fogera, Raya, Horro, Abigar, Shekko, Arssi) are relatively resistant to GI worms than exotic breeds (Frisch and O'Neill, 1998; Negussie et al., 2000; Tibbo, M. 2006; Menkir et al., 2007; Solomon et al., 2009; Kebede et al., 2012). In addition, Moti et al. (2013) reported that Physiological status of ruminants like level of host immunity to the parasites is subjected to the number of eggs produced by adult female helminthes. In the same way, the females are readily infected and existing worm burdens become more active and increase egg spassed in the feces and develop Larvae (L_3) on the pasture (Woldemariam, 2005).

Environmental factor

As far as Ethiopia is among the tropical African countries, the temperature is permanently favorable for larval development in the environment. The favorable environment for larvae development is ranged at temperature about 10–36 °C and humidity proportion of 85% (Debela, 2002). In the arid tropical climates of lowland areas of the country has an environment which ranges from extensive pasturelands and browse plants to intensive grazing areas (Nejib et al., 2016). This environment is ranged from harsh to favorable for growth and survival of free-living stages of the GI helminthes (Tilahun, 1995; Debela, 2002).

Pathogen factor

The epidemiology of GI helminthes is also strongly influenced by host-parasite biology after infection has been occurred (Abebe et al., 2010; Diriba et al., 2013). Hypobiosis has been undergone by GI helminthic the abomasal or intestinal mucosae of the host (Cheru et al., 2014). Whereas, Abebe and Esayas (2001) stated that the immune status of the host influenced the rates of hypobiosis and usually arrested during external environments are unfavorable for the development and survival of eggs and larvae. Such development cycle usually coincides with the onset of rainy seasons and favourable period for larval development and transmission (Kumsa and Wossenie, 2006; Feyisa et al., 2010; Hailu et al., 2011; Nejib et al., 2016).

Currently applied diagnostic techniques

The diagnosis of helminthes of ruminants is based on demonstrating the presence of eggs or larvae in fecal samples or parasites recovered from the digestive tracts or other viscera of the animals (Hailu et al., 2011; Addisu and Berihun, 2014). Although a great variety of methods and modifications have been described fordiagnosis, standardized techniques such as egg or larval counts, worm counts and pasture larval counts did not exist. Therefore, most diagnostic laboratories

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as well as teaching and research institutions were applied their own set of protocols and test procedures (Kassai, 1999). Common diagnostic procedures for helminthes infections in Africa in general and in Ethiopia in particular are simple flotation, sedimentation, modified McMaster and faecal culture methods (Hansen and Perry, 1994; Kassai, 1999; Waller, 1997). Some nematode genera such as strongyloides produce eggs that are identical in appearance which couldn't be identified easily by faecal examination alone. So to identify these faecal cultures are required (Hansen and Perry, 1994; Urquhart et al., 1996; Kassai, 1999; Van Wyket al., 2004). However, *Nematodirus*, Strongyloides and Trichurisspecies have eggs that can be differentiated by their distinctmorphological features. Post-mortem examinations and identification of adult worms and arrested larvae in animals are the definitive means of identifying the parasite. Similar to faecal egg counts; there are many procedures that are described for post-mortem examination for nematode parasites (Hansen and Perry, 1994; Urquhart et al., 1996; Kassai, 1999).

Chemotherapies and control options

Effective helminthic control is a major element in ensuring the sustainability of animal production. The main aim of control is therefore to ensure that the biotic potential of a parasite is restrained at a level compatible with the biological requirements of economic livestock production (Waller, 1997). Since eradication of gastrointestinal parasites is not practical, only integrated control methods can be envisaged. Some of the basic principles include grazing management, acquisition of natural or artificially induced immunity, biological control and the judicious use of anthelmintic (Hazelby et al., 1994).The main methods for control of helminthic parasites are prophylactic treatment with antihelmintics combined with grazing management (Van Wyk et al., 1999). Despite the accumulation of drugs in animal products and undesirable effects on non-target organisms in the environment, together with an increase in anthelmintic resistance, the use of antihelmintics still remains the corner-stone of helminth control (Waller, 1997; Van Wyk et al., 1999; Bersissa and Girma, 2009). Since animals are often infected with a wide range of helminths, the need for broad-spectrum compounds against trematodes, cestodes and nematodes, and their larval stages is obvious (Hazelbyet al., 1994). The epidemiological information on GI helminths parasites of domestic ruminants gathered in Ethiopia can be used to design appropriate control measures. In principle, control should aim at the reduction of transmission rates. Several control methods, which include cultural husbandry, chemical, biological, ethno-veterinary medicine and immunological control, have been proposed (Nejib et al., 2016).

Husbandry pattern, control and prevention

A thorough husbandry practices such as controlling stocking rates, rotational grazing, and providing hygienic grazing can be considered as an alternative husbandry control technique (Diriba and Birhanu, 2013; Abebe et al., 2015; Temesegen and Walanso, 2015). The best way to prevent GI helminthes is to keep animals away from potentially dangerous environment. An absolute separation of stock from intermediate host zone is only practical in intensive farming husbandry systems (Woldemariam, 2005) which the country has a limited effort to do so. However, in communal grazing condition which is very common and traditional in Ethiopia, animals are communally grazed and therefore; practices such as rotational grazing and provision of clean pastures would not be feasible (Menkir et al., 2007; Cheru et al., 2014).

Chemotherapeutic interventions

Nowadays, the control of GI parasites of livestock in Ethiopia is mainly based on the use of anthelmintics. The most commonly used generic broad spectrum antihelminthics that are available in Ethiopia are the Benzimidazoles, Imidazothiazoles and Macrocyclic lactones which consist albendazole, levamisole and ivermectin, respectively (Woldemariam, 2005; Kumsa, and Wossene, 2006; Menkir et al., 2007). It is readily available wherever in the country since it has been imported massively by the government and non-government institutions; and used by every individual including farmer. However, the use of antihelmintics at regular intervals for a long period of time and treating by mass whenever an animal manifest clinical syndrome has become the major issue for the development of multiple resistances; for instance against benzimidazoles (Woldemariam, 2005). Targeted or selective application of anthelmintic treatment might be an important tool to keep susceptible GI nematode strains in livestock and to delay this case. An alternative approach for selective anthelmintic treatment was studied using experimental small ruminants for the management of haemonchosis by using the FAMACHA© method in the Mid-Rift Valley of Ethiopia by Woldemariam (2005) and Menkir (2007). Such system can be used by the farmers themselves by checking their animals for signs of anaemia (VanWyketal, 2004). Unfortunately, it was not practiced very well in Ethiopia where mixed parasitc infection, and where traditional feeding practice is followed (VanWyk et al., 2004; Kumsa and Wossene, 2006). A cost-effective preventive control programs for helminth infection in ruminants is based on sound epidemiological knowledge of the time relationship between contamination of pastures and the seasonal availability of infective larvae in a given geographic area. Epidemiological knowledge, its application in grazing management whenever feasible and access to antihelmintics of high efficacy are key factors for the success of controlling helminth infections in domestic ruminants (Aynalem et al., 2009; VanWykand Mayhew, 2013). Projecting models derived from more complete information on the ecology of GI helminth infection and anthelmintic resistance, climate and local management factors provide a basis for improved control schemes based on chemotherapy, management and immunization within similar climate (VanWyk and Mayhew, 2003; Woldemariam, 2005; Menkir et al, 2007).

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Ethno-veterinary practices

As a result of the gradually increasing anthelmintic resistance, residual effect on animal products, environmental pollution, scarcity and high cost of such drug especially to poor farmers have enforced to reconsider other alternative helminthic control technique in the country (Bersissa and Girma, 2009). Of these, Ethno-veterinary medicine has become a substantial and most expanding interest of options for Ethiopian farmers. Although such a kind of conventional veterinary medical system was yet very poor in the country, a very limited effort have been done to encourage the widely used ethno-veterinary plants in the country (Lulekal et al., 2008; Fullas, 2010; Asfaw and Fentahun, 2020). In order to do so, several Ethno-veterinary surveys were conducted so far in the country which indicated as if several traditional healers use medicinal plants for de-worming livestock (Jemal et al., 2011). To mention few examples, Herbal preparations from fresh leaves of *Dodonea viscose*, *Albizia gummifera* and *Vernonia amygdalina* against mixed natural infections in sheep was evaluated by Biffa et al. (2004) to show the anthelmintic activities. In addition, an In-vitro antihelmintics activity study from *Rhus glutinnosa*, *Syzygium guineensa*, *Albizia gummifera*, *Croton macrostachyus*, *Ekebergia capensis, Acacia nilotica and Terminalia schimperiana* against *Haemonchis contortus* have been reported by Eguale et al. (2006) and Jemal et al. (2011). Furthermore, anthelmintic activity of plants such as *Allium sativum, Zingiber officinale, Cucurbita mexicana, Ficus religiosa, Artemisia brevifolia, Calotropis procera, Nicotiana tabacum, Butea monosperma*, *Coriandrum sativum*, *Ocimum*, *Thymus schimperi* and *Echinops kebericho* have been reported by Abera (2003), Biffa et al. (2004) and Giday et al. (2007).

