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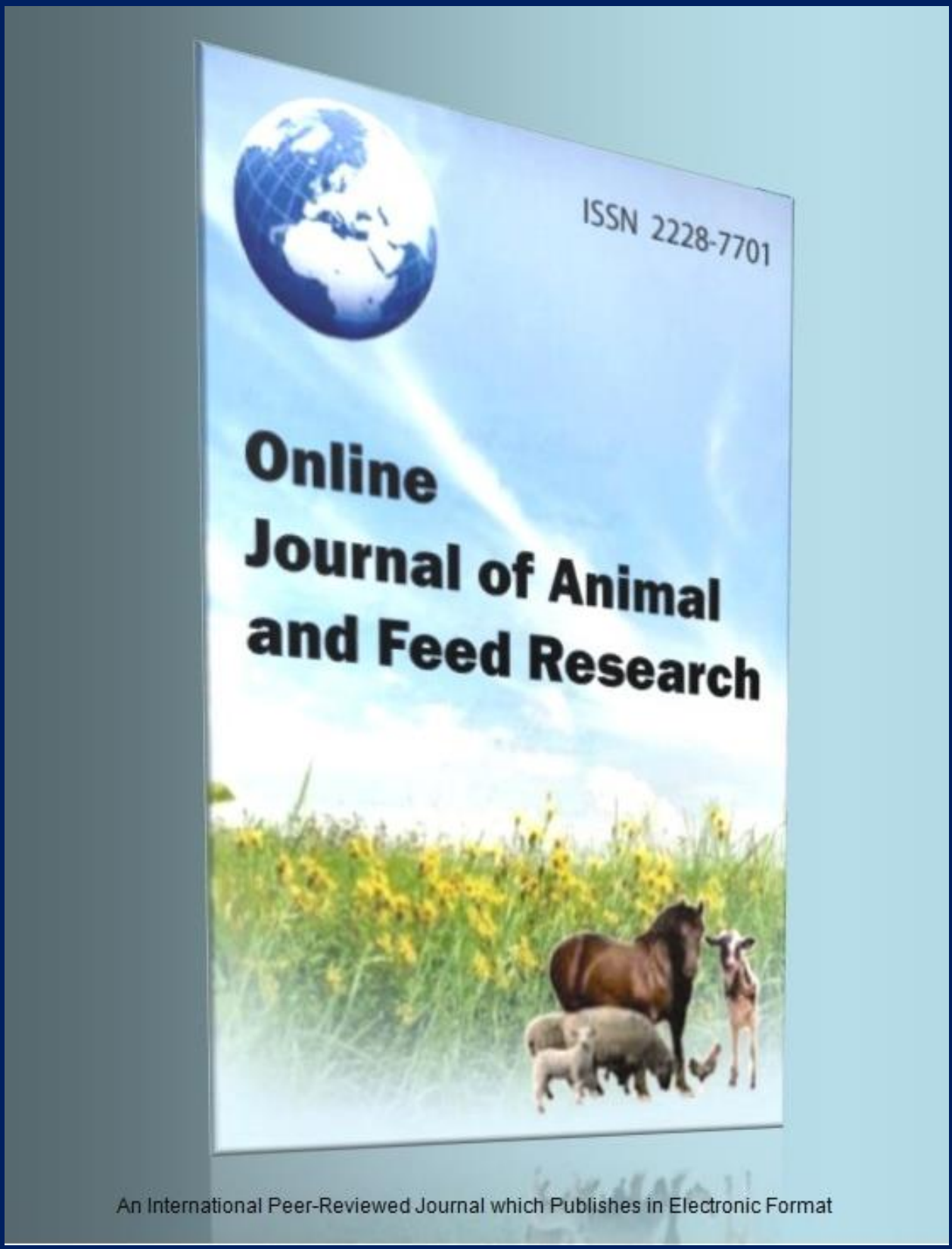

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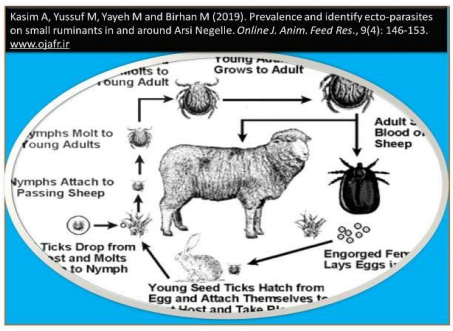
Volume 9 (4); July 25, 2019

Review



Prevalence and identify ecto-parasites on small ruminants in and around Arsi Negelle.