CONCLUSION

This review work assessed the GI helminthes in Ethiopia and provides a clue on perspectives and constraints encountered in researches which were done on GI helminths in ruminants. Twenty three (23) GI helminthes species that belong to all the three major classes of helminths have been found to occur in domestic ruminants in Ethiopia. In addition, nematodes are the most commonly encountered GI helminths while Cestodes are the least. In most reports, a higher rate was recorded in small ruminants. The most prevalent genera of GI helminths reported in order of prevalence are *Haemonchus*, Trichostronglyus, *Oesophagostomum*, *Nematodirus*, *Cooperia*, *Toxocara* and *Bunostomum* from Nematodes; *Moneziaand* *Cyticercus* from Cestodes. Whereas, *Fasciola*, *Paramphistomum* and *Shistosoma* are found from Trematode category. Both the abattoir and coprological studies have indicated that infection by GI helminthes in ruminants is highly prevalent and widespread in all agro-ecologies and livestock production systems in Ethiopia. Fecal diagnostic techniques such as simple flotation, sedimentation, modified McMaster have been used routinely in Ethiopia. It has been also shown that prevalence of GI helminthes parasites was related to the agro-climatic conditions such as quantity and quality of pasture, temperature, humidity and grazing behavior of the host and the susceptibility of any intestinal helminthic parasites were also influenced by age, breed, species, health status, physiological factors and previous exposure to parasites.

Due to the lack of effective helminthes control strategies in Ethiopia, antihelmintics are exclusively used. Though Ethiopia has a huge amount of small and large ruminants population, the country is facing a direct and indirect economic lose as a result of GI helminthes infection. Hence, immediate remedies shall be taken into action on control and prevention methods against such anthelmintic resistant GI parasites. In order to this, it is advantageous to collect and looking over the previous researches done so far to reconsider their gaps for the future short and long term actions on prevention and control strategies. So, all-inclusive and well organized documentation about GI helminthes of ruminants in the country is essential to support researchers and policy makers to develop such remedies. Finally, applicable field diagnostic technique should be introduced as far as mixed parasitic infection and traditional feeding practice is common in the country which can aggravate GI helminthes infection.

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Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

Data will be made available up on request of the primary author

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INFLUENCE OF PELLETED BROWSE-BASED SUPPLEMENTS FED WITH A BASAL DIET OF *Andropogon gayanus* HAY ON INTAKE, DIGESTIBILITY, GROWTH AND HAEMATO-BIOCHEMICAL INDICES IN WEST AFRICAN DWARF GOAT

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Supporting Information

ABSTRACT: This study evaluated the nutritional value of pelleted supplements based on four major feed resources fed to small ruminants by smallholder farmers in the Accra Plains. Leaves of *Samanea saman, Acacia auriculiformis* and *Ficus exasperata,* and cassava peels were dried, milled, mixed with other ingredients and pelleted to form *Samanea saman* (SS-S), *Acacia auriculiformis* (AA-S), *Ficus exasperata* (FE-S) and cassava peel (CP-S) based supplements. The supplements were fed to twenty West African Dwarf goats on a basal diet of *Andropogon gayanus* (Gamba grass) hay in a completely randomised design experiment. Voluntary intake of dry matter did not differ (P>0.05) by the type of supplement. However, crude protein intake was higher (P<0.05) in goats fed FE-S than those fed CP-S. Acid detergent fibre intake was higher (P<0.05) for SS-S, AA-S and FE-S than CP-S. Digestibility of dry matter, organic matter, crude protein, acid detergent fibre and neutral detergent fibre were lowest (P<0.05) for goats on CP-S. Average daily weight gain and Feed conversion ratio were not affected (P>0.05) by dietary treatments. Also, dietary treatment did not affect (P>0.05) the concentrations haematological and blood biochemical constituents determined except urea which was higher (P<0.05) in goats fed SS-S than the other treatments. In conclusion, the above results suggest that browse-based and cassava peel-based supplements could be fed to confined goats on roughage diets especially in the dry season without any deleterious effects on intake, growth and physiology of goats.

Keywords: Accra plains, Performance, Shrub Leaves, Smallholder Farmers, Supplementation

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Abbreviations: AA-S: *Acacia auriculiformis*-based supplement; ADMD: Apparent digestibility of dry matter; ANOVA: Analysis of variance; AOAC: Association of Official Analytical chemists; CP-s: cassava peel-based supplement; FE-B*: Ficus exasperata*-based supplement; LIPREC: Livestock and Poultry Research Centre; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; PCV: packed cell volume; RBC: red blood cell; SEM: standard error of mean; SS-S: *Samanea saman*-based supplement; T. Cholesterol: total cholesterol; WBC: white blood cell

INTRODUCTION

Small ruminant production is a major livelihood diversification strategy among smallholder farmers in Ghana. Often, animals are grazed extensively on natural pasture with little or no feed supplementation coupled with minimal health care (Baiden and Obese, 2010). However, restrictions in animal movement, especially during the cropping seasons, are now compelling many small ruminant keepers to fully or partially confine their animals. Feeding of confined small ruminants is often inadequate and such animals perform less than their free-roaming counterparts (Baah et al., 2012). The challenges associated with confinement in the cropping season, coupled with the scarcity of quality feed during the dry season severely constrain small ruminant production in Ghana (Adjorlolo et al., 2016) affecting income generation. Slow growth rate, unstable weight gains, lowered resistance to diseases and reproductive problems of small ruminants have been reported in these instances (Annor et al., 2007, Konlan, 2010). The provision of appropriate supplementary feed which can supply substantial amounts of dietary energy, protein and minerals would be an important step in enhancing the productivity of ruminants in Ghana.

Recently, Adjorlolo et al. (2020) fed pelleted *Samanea saman, Acacia auriculiformis* and *Ficus exasperata leaf meal-based diets* and cassava peel meal- based diet as supplements to West African Dwarf sheep fed on a basal diet of *Andropogon gayanus* (Gamba grass) hay and concluded that they were acceptable to sheep and could help improve performance on low quality forages.

Currently, there is lack of information on the utilization of browse-based pelleted feed supplements on the growth and physiology of the West African Dwarf goat the most popular goat breed in Ghana. This study therefore evaluated the effects of pelleted *Samanea saman (SS-S), Acacia auriculiformis (AA-S), Ficus exasperata* (FE-S) and cassava peel-based

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(CP-S) supplements on feed intake, growth rate and blood profiles of West African goats fed basal diet of *Andropogon gayanus* (Gamba) grass hay.

MATERIALS AND METHODS

Study area

The study was conducted at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana (05^{0}68' N, 00^{0}10' W) in the Coastal Savannah belt of Ghana, West Africa. Annual rainfall averages 881 mm per annum but with a high degree of variability. The rainy season was from April to June, the minor season was from September to October, and the dry season from November to March (Adjorlolo et al., 2014). `

Experimental animals and their management

West African Dwarf goats were housed in individual pens with concrete floors. The housing unit had roofs made of corrugated iron sheets. The pens were 3m × 1.5m in dimension. Each pen had one wooden feeding trough for the basal diet and two plastic troughs, one for the supplement and the other for water, similar to the study by Adjorlolo et al. (2020). The animals were treated against external parasites with pour-on acaricide and dewormed with Albendazole (10%), a broad-spectrum anthelminthic. All the procedures in this study were approved by the Noguchi Memorial Institute for Medical Research Institutional Animal Care and Use Committee (NIACUC), University of Ghana (NIACUC Protocol No: 2017-03-2R).

Preparation of experimental diets

Leaves of three browse plants, namely, *Samanea saman, Acacia auriculiformis* and *Ficus exasperata* were harvested from trees and shrubs within the study area and shade-dried. Cassava peels were bought from *gari* producers and sun-dried. The dried leaves and cassava peels were ground with a hammer mill (1-mm screen) into meals and each mixed with other ingredients (Table 1) and pelleted. *Andropogon gayanus* (Gamba grass) was harvested at the flowering and seeding stage, sun-dried and tied in bundles for storage.

Table 1 - Ingredient composition of supplements used in the study

Ingredients: (g/kg)	Supplements			
	SS-S	AA-S	FE-S	CP-S
Maize	159	124	165	0
Wheat bran	120	135	108	650
Mineral salt	5	5	5	5
Dicalcium phosphate	5	5	5	5
Sulphate of ammonia	5	5	5	5
Urea	6	26	12	15
Cassava peels	0	0	0	32
Samanea saman	700	0	0	0
Acacia auriculiformis	0	700	0	0
Ficus exasperata	0	0	700	0
Total (Kg)	1000	1000	1000	1000
Crude protein (Calculated)	160.6	160.1	160.7	160.7

CP-S: cassava peels-based; SS-S: *Samanea saman-based*; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperate* based supplements

The pelleted supplements were formulated to be isonitrogenous using literature values of nitrogen concentrations in the browses and cassava peels. The dietary treatments were as follows:

SS-S = Gamba grass hay + *Samanea saman* leaf meal-based supplement
AA-S = Gamba grass hay + *Acacia auriculiformis* leaf meal-based supplement
FE-S = Gamba grass hay + *Ficus exasperata* leaf meal-based supplement
CP-S = Gamba grass hay + Cassava peel meal-based supplement

Voluntary feed intake and growth study

The voluntary feed intake and growth studies were carried out using twenty young West African Dwarf goats with an initial average live weight of 10.7 ± 2.3 kg. They were allocated randomly to four experimental diets with each treatment having five replicates (five goats per treatment) in a completely randomized design. Animals in each treatment group were offered Gamba grass hay as basal diet and either of the three browses or cassava peel- based supplements. Supplements were offered at about 08:00 hours each day. Supplement allowance was about one percent of each animal's body weight (about 25% of voluntary intake). After each goat had consumed all the supplement provided, the basal diet was offered *ad libitum*. Animals were allowed 14 days to adjust to the diet after which daily feed intake and fortnightly body weights were taken for eleven weeks (77 days). Feed intake was determined daily as the difference between weight of feed offered and refusals.

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Digestibility study

For the digestibility studies, faecal collection bags were used to collect faecal samples from goats on the feeding trial. Faecal samples were taken from two goats per treatment for six days during the final week of the feeding trial. The faecal samples were stored in a refrigerator after collection. They were bulked for each goat and oven-dried at 55℃ to a constant weight for dry matter (DM) determination. The dried faeces were ground through a 1.0mm sieve using a laboratory mill and bagged pending further analysis. Apparent digestibility of dry matter (ADMD%), was calculated as:

ADMD (%) = $(\frac{DM\ intake - Faecal\ DM}{DM\ intake}) \times 100)$

Similar calculations were followed to determine apparent digestibility of organic matter, crude protein, neutral detergent fibre, acid detergent fibre.

Chemical analysis of feed and faeces

Feed and faecal samples were analysed for dry matter, organic matter, crude protein and total ash according to the methods of AOAC (2004). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined according to the method of Van Soest et al. (1991).

Blood sampling

Blood samples were collected every two weeks (week 1, 3,5, 7 and 9) from the jugular vein of each sheep using a vacutainer needle. Sampling was done in the morning, between 07:30 and 08:00 hours. A total of 10 ml of blood sample was collected and 4 ml transferred into a glass vacutainer tube containing the anticoagulant tripotassium ethylene diamine tetra acetic acid (K3.EDTA). The tubes were placed on ice and transported immediately to the Laboratory for haematological analysis. The remaining 6 ml was transferred into glass vacutainer tubes containing clot (Gel) activator. This was placed on ice pack and also transported to the Laboratory where it was centrifuged at 3000 rpm for 10 minutes at 4^0C. The sera obtained were gently harvested into Eppendorf tubes and stored at -20^0C until the analyzed for biochemical parameters.

Haematological analysis

The haemoglobin concentration was determined by the cyanmethaemoglobin method (Gillet et al., 2009), while PCV was estimated by the microhaematocrit method (Samour, 2006). The RBC and WBC counts were determined using the haemocytometer. Total RBC count was determined using the formula given by Samour (2006):

RBC (10^{12} /L) $= \frac{N}{100}$, Where: L= Litre ; N = Number of cells counted in 160 small squares.

Total WBC counts was estimated using the formula given by Campbell (1995): WBC (10^9 /L) $= \frac{N \times 10 \times 200}{9}$, where:

L= litre; N = number of cells counted in nine small squares.

The RBC indices were computed using the formulas provided by Reece and Swenson (2004) below:

MCV(fL) = $\left(\frac{PVC}{RBC}\right)$ x 10

MCH(pg) = $(\frac{Hb}{RBC})$ x 10

MCHC(g/dL) = $(\frac{Hb}{PCV})$ x 100

In determining the differential WBC counts, thin smears of blood were made from blood samples obtained from venipuncture, on well ethanol-cleaned, grease-free microscope slides. They were air-dried, fixed in absolute methanol and stained with Giemsa stain. Stained slides were studied under oil immersion objective at 1000X magnification. Percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils were all determined based on observation of 200 WBC per film.

Blood biochemical analysis

The concentrations of glucose, total proteins, albumin, total cholesterol, urea, sodium and potassium were determined in the serum at weeks 1, 3, 5, 7 and 9 using the Mindray BA -88A Semi-Auto Chemistry Analyzer (Nanshan, China). Globulin concentration was computed as the difference between total protein and albumin concentrations.

Statistical analyses

Data from the feed intake, growth and digestibility studies were subjected to Analysis of variance procedure (ANOVA) of GenStat Release 12th Edition (VSN International, 2009), whilst that of the blood parameters was analyzed using repeated measures analysis of variance procedure of GenStat (VSN International, 2009). The Least significant difference procedure of GenStat was used to separate the means at 5% level of significance.

RESULTS

Chemical composition of feed ingredients and supplements

The chemical composition of the basal diet (Gamba grass hay), the three browses (Samanea, Acacia and Ficus) and cassava peels are presented in Table 2. The basal diet, the leaf meals of the three browses and cassava peels had comparable dry matter contents (88.3 to 91.4%) and organic matter (81.1 to 85.3%) contents. Apart from cassava peels

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all the leaf meals of the three browses had higher crude protein than Gamba grass hay. Also, all the leaf meals of the browses and cassava peel-meal had lower neutral detergent fibre and lignin contents than Gamba grass. The chemical composition of the experimental supplements are shown in Table 3. AA-S had the highest crude protein content (22.3%) while CP-S had the least (15.9%).

Influence of supplements on voluntary intakes in West African Dwarf goat

Intakes of crude protein and acid detergent fibre were influenced by the type of supplement offered (Table 4). Sheep fed FE-S had higher (P<0.05) crude protein intake than those fed CP-S. Acid detergent fibre intake was higher (P<0.05) for SS-S, AA-S and FE-S than CP-S. The dry matter, organic matter, neutral detergent fibre and lignin intakes were however similar across dietary treatments.

Digestibility of nutrients by West African Dwarf goat

Digestibility of dry matter, organic matter, crude protein and detergent fibre were all influenced by the type of supplement fed (Table 5). Dry matter digestibility was similar for SS-S, AA-S and FE-S but higher (P<0.05) than CP-B. Crude protein and neutral detergent fibre digestibilities also followed a similar tend to that of dry matter digestibility. Organic matter digestibility was higher (P<0.05) for sheep fed SS-S and FE-S than those fed CP-S. Also, the acid detergent fibre digestibility in goats fed SS-S or AA-S were higher (P<0.05) than those fed CP-B.

Daily weight gain and feed conversion ratio

Average daily weight gain and feed conversion ratios did not differ (P>0.05) with the type of supplement (Table 6). None of the supplements led to weight loss in the goats.

Table 2 - Chemical composition of leaf meals of browses, cassava peel meal and *Andropogon gayanus* hay

Fraction (%)	Grass hay	Samanea	Acacia	Ficus	Cassava Peels
Dry matter	91.4	90.9	90.7	89.8	88.3
Crude protein	6.2	22.6	14.5	14.4	5.2
Organic matter	81.1	84.6	83.2	85.3	82.9
Neutral detergent fibre	72.2	53.8	62.1	54.4	46.9
Acid detergent fibre	43.5	36.6	47.8	42.5	28.7
Lignin	7.1	5.5	7.1	5.8	3.8
Total ash	11.4	6.5	8.5	6.4	5.1

Table 3 - Chemical composition of the experimental supplements

Fraction (%)	Supplement			
	SS-S	AA-S	FE-S	CP-S
Dry matter	89.9	90.4	90.8	89.7
Crude protein	19.1	22.3	21.7	15.9
Organic matter	85.2	84.1	82.4	84.1
Neutral detergent fibre	46.5	49.1	46.3	42.9
Acid detergent fibre	28.5	33.8	19.4	31.8
Lignin	4.1	4.4	5.3	3.9

CP-S: cassava peels-based; SS-S: *Samanea saman-based*; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements

Table 4 - Influence of supplements on voluntary intakes in West African Dwarf goat

Parameter (g/day)	Treatments				*SEM*	*P-value*
	SS-S	AA-S	FE-S	CP-S		
Dry matter intake	321.2	306.3	388.7	361.9	24.5	0.084
Organic matter	297	282	353	334	22.5	0.109
Crude protein intake	47.3^{ab}	50.3^{ab}	54.2^{a}	45.1^{b}	2.84	0.011
Neutral detergent fibre intake	54.3	55.6	69.0	58.5	5.63	0.212
Acid detergent fibre intake	36.9^{a}	40.0^{a}	41.4^{a}	25.5^{b}	3.59	<0.001
Lignin intake	27.9	31.4	30.2	21.0	3.63	0.119

^{a,b,} Means within a row with different superscripts differ significantly at P<0.05; SEM: standard error of mean; CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements

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Table 5 - Digestibility of components of feed as influenced by supplementation

Fraction (%)	Supplements				*SEM*	*P-value*
	SS-S	AA-S	FE-S	CP-S		
Dry matter	57.5[a]	56.8[a]	62.7[a]	47.7[b]	2.85	<0.012
Organic matter	52.4[a]	51.2[ab]	58.6[a]	43.1[b]	3.10	<0.018
Crude protein	46.4[a]	47.2[a]	46.5[a]	38.7[b]	1.36	<0.001
Neutral detergent fibre	38.4[a]	40.2[a]	36.2[a]	30.7[b]	1.34	<0.001
Acid detergent fibre	33.4[ab]	34.2[a]	29.5[bc]	26.8[c]	1.37	<0.004

[a,b,c,] Means within a row with different superscripts differ significantly at P<0.05.; SEM: standard error of mean; CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements

Table 6 – Effect of supplementation on growth parameters in West African Dwarf goats

Parameter	Supplements				*SEM*	*P-value*
	SS-S	AA-S	FE-S	CP-S		
Initial weight (kg)	10.9	9.75	12.00	10.75	1.33	0.436
Final weight (kg)	11.8	10.63	12.80	11.88	1.23	0.399
Average daily gain (g/d)	10.8	10.54	9.64	13.55	2.96	0.612
Feed intake (g)	321.2	306.3	388.7	361.9	32.7	0.084
Feed conversion ratio	33.6	35.1	50.7	27.9	12.7	0.330

[a] SEM: Standard error of mean; CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements

Haematological and serum biochemical parameters in West African Dwarf sheep

At the end of the study, dietary treatment did not affect all the haematological and serum biochemical indices measured except serum urea concentrations which was higher (P<0.05) in goats fed SS-S than those fed AA-S, FE-S and CP-S (Table 7). Generally, the concentrations of most of the haemato-biochemical indices remained relatively stable and showed similar trends across dietary treatments during the period of study (Figures 1 and 2).