Kasim A, Yussuf M, Yayeh M and Birhan M.

Online J. Anim. Feed Res., 9(4): 146-153, 2019; pii: S222877011900019-9

Abstract

A cross sectional study was conducted to determine the prevalence and identify Ecto-parasites on small ruminants. A total of 422 small ruminants; sheep (n=246) and goats (n=176) were included in and around Arsi Negelle. Simple random sampling method was used to determine clinical infestated animal from healthy animals and data was analysed using SPSS 16 software for the description of p-value and chi-square. The result indicated that overall prevalence of ectoparasite in small ruminant was 126 (29.9%). Of which the prevalence of ectoparasite in sheep and goat was about 77 (31%) and 49 (27%), respectively. The most common ectoparasite encountered in order of their predominance were, lice (31.7%), flea (24.6), tick (19%), mixed (12.7%) and mite (12%). The statistical analysis indicated that sex (X2=13.774, P=0.0001), body condition (X2=40.463, P=0.0001) and age (X2=6.129, P=0.013) were some of the important factors showed significant difference to ectoparasite infestation of small ruminants. No statistical significant difference (P>0.05) were found between the species of small ruminants and ectoparasite infestations. The major ectoparasites at genus level identified on sheep and goat, were Ctenocephalides spp (23%), Bovicola (20.6%), Linognathus species (11.9%) Rhipicephaluses (8.7%), Amblyomma (7.1%), sarcoptes (7.1%), Hyalomma (5%) and Demodex (4.8). This study was conducted to identify the major ectoparasite and their prevalence on the small ruminants in study area. The most important ectoparasite identified, were lice, flea, ticks and mite. Therefore based on the findings following recommendations are forwarded: Strategic treatment of small ruminants with insecticides should be practiced in the study area to minimize the impact of ectoparasite on the health of animals; Awareness creation for the local farmers about the control of ectoparasite is essential and Further detailed study on economic loss associated with ectoparasite should be conducted.

Keywords: Ectoparasite, Prevalence, Small Ruminant, Arsi Negelle

[Full text-PDF]

Research Paper

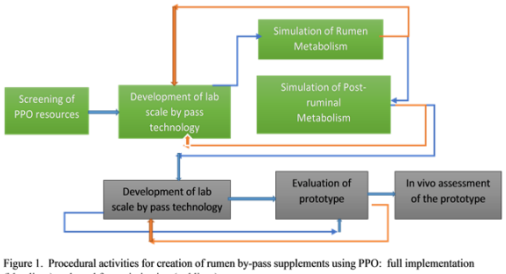


Figure 1. Procedural activities for creation of rumen by-pass supplements using PPO: full implementation (blue lines) and need for optimization (red lines).

Polyphenol oxidase containing extract: sources and contributions in animal nutrition.

Chanie D, Fievez V and Tegegne F.

Online J. Anim. Feed Res., 9(4): 154-157, 2019; pii: S222877011900020-9

Abstract

Polyphenol oxidase (PPOs) which are found naturally from plants, fruits, and vegetables and agricultural by products are getting considerable attention in food processing industries and in animal nutrition due to their antioxidant activity. Even though synthetic antioxidants have been used widely; they become a major cause for nutritional losses and quality deterioration which subsequently results in health problem in humans. To substitute synthetic antioxidants with natural Polyphenol oxidase (PPOs) containing extracts, many researches are being conducted on characterization of PPO sources and on protection of polyunsaturated oils and protein against ruminal degradation using PPO. Among many sources of PPO, Apple (malus sylevestris var domestica), Banana (*Musa cavendishii*) Mango (*mangifera indicacv.manila*) and Red clover (*Trifolium pretense L.*), Tomato stems (*Solanum lycopersicum L.*), Potato (*Solanum tuberosum L.*) are mentioned by different researchers from fruit sources and other plant sources, respectively. The contribution of PPO for protein and lipid protection is also being investigated. The main aim of this paper is to document the existing information on different sources and contribution of PPO as a baseline for further investigation. Further laboratory investigation on the role of polyphenol oxidase is crucial to improve the productivity of livestock with minimum feed cost.