Table 7 - Haematological and serum biochemical parameters of West African Dwarf goat fed basal diet of *Andropogon gayanus* hay and supplements

Parameters	Treatments				SEM	P-value	Reference Range[1]
	SS-S	AA-S	FE-S	CP-S			
Haematological Indices							
Haemoglobin (g/dL)	10.3	10.1	10.0	10.1	0.122	0.265	8 – 12
PCV (%)	26.7	24.6	23.1	24.9	1.520	0.350	22 – 38
RBC (x10^{12}g/L)	13.6	12.2	11.6	12.5	0.865	0.351	8 – 18
MCV (fL)	20.0	20.4	20.6	20.1	0.701	0.910	16 – 25
MCH (pg)	7.85	8.47	9.23	8.30	0.584	0.344	5 – 8
MCHC (g/dL)	39.2	41.8	44.9	41.3	2.29	0.317	30 – 36
WBC (x10^{9}/L)	11.7	12.0	11.9	11.5	0.639	0.934	4 – 13
Neutrophils (%)	50.8	48.6	45.4	44.0	2.45	0.189	30 – 48
Lymphocyte (%)	46.9	50.1	51.6	53.0	2.64	0.360	50 - 70
Eosinophils (%)	0.6	0.90	1.84	1.40	0.372	0.091	1 – 8
Monocytes (%)	1.68	0.50	1.08	1.15	0.480	0.372	0 – 4
Basophils (%)	0.00	0.00	0.00	0.00	0.000	0.000	0 – 1
Serum Biochemical Indices							
Glucose (mmol/L)	1.20	1.30	1.50	1.49	0.130	0.261	2.78 - 4.16
Total protein (g/L)	60.3	55.7	55.6	59.4	0.260	0.145	60 – 70
Albumin (g/L)	29.8	29.3	28.7	28.1	0.090	0.519	27 – 39
Globulin (g/L)	30.6	26.4	26.9	31.3	0.220	0.291	27 – 41
Total cholesterol (mmol/L)	3.43	3.37	4.00	4.28	0.461	0.097	2.07 – 3.37
Urea (mmol/L)	9.39	5.74	6.51	6.17	0.810	0.016	3.6 – 7.1
Sodium (mmol/L)	162	154	159	156	4.62	0.568	139 – 149
Potassium(mmol/L)	5.97	6.18	6.17	6.09	0.290	0.937	4.5 – 6.7

[a,b] Means within a row with different superscripts differ significantly at P<0.05.; SEM: Standard error of mean; CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements. [1]Merck Manual (2012).

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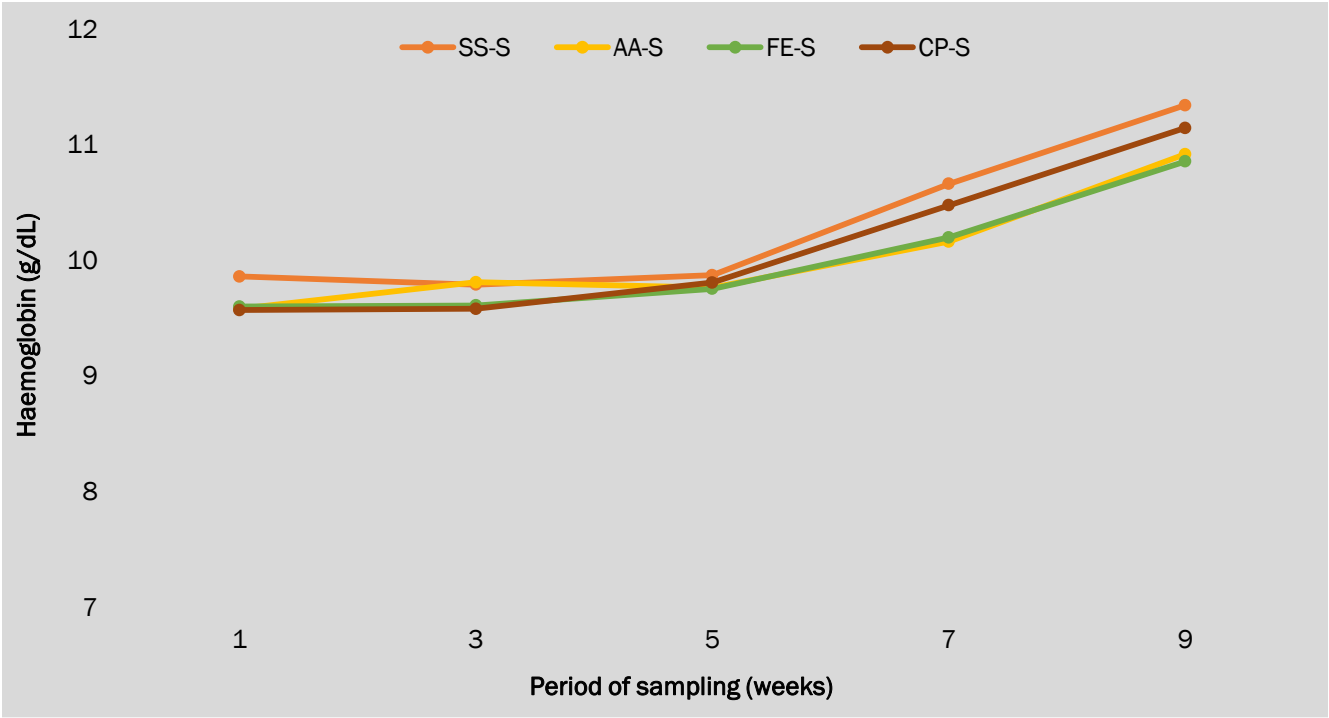

Figure 1 - Changes in haemoglobin concentration in West African Dwarf sheep. CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements.

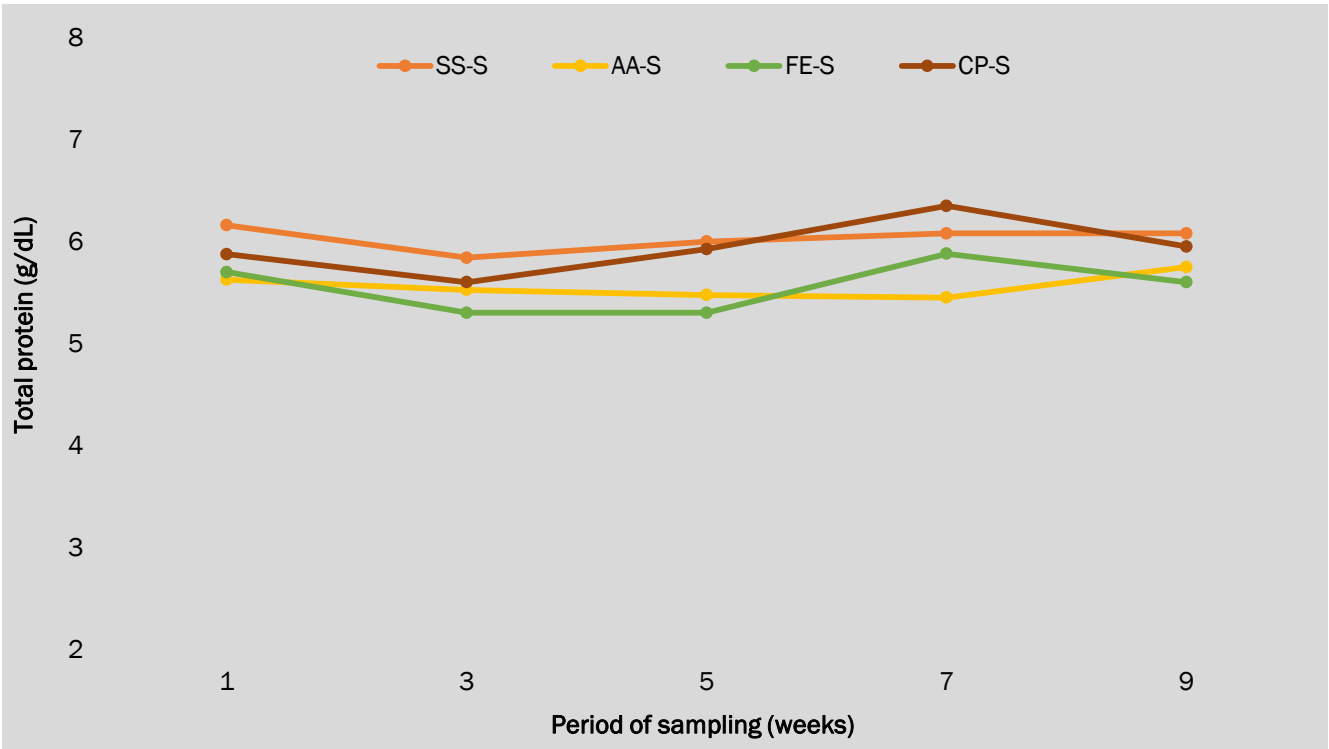

Figure 2 - Changes in total protein concentration in West African Dwarf sheep. CP-S: cassava peels-based; SS-S: *Samanea saman* based; AA-S: *Acacia auriculiformis*-based and FE-S: *Ficus exasperata*-based supplements.

DISCUSSION

The crude protein content of Samanea, Acacia and Ficus were higher than the mean values reported in the literature (Abdu et al., 2012; Bello et al., 2014; Delgado et al., 2014) probably due to varietal or environmental differences. Sheep fed FE-S had higher ($P<0.05$) crude protein intake than those fed CP-S. This may be due to the higher crude protein content of FE-S resulting from the higher crude protein content of the Ficus leaf meal used (14.4%) compared with values as low as 6.9% reported by Bello et al. (2012). The crude protein intake range of 45.1 to 54.2 g/day in this study was

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lower than the 59.6 to 67.0 g/day reported in an earlier study when the same supplements were fed to sheep (Adjorlolo et al., 2020).

The lower dry matter digestibility in sheep fed CP-S compared to the other treatments could be attributed to lower crude protein intake of this supplement. This suggests that for goats on grass hay nitrogen is the more limiting nutrient for the rumen microbes, compared with starch which is high in the cassava peels. Also, anti-nutritional factors such as cyanogenic glycosides in the cassava peels might have slowed down microbial action and thereby decreased dry matter digestibility. Anti-nutritional factors are known to interfere with normal digestion, metabolism and absorption of nutrients (Gilani et al., 2005). Crude protein and neutral detergent fibre digestibility also followed a similar tend to that of dry matter digestibility. The higher crude protein intake of goats fed SS-S, AA-S and FE-S based diets over CP-B diets could have enhanced the digestibility of crude protein and neutral detergent fibre in these supplements than the CP-B. The leaves of trees and shrubs are high in readily degradable nitrogen and some by-pass protein. Inclusion of such browses in ruminant diets will cause faster fermentation rate and substrate degradation hence increasing dry matter intake. The dry matter and crude protein digestibility obtained in the present study were comparable to the 54.7 to 68% and 44.0 to 59.0% respectively reported when Red Sokoto goats were fed elephant grass (*Pennisetum purpereum*) ensiled with varying proportions of cassava peels (Olorunnisomo, 2011).

The high organic matter digestibility for AA-S and FE-S diets than CP-S could be due to the provision of adequate nutrients to the rumen microbes with consequent improvement in organic matter intake whilst higher levels of cyanogenic glycosides in CP-S adversely affected rumen microbial activity resulting in lower organic matter digestibility. Also, the lower crude protein digestibility in goats fed CP-S may account for their lowest organic matter digestibility. The neutral detergent fibre digestibility was higher for SS-S, AA-S and FE-S than CP-B probably due to moderate concentrations of secondary metabolites in the Samanea, Acacia and Ficus leaf meals that might have had positive influence on rumen microbes in accordance with some reports that low or moderate concentrations of secondary metabolites positively impacts rumen fermentation (Salem et al., 2006; Jiménez-Peralta, 2011). The low crude protein level in CP-S could have inhibited rumen activity thus decreasing digestibility of neutral detergent fibre of goats fed that diet.

Similarity in weight gain for goats on CP-S to the other treatments, in spite of the differences in digestibility, may suggest similar metabolisable energy intake due to higher level of digestible starch in cassava peels. Daily weight gain in this study ranged from 9.64 to 13.6 g/day and feed conversion ratio ranged from 27.9 to 50.7 respectively. The average daily weight gains were comparable to the 10.4 to 18.7 g/day obtained when Philippine native goats were fed concentrates with different inclusion levels of *Samanea Saman* (Morais et al., 2018) but lower than in other studies when goats were fed grass- hay basal diets or grass and silage diets supplemented with browse tree leaves or leguminous tree foliage (Okoruwa, 2020; Okoruwa and Ikhimioya, 2020).

Blood indices serve as useful indicators of nutritional, physiologic, metabolic and health status of farm animals (Mirzadeh et al., 2010; Onasanya et al., 2015) and hence essential in evaluating the suitability of introduced feed resources. The non—significant but similar concentrations of haematological parameters measured in the present study suggest similar ability of the dietary treatments in enhancing the production of haemoglobin for efficient transportation of gases, normal synthesis of RBCs and production of enough WBCs to adequately defend the body against infections. The inclusion of the supplements did not adversely affect the health of the goats indicating that the quality of the supplementary diets was good to help sustain growth of goats during periods when animals rely on poor quality fodder. The levels of the haematological parameters measured were within the normal physiological ranges reported for goats (Merck Manual, 2012) and were also comparable to the values reported by Baiden et al. (2007), when West African Dwarf goats were fed varying levels of cassava pulp as a replacement for cassava peels.

The higher serum urea concentrations in goats fed SS-S than those fed AA-S, FE-S and CP-S might be due to the higher crude protein levels in the Samanea leaf meal than the Acacia and Ficus leaf meals and the cassava peel meal. Most concentrations of the serum biochemical indices determined were within the normal physiological range reported for goats (Merck Manual 2012) suggesting feeding the supplements did not have adverse effects on the physiology of the West African Dwarf goats. The concentration of the biochemical parameters measured compared favourably to the values obtained by Hassan et al. (2015) when they fed some forage shrubs made up of Acacia, Leucaena and Moringa to goats during the dry season.

CONCLUSION

Feeding the supplements influenced feed intake and growth performance to a similar extent and did not have any deleterious effect on the physiology and health of the goats. Therefore, all the four dietary supplements could be fed to confined goats on roughage diets especially in the dry season to overcome the adverse effects of seasonal fluctuation in feed quality on growth and health of goats.

DECLARATIONS

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Authors' Contribution

LA conceived the study, participated in the design of the study, contributed to data analysis and the write up of the manuscript, EA participated in the data collection and contributed in data analysis and the write up of the manuscript, AM was in involved the design and data analysis of the study and contributed to the write up of the manuscript. FO participated in the design and coordination of the study, contributed to data analysis and the write up of the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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PHENOTYPIC CHARACTERIZATION OF GENETIC RESOURCES OF INDIGENOUS CATTLE IN WEST GONDAR ZONE OF ETHIOPIA

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study was conducted to identify and phenotypic characterization of the existing indigenous cattle genetic resources in the three districts of West Gondar zone of Amhara Region based on their descriptive morphological characteristics. A total of six sampling sites were purposively selected based on the distribution of unique cattle types. Key informant and focus group discussions were used to identify the new genetic resources. A total of 180 adult male and 720 female cattle were selected for 20 morphological and 9 biometrical traits. The focus group discussions revealed that, diversified indigenous cattle were found (Fellata in Quara, Rutana in Metema, Miramir in West Armachiho and Qocherie from all Districts). The overall mean linear body measurements of male cattle for horn length, body length, heart girth, canon bone length and cannon bone circumferences were 23.92 cm, 127.97 cm, 161.15cm, 20.37 cm and 19.81 respectively. Females had horn length (25.86 cm), body length (122.85 cm), pelvic width (37.47 cm) and canon bone length (19.26 cm), canon bone circumferences (18.36 cm) and mouth circumferences (36 cm). In a discriminate analysis, all sampled populations were classified with the overall hit rates of 75% for males and 60% for females. The shortest and longest mehalanobis distances were between Abrajira with Dubaba (0.762) and Gendawuha Birshign with Mirt Gelegu (37.88) for male and Gendawuha Birshign with Dubaba (0.358) and Gendawuha Birshign with Mirt Gelegu (29.37) for females, respectively. In stepwise discriminate analysis the top traits which were highly discriminated male cattle populations were cannon bone length, horn length and cannon bone circumferences. This was similar in females except the replacement of cannon bone circumferences with mouth circumferences. Three clusters were formed (Fellata from Mirt Gelegu in cluster one and Qocherie from Gendawuha Birshign, Dubaba and Abrajira in cluster two and Rutta from Lominat Forgena and Miramir from Gilal Wuha in cluster three. Three indigenous cattle types were identified with their phenotypic features and further molecular characterization should be done to confirm their genetic distinctiveness.

Keywords: Cattle, Characterization, Genetic Resources, Phenotype, West Gondar Region.

INTRODUCTION

Ethiopia is rich in animal genetic resources both in diversity and population number in Africa (Endashaw, 2010). The current national estimated number of livestock population except for the three zones of Afar and six zones of Somali region; 59.5 million cattle, 30.7 million sheep 30.2 million goat, 1.21 million camel, 56.53 million poultry, 2.16 million horses, 0.41 million mules and 8.44 million donkeys (CSA, 2017). According to FAO (2010) at the level of national economy average livestock production contributes about 30-35% of agricultural gross domestic product (GDP) and 1-16% of the overall national GDP. The same source indicated that cattle have the highest figure and most important sources which contributed about 35% in agricultural GDP and 15% in total GDP. Among livestock population, cattle contribute a lot to improve the wellbeing of the farm family through food supply, balancing nutrition, income, savings, insurance, ritual and other social purposes.

The contribution of livestock to the country's economy is too low when compared with the population size. This might be due to weak estimation methodology or weak analysis of economic values of livestock products and poor plane of nutrition, prevalence of disease and lack of appropriate breeding strategies (Mezgebe et al., 2018; Sasaki et al., 2019). To divert this challenges; identification and phenotypic characterization of cattle genetic resources are necessary in order to develop breed improvement programs (FAO, 2007). However, based on the previous work at national level, there is insufficient information about breed characterization at phenotypic and genetic levels (DAGRIS, 2009; Kemp et al., 2007). Similarly, no one has conducted a research work on phenotypic characterization of cattle genetic resources in the border of Sudan in the Western part of West Gondar zone. Therefore, with this gap the specific objective was initiated to identify and phenotypically characterize cattle genetic resources in West Gondar zone, Amhara Region, Ethiopia.