Keywords: Polyphenol oxidase, Rumen, Protein degradation, Lipid degradation

[Full text-PDF]

Research Paper

Study on dystocia with history of uterine infections and calving hygiene along with subclinical endometritis in dairy cows in and around Gondar, NW Ethiopia.

Moges N.

Online J. Anim. Feed Res., 9(4): 158-161, 2019; pii: S222877011900021-9

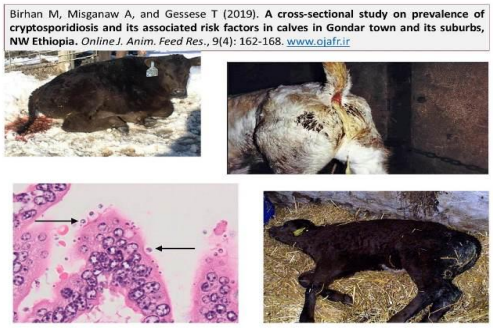
Abstract

The aim of this study was to assess dystocia with history of uterine infections and calving hygiene on the characteristics of the intrauterine environment in dairy cows by cytological tests were performed on intrauterine perfusion fluid along with subclinical endometritis conducted between January 2016 to September 2017. The percentage of polymorphonuclear cells (neutrophil) was calculated. It was found that increase in the number of neutrophils correlated with increase subclinical endometritis. During the study period n=147 apparently healthy 3rd trimester pregnant cows were selected in dairy farms in and around Gondar, North Western Ethiopia. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Abnormal parturition, uterine infections and calving hygiene were statistically significant (P<0.05) for subclinical endometritis. In conclusion, subclinical endometritis in postpartum dairy cows resulting in substantial economic losses due to decreases in both milk production and fertility.

Keywords: Calving hygiene, Cow, Dystocia, Gondar, Subclinical endometritis, Uterine infection

[Full text-PDF]

Research Paper



A cross-sectional study on prevalence of *cryptosporidiosis* and its associated risk factors in calves in Gondar town and its suburbs, NW Ethiopia.

Birhan M, Misganaw A, and Gessese T.

Online J. Anim. Feed Res., 9(4): 162-168, 2019; pii: S222877011800022-9

Abstract

Cryptosporidiosis is a common gastrointestinal disorder in humans and animals caused by various *Cryptosporidium* species. The present study was carried out to determine the prevalence of *cryptosporidium oocyst* and its potential risk factors in calves less than one year of age in and around Gondar town. For this purpose, 384 fecal samples (n=384 calves) from different dairy farms were collected and screened by using modified Ziehl-Neelsen staining technique. The overall prevalence of *Cryptosporidium* oocysts was 21.4 % (82/384). The association between different risk factors and prevalence of *Cryptosporidium* oocysts was assessed. There were significant associations (P<0.05) between prevalence of *Cryptosporidium* oocysts and age of calves, fecal consistencies, daily cleaning of the farm and water source. On the other hand, there was no significant association between prevalence of *Cryptosporidium* oocysts and sex, breed, and body condition of the calves and also provision of colostrums to the calves (P>0.05). In conclusion, this study demonstrated host factors and management factors greatly affect the prevalence of Cryptosporididosis in calves. Therefore, the current study reported the role of host factors (age and sex) and management factors (water source and daily cleaning of the farm) needed to be clearly recognized by all stakeholders in order to understand their effects on the disease occurrences as well as in control and prevention of in calves.

Keywords: Calves; Cryptosporidiosis; Dairy farm; Oocyst; Ziehl-Neelsen

[Full text-PDF]

Research Paper

Cytological Study of subclinical endometritis with respect to age, parity, farm scale and body condition score in dairy cows.

Moges N.