MATERIAL AND METHODS

Description of the study area

The study was conducted in the three districts namely Quara, Metema and West Armachiho of West Gondar zone. Exploratory approach and purposive sampling techniques were used to characterize the unique and diversified cattle

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population in the zone. To know the distribution of cattle genetic resources and establish the sampling frame, rapid preliminary survey and discussions were held with zonal, district and rural development agents of livestock experts. Based on the information, six representative sites were purposively selected. Sampling frame, from each mature age cattle breed types n=150 (30 males and 120 females) were randomly selected for administration of morphological and biometrical measurements (FAO, 2012). Therefore, a total of 180 male and 720 female cattle were randomly selected for phenotypic characterization.

Data types

Twenty categorical traits were recorded and documented from live adult cattle by visual observation and nine quantitative traits were considered and measured using mark off measuring tape in cm with a precision of 0.5 from cattle with wear off one and above pair of permanent teeth (FAO, 2012).

Data analysis

Univarite analysis. General linear model procedures (PROC GLM) were employed for quantitative variables to detect statistical differences among sample cattle population. The model was developed to evaluate the linear body measurements with the fixed effect of sex and sites.

$Y_{jk} = \mu + S_j + L_k + e_{jk}$

Yjk: Linear body measurements in each the j^{th} sex and k^{th} location; μ: Overall mean, for both sexes separately; S_j: the effect of j^{th} sex (j: 1=male, 2= female); L_k: the effect of k^{th} location (k: 1=Dubaba, 2=Mirt Gelegu, 3=G.Birshign, 4=Lominat Forgena, 5=Abrajira, 6=Gilal Wuha); e_{jk}: residual random error associated with $_{jk}{}^{th}$ observation.

Multivariate analysis

Quantitative variables from male and female animals were separately subjected to discriminate analysis (PROC DISCRIM) to classify the sampled populations into homogenous/distinct breeds as a breed in their home areas. Procedure of canonical discriminate analysis (PROC CANDISC) was used to determine the existence of population level phenotypic differences among the sample cattle populations/the study sites. The step wise discriminate analysis procedure (PROC STEP DISC) was used and run to rank the variables by their discriminating power. Correspondence analyses were considered for association of different categorical variables for each study sites/cattle population. Finally, to classify the sampled cattle population cluster analysis were carried out by using quantitative variables and then classification trees were constructed based on results of the analysis and showed groups of the identified breed types.

RESULT AND DISCUSSION

Univarite analysis

Quantitative Variation for male population. Almost all continuous variables were highly significant ($p<0.0001$) and affected by site except ear length ($p<0.99$; Table 1). This might be due to the study areas were occupied by different breed types with varied quantitative traits. The smallest and highest coefficient of determination (R^2) was calculated for ear length and for heart girth, respectively. Whereas, for the coefficient of variation (CV) was heart girth and horn length, respectively. The least square means comparison among the study sites revealed that male population from Gilal Wuha, Lominat Forgena and Mirt Gelegu had the largest values than the remaining sites for most variables. While, the lower least square mean values were obtained from the remaining three sites (Dubaba, Gendawuha Birshign and Abrajira).

Quantitative variation for female population. Like male population except ear length all sampled continuous variables were highly significant ($p<0.0001$) for sample sites of female cattle population (Table 2). The coefficients of variation ranged from 2.39% for heart girth to 34.9% for horn length. Traits which had high coefficient values were possible for source of variation in the population. The highest least square mean values for most measurable traits in female sampled population were observed in Gilal Wuha, Lominat Forgena and Mirt Gelegu sites. This indicated that the cattle type from these sites had phenotypic superiority than the remaining site cattle types. This agrees with Monastery and Begait cattle type documented by Zewdu (2004); Mulugeta (2015), respectively. Conversely, the lowest least square mean values were recorded from Dubaba, Gendawuha Birshign and Abrajira. These three sites had almost similar measurement values. This might be due to their continual interbreeding or same phenotypic features. These findings are similar with Zewdu (2004), Fasil (2006); Tadesse (2015) who reported for North Gondar lowland, Gojjam highland and Horro cattle types, respectively.

Multivariate analysis

Discriminate analysis. The correct classification percentages for male population were ranged from 46.67% to 93.33% for site 1 (Dubaba) and site 2 (Mirt Gelegu), respectively. The remaining reclassification values of Dubaba were explained in Gendawuha Birshign (16.67%) and Abrajira (36.67%) sites. This indicated that males from Dubaba were shared high number of cross classification hit rates with Gendawha Birshign and Abrajira sites. Among the study sites male cattle population from Mirt Gelegu scored the highest classification percentages (93.33%). This indicated that male cattle from Mirt Gelegu were more heterogeneous and distinct from other site population. Similarly, male cattle

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population in Lominat Forgena had correct classification of 76.67% in their sites and the incorrect classification (23.33%) was explained in Gilal Wuha location. This revealed that males from these sites had common characteristics. The overall correct and incorrect classification percentages of male populations in all the study sites were 75% and 25%, respectively. This correct hit rate classification higher than (Zewdu, 2004) for north Gondar cattle (73.8%) but lower (Tadesse, 2005) for Horro cattle (82.72%) and (Fasil, 2006) for Gojjam highland cattle (80.85%), respectively. Similarly, the correct classification percentages of female sampled population for all sites were ranged from Gendawuha Birshign (42.5%) to Mirt Gelegu (98.33%) sites. The remaining classification percentages of Gendawuha Birshign sites were incorrectly classified in Dubaba (23.33%), Abrajira (33.33%) sites. Correspondingly, the correct and incorrect classification values of female population from Lominat Forgena were 47.5% and 37.5%, respectively. This incorrect classification percentage was explained in Gilal Wuha. This indicated that the two sites had similar phenotypic features. The overall correct classification hit rates for female sample population were 60%. The overall error count estimation in male sample population was better than female population (as the average hit rate was 75% than 60%).

Canonical discriminate analysis. It was performed by CANDISC procedures separately for male and female sample population. As per Table 5, all mahalanobis distances obtained from the sites for male population were highly significant ($p<0.0001$). Therefore, male population from each site has their own measurable differences. The nearest distances were observed in Abrajira and Dubaba sites with the value of 0.76185. This might be due to the two sites had phenotypically similar male cattle population. Whereas, the longest distances (37.88) was observed between Gendawuha Birshign and Mirt Gelegu sites. Therefore, these two sites had their own distinct and different male cattle types. In case of female sample population, the Mahalanobis distance between sites was slightly lower than that of male population. Mahalanobis distances obtained for female population was significant for all sites. The nearest distance was observed in between Gendawuha Birshign and Dubaba followed by Abrajira and Gendawuha Birshign with the values of 0.35833 and 0.51030, respectively. The largest distance was observed between site 3 (Gendawuha Birshign) and site 2 (Mirt Gelegu) with the value of 29.38. The narrower and wider differences were resulted from phenotypic similarities and disparities between the sample populations.

Stepwise discriminate analysis. All 9 continuous variables for both sexes were separately subjected to the STEPDISC procedure of (SAS, 9.4) and all 9 variables for males and 8 variables for females were identified as best discriminating variables (Table 7). Variables used for discriminating male breed types were ordered based on their discriminating powers namely cannon bone length, horn length, cannon bone circumferences and body length. Similarly, top variables, which discriminate female population, were cannon bone length, horn length, mouth circumferences, body length, ear length, and cannon bone circumferences. Whereas, height at wither was weak for separating female sample population and was removed.

Correspondence analysis for qualitative variables. Twenty different categorical variables for all the study sites were considered for correspondence analysis (Figure 1). Two dimensions were observed for the clear relationships of the traits with study sites. Categorical variable lies on the middle line were predominantly found in all the population and represented low variation among the population. Small hump size, and lateral ear orientation in (sites 1 and 2) and, erect horn shape, convex facial profile, medium hump size and medium body condition both in site 3, 5 and site 4, 6 had very close to the middle line. And, for all population large tail length, medium body condition score, sloppy rump profile, short and curved horn shape and black white color variables represented very low variations. Whereas, categorical variables far from the middle line were distinguished the 6 studied populations namely coat color type, navel flap, dewlap width, udder size and teat length in one hand and the genetic groups were in the other hands.

Cluster analysis. Cluster analysis is a multivariate technique used to group individuals based on common characteristics in to homogeneous and distinct groups (Peter, 1997). Due to this; the population in one cluster shared certain common characters more than the population in other cluster. According to cluster analysis 3 similar clusters were formed for both sexes in figure 2. Hence, the first cluster consisted of sample population of site 2 (Mirt Gelegu) which, represent Fellata cattle type, came out distinctly far from the rest group breed types in both cases. The second cluster contained three sites namely; site 1 (Dubaba), site 3 (Gendawuha Birshign) and site 5 (Abrajira). Moreover, the cattle type from site 4 (Lominat Forgena) and site 6 (Gilal Wuha) grouped under the third clusters.

Cluster 1: Fellata cattle type. This cattle type is unique in many aspects from the rest of the population. They are aggressive in their temperament and large in body size. Their coat colour pattern is plain (76.0%) and patchy (14.7%) and spotted (9.3%). The hair types were shiny (69.3%) with dull (26.0%) and smooth (9.3%). Most of the animals have exceptionally longer horns (50.95 cm in females and 47.06 cm in males) with white in colour and lyre (54%), curved (26.7%) and straight (19.3%) in its shape. Mostly flat (58%) facial profiles were observed in both sexes and rarely concave with thin neck in females and regularly convex in males. Ear is medium to big in size with mainly straight and pendulous shapes. Humps and dewlaps are well developed in males with good height at wither but small humps with erected shapes and almost non dewlaps in females (their unique features). Body condition scoring of this cattle was medium (78.7%), lean (16.0%) and fat (5.3%) in both sexes. Tail is medium to long in females and long with bushy ends in males; important for prevention of wild flies. Cows have longer navel length, large udder and teat size of all the breed types in the study location. These characteristics were considered for selection criteria in the study location. Similarly, perpetual sheath and testicular lengths are very long in males. These traits were also highly considered in the communities for selection criteria's of breeding bull. These cattle types mainly serve for the community through reproduction, milk production and

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for income generation, and draft power. According to key informants; this cattle type is believed to be found only in Quara district of specific location.

Cluster 2: Qocherie /Korrer cattle type. These are medium body size cattle types with good temperament. Their coat colors are dominantly plain of red, white, black, grey and patchy of black brown, with a very small proportion of spotted of red white, white and red sora pattern and types, respectively. Their facial profile is dominantly concave in females and convex in males with moderate ear length of straight and slightly pendulous in shapes and forward, lateral in orientation. In both sexes, horns are medium to large in size; straight, curved in shapes with forward and upward in orientation. Hump and dewlap sizes are relatively large in males with moderate heart girth and small to medium size in females. They have small to medium length of navel flap with medium to large udder and teat size with good taste of milk. Perpetual sheath and testicular circumferences are medium to large in males. Tail is dominantly medium to long in its length in females and long (below hocks) in males. As group discussants revealed that main attributes of these cattle types are milk production, reproduction and income purposes. Based on the above characteristics these dominant Qocherie cattle types are grouped under Small East African Zebu cattle types.

Cluster 3: Ruttana/Miramir cattle type. They are huge in body size when compared with other cattle types and able to communicate with language. These cattle types are almost similar with the characteristics of large East African Zebu and not documented in literature as Rutana/Miramir cattle in the country. They are aggressive in temper but it is desired by their owners (cannot be stolen by theft). Their coat color pattern is spotted and patchy composed of white- black, black-white and gray type; rarely plain of black, white and grey. Mostly they have shiny, dull and the rest smooth hair types. Horns are medium to large in both sexes with straight, curved and lyre shapes and some cattle also have no horns. Females' have predominately flat and slightly concave facial profile and male have convex profiles. Ear is medium to large in size and mainly straight shapes and they are good in body conformation and scored mainly medium and rarely fat condition with droopy, sloppy and flat rumps. Hump sizes are small to medium in females and well developed in males with commonly erected and droopy shapes with cervico-thoracic in its position. Similarly, dewlap sizes are large in males but very small in females. These cattle types are known with longest sheath and testicular circumferences. Medium to large udder and teat size and long navel flaps. Majorities have long tail length and in small proportion has medium lengths. Milk, reproduction, income generation, traction power and beef are among the most important of functions of this cattle type. According to key informants Rutana-Miramir cattle types are found only in specific location of Metema and West Armachiho districts border to Sudan, respectively. Except body size (little big in Miramir), name of cattle type and their location, all the rest characters are almost similar in phenotype for these cattle type and grouped under one cluster. As per the above, characteristics indicated that these cattle types are a unique.

Table 1 - Least square means and pair wise comparisons of body measurements for males by site (cm)

Variables	Dubaba	M.G	G.B	L.F	Abrajira	G.Wuha	R^2	CV	Site
Horn length	18.81^{b}	47.06^{a}	16.41^{b}	21.28^{b}	17.48^{b}	22.51^{b}	64.32	33.3	p<.0001
Ear length	22.12^{a}	22.02^{a}	20.99^{a}	21.75^{a}	22.17^{a}	22.34^{a}	40.48	5.52	0.999
Body length	119.41^{b}	136.41^{a}	117.63^{b}	138.37^{a}	117.84^{b}	138.15^{a}	83.70	3.97	p<.0001
Height W.	113.38^{b}	132.53^{a}	110.19^{b}	135.23^{a}	114.26^{b}	136.49^{a}	80.89	5.05	p<.0001
Canon BL	16.64^{b}	24.59^{a}	16.17^{b}	23.47^{a}	16.83^{b}	24.53^{a}	79.98	9.88	p<.0001
Canon BC	16.64^{b}	22.26^{a}	17.42^{b}	22.64^{a}	16.19^{b}	23.69^{a}	77.09	9.17	p<.0001
Mouth C	38.63^{b}	39.89^{ab}	38.64^{b}	39.85^{b}	38.71^{b}	41.42^{a}	55.78	5.04	p<.0001
Pelvic width	35.71^{b}	41.16^{a}	36.70^{b}	41.51^{a}	36.56^{b}	42.20^{a}	70.16	5.46	p<.0001
Heart girth	151.98^{b}	170.48^{a}	148.86^{b}	172.94^{a}	152.35^{b}	170.32^{a}	85.49	3.28	p<.0001

$^{a.b.c.e.f}$ Means with the same superscript letter on the same row are not significantly different, R^2= Coefficient of determination, CV= Coefficient of Variation; M.G= Mirt gelegu, G.B=Gendawuha Birshign and L.F=Lominat forgena.

Table 2 - Least square means and pair wise comparisons of body measurements for females by site (cm)

Variables	Dubaba	M.G	G.B	L.F	Abrajira	G.wuha	R^2	CV	Site
Horn length	20.63^{b}	50.95^{a}	20.95^{b}	19.75^{b}	19.92^{b}	22.97^{b}	62.92	34.9	p<.0001
Ear length	21.23^{a}	21.03^{a}	21.51^{a}	21.22^{a}	21.13^{a}	21.56^{a}	42.02	6.22	0.999
Body length	116.63^{b}	129.79^{a}	116.65^{b}	127.90^{a}	114.82^{b}	130.49^{a}	74.21	4.03	p<.0001
Wither Height	110.83^{b}	122.65^{a}	110.32^{b}	121.33^{a}	108.81^{b}	124.17^{a}	68.48	4.85	p<.0001
Canon BL	17.07^{c}	22.26^{a}	16.47^{c}	21.31^{b}	16.32^{c}	22.13^{ab}	78.71	8.00	p<.0001
Canon BC	16.46^{b}	20.41^{a}	16.03^{bc}	20.64^{a}	15.36^{c}	21.36^{a}	76.20	8.47	p<.0001
Mouth C.	36.35^{ab}	34.51^{c}	36.52^{b}	36.16^{b}	35.71^{b}	36.78^{a}	44.31	4.97	p<.0001
pelvic width	35.63^{b}	38.49^{a}	36.15^{b}	38.98^{a}	36.16^{b}	39.42^{a}	64.43	5.65	p<.0001
Heart girth	148.61^{d}	152.32^{c}	147.57^{d}	155.03^{b}	147.57^{d}	157.29^{a}	79.74	2.39	p<.0001

$^{a.b.c.d.e.f}$ Means with the same letter on the same row are not significantly different; R^2= Coefficient of determination, CV=Coefficient of Variation;M.G= Mirt gelegu,G.B=Gendawuha Birshign and L.Forgena=Lominat Forgena.

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Table 3 - Number of observation and percentages (in bracket) for male sample population using discriminant analysis in each study sites

Site	Dubaba	M.Gelegu	G.Birshig	L.Fogena	Abrajira	G.Wuha	Total
Dubaba	14(46.67)	0	5(16.67)	0	11(36.67)	0	30(100)
M.G	0	28(93.33)	0	1(3.33)	0	1(3.33)	30(100)
G.B	2(6.67)	0	24(80.00)	0	4(13.33)	0	30(100)
L.F	0	0	0	23(76.67)	0	7(23.33)	30(100)
Abrajira	4(13.33)	0	2(6.67)	0	24(80.00)	0	30(100)
G.Wuha	0	0	0	8(26.67)	0	22(73.33)	30(100)
*TECE	0.5333	0.0667	0.2000	0.2333	0.2000	0.2667	25(100)

M.G=Mirt Gelegu, G.B=Gendawuha Birshign, L.F=Lominat Forgena, G.W=Gilal Wuha and*TECE=total error count estimate.

Table 4 - Number of observation and percentages (in bracket) for female sample population using discriminate analysis in each study sites

Sites	Dubaba	M.Gelegu	G.Birshign	L.Fogena	Abrajira	G.Wuha	Total
Dubaba	56(46.67)	0	33(27.5)	3(2.5)	28(23.33)	0	120(100)
M.G	1(0.83)	118(98.33)	0	1(0.83)	0	0	120(100)
G.B	28(23.33)	1(0.83)	51(42.50)	0	40(33.33)	0	120(100)
L.F	12(10.00)	1(0.83)	3(2.50)	57(47.50)	2(1.67)	45(37.50)	120(100)
Abrajira	29(24.17)	0	24(20.00)	1(0.83)	66(55.00)	0	120(100)
G.Wuha	1(0.83)	2(1.67)	1(0.83)	32(26.67)	0	84(70.00)	120(100)
*TECE	0.5333	0.0167	0.5750	0.5250	0.4500	0.3000	40(100)

M.G=Mirt Gelegu, G.B=Gendawuha Birshign, L.F=Lominat Forgena, G.W=Gilal Wuha and*TECE=total error count estimate.

Table 5 - Mahalanobis distances between sites for male sample population

Sites	Dubaba	M.Gelegu	G.Bishign	L.Forgena	Abrajira	G.Wuha
Dubaba	***					
M.Gelegu	34.52597	***				
G.Bishign	2.77802	37.88397	***			
L.forgena	31.35831	14.73973	29.23378	***		
Abrajira	0.76185	35.01792	4.28409	31.60054	***	
G.Wuha	30.62582	14.68674	27.05451	2.28319	30.97339	***

***=p<.0001, It is correlation type/mirror reflection of each other that is why I used semi diagonal matrix.

Table 6 - Mahalanobis distances between sites for female sample population

Sites	Dubaba	M.Gelegu	G.Bishign	L.forgena	Abrajira	G.Wuha
Dubaba	***					
M.Gelegu	25.76750	***				
G.Bishign	0.35833	29.37554	***			
L.forgena	11.26041	15.30541	14.40911	***		
Abrajira	0.92079	28.48766	0.51030	14.51182	***	
G.Wuha	14.53294	14.26129	18.27180	0.58177	18.33785	***

***=p<.0001

Table 7 - Stepwise selection summary table for male and female population

Steps in discriminating power		Entered Traits	Partial R^2	F-statistics	Pr > F	Wilks' Lambda	Pr< Lambda	Average Squared Canonical Correlation	Pr>ASCC
Male population	1	CBL	0.763	112.17	<.0001	0.237	<.0001	0.153	<.0001
	2	HL	0.488	32.93	<.0001	0.121	<.0001	0.244	<.0001
	3	CBC	0.329	16.87	<.0001	0.081	<.0001	0.298	<.0001
	4	BL	0.289	13.96	<.0001	0.058	<.0001	0.323	<.0001
	5	EL	0.264	12.17	<.0001	0.043	<.0001	0.336	<.0001
	6	HW	0.121	4.67	0.0005	0.037	<.0001	0.349	<.0001
	7	HG	0.102	3.81	0.0027	0.034	<.0001	0.367	<.0001
	8	PW	0.099	3.71	0.0033	0.030	<.0001	0.383	<.0001
	9	MC	0.082	2.96	0.0138	0.028	<.0001	0.389	<.0001
Female population	1	CBL	0.71	349.9	<.0001	0.299	<.0001	0.142	<.0001
	2	HL	0.52	153.87	<.0001	0.139	<.0001	0.245	<.0001
	3	MC	0.25	46.72	<.0001	0.105	<.0001	0.266	<.0001
	4	BL	0.12	19.94	<.0001	0.092	<.0001	0.274	<.0001
	5	EL	0.099	15.73	<.0001	0.083	<.0001	0.279	<.0001
	6	CBC	0.093	14.45	<.0001	0.075	<.0001	0.289	<.0001
	7	PW	0.048	7.12	<.0001	0.071	<.0001	0.298	<.0001
	8	HG	0.016	2.34	0.0402	0.070	<.0001	0.299	<.0001

The P-values for both Wilks' Lambda and ASCC were highly significant (P<0.0001) for both sexes, HL= horn length, EL= ear length, BL=body length, height at wither, CBL=canon bon length, CBC=canon bon circumference, MC=mouth circumference, PW=pelvic width and HG= heart girth

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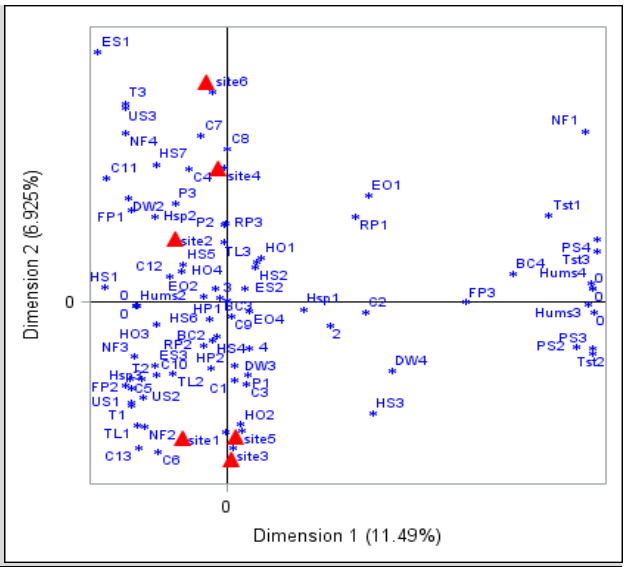

Figure 1- Associations among qualitative variables revealed by multiple correspondence analyses.

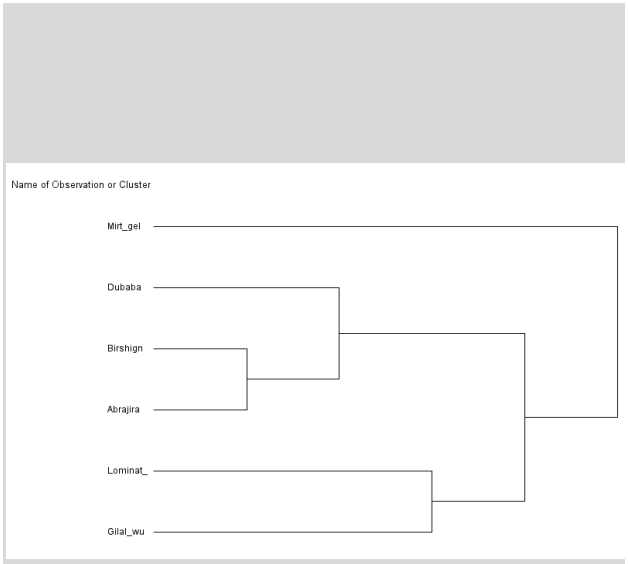

Figure 2 - Cluster trees for cattle population by study site.

Figure 3 - Fellata cattle type.

Figure 4 - Breeding female to the left and breeding male to the right in Fellata cattle.

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Figure 5 - Qocherie cattle type.

Figure 6 - Breeding female to the left and breeding male to the right in Qocherie cattle.

Figure 7 - Rutana or Miramir cattle type.

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Figure 8 - Breeding female to the left from Rutana and right from Miramir.

Figure 9 - Breeding male to the left from Rutana and right from Miramir.

CONCLUSION

The key informant and focus group discussions are found to be a useful individual to identify distinct animal genetic resources of Qocherie/Korer, Fellata, Qocherie/Habesha, Rutana, Qocherie/Gobie and Miramir from the six sites of West Gondar zone. Phenotypically Fellata, Rutana and Miramir cattle had longer and larger body length, wither height, heart girth and pelvic width than Qocheire/korer/Gobie cattle types. In addition, characterization of the phenotypic diversity of cattle based on their morphologies, performances with univariate and multivariate analysis has given the powerful evidences on the general uniqueness of the three traditional cattle breed types (Qocherie/Korer/Gobie, Fellata and Rutana/Miramir).

Recommendation

The country, Amhara region and university of Gondar farm should try to introducing; Fellata and Rutana/Miramir cattle breed to on station characterization for breed improvement programs. Further, confirmatory and exploratory study should be employed other parts of Ethiopia to investigate the remaining cattle in the country. In-depth molecular characterization using genetic markers should be under taken to confirm the level of genetic variations and relationships among the identified and other indigenous cattle types in the country.

DECLARATIONS

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Authors' Contribution

All authors had similar role in writing and conducting of study.

Conflict of interests

The authors have not declared any conflict of interests.

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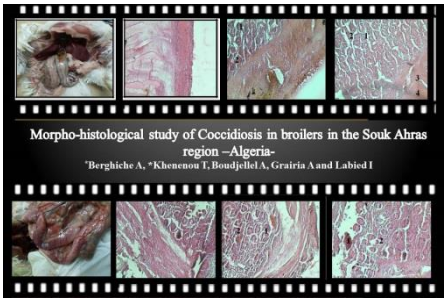

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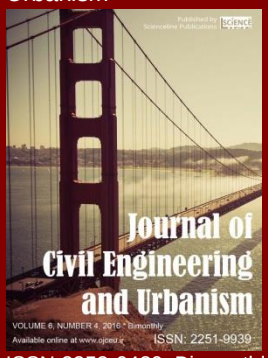

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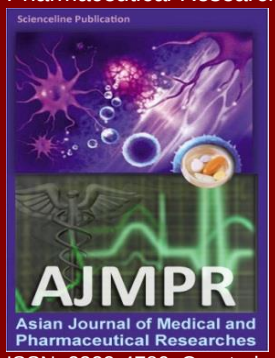

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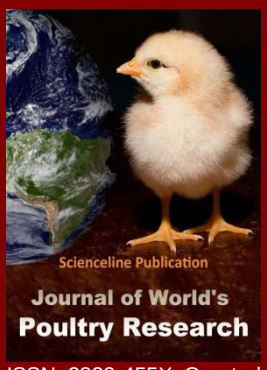

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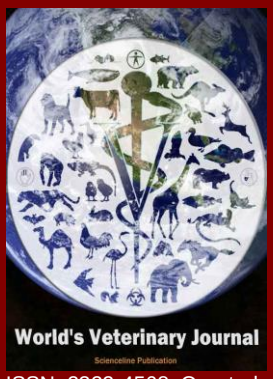

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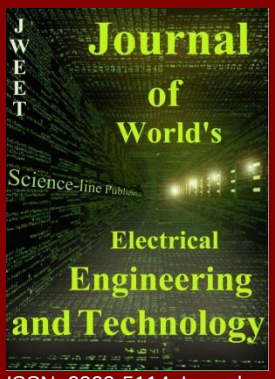

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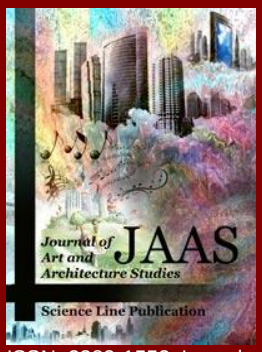

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