Online J. Anim. Feed Res., 9(4): 169-172, 2019; pii: S222877011800023-9

Abstract

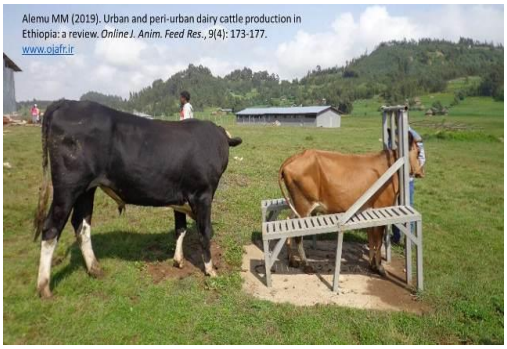
The objective of this study was to investigate the incidence of subclinical endometritis with respect of age, parity, farm scale and body condition score in dairy cows. A total n= 147 of apparently healthy 3rd trimester pregnant cows were selected with no signs of

clinical endometritis were examined from January 2016 to September 2017. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Age and parity were statistically significant (P<0.05) for subclinical endometritis. Older cows greater than 6 years were more affected sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) (χ2=51.97; P<0.05), the difference was statistically significant. The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) (χ2=14.48; P<0.05), the difference is statistically significant. However, body condition score and farm scale showed no significant variation with regard to subclinical endometritis.

Keywords: Age, Body condition score, Cytology, Gondar, Parity, Subclinical endometritis

[Full text-PDF]

Review



Urban and peri-urban dairy cattle production in Ethiopia: a review.

Alemu MM.

Online J. Anim. Feed Res., 9(4): 173-177, 2019; pii: S222877011800024-9

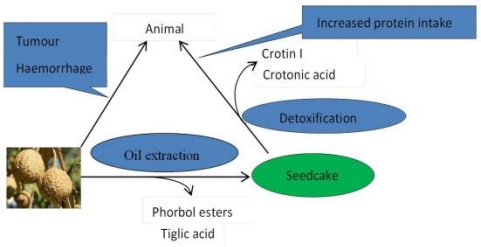
Abstract

Dairy production is among the developing agricultural sector in the urban and peri-urban areas of Ethiopia. This paper reviews the status of the current urban and peri-urban dairy cattle production systems and indicates possible recommendations. Hence management practices which includes feeds and feeding, breeding, housing and cattle holding are assessed based on different findings. Besides milk production potential, change drivers of the sector and major challenges are reviewed. The major feeds available for cattle are hay, crop residue and agro-industrial byproducts with stall feeding being the dominant system. In all reviewed findings both natural mating and AI is used though the preference is mostly affected by accessibility. Cities with highest population and better market and input access hold higher number of cattle per house hold than with lower population and market access. The average daily milk yield is higher in and around Addis Ababa followed by other big regional cities. High rate of urbanization, population growth, change in life style together with better access to inputs are behind the expansion of the sector. But shortage and cost of feed, shortage of land and waste disposal are the major challenges. Therefore, there is a need to organize the current status of the sector and look for solutions to cope up with the growing need of milk and milk products around the cities.

Keywords: Cattle Feeding, Dairy Breeding, Milk Production, Peri-Urban Dairy, Urban Dairy

[Full text-PDF]

Review



Owade JO, Gachuiri ChK, Abong GO (2019). **Utilization of croton seed as a possible animal feed: a review.** *Online J. Anim. Feed Res.*, 9(4): 178-186.
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Utilization of croton seed as a possible animal feed: a review.

Owade JO, Gachuiri ChK, Abong GO.

Online J. Anim. Feed Res., 9(4): 178-186, 2019; pii: S222877011800025-9

Abstract

Croton is one of the largest genera in the Family Euphorbiaceae. Its species are distributed in a wide range of environmental and climatic conditions. The plants have for a long time been exploited for medicinal purposes but still hold the potential for nutritional purposes. The seeds of the plant have been exploited in oil extraction to produce biofuel but the residual seedcake has been less utilized. The use of croton seed and seedcake as feed has been in practice in Kenya and other countries but questions on its safety have been raised. Croton seeds are known to contain various phytochemicals and toxins such as crotin I, crotonic acid and tiglic acid that have deleterious health effects on animals. Notwithstanding the rich nutritional composition of the croton seeds, its safety concerns have limited their utilization as feeds. The croton seeds are rich in both essential fatty acids and protein whereas the residual seedcake is only rich in protein. However, the seedcake has toxic phytochemicals that include cardiac glycocides, alkaloids, phorbal esters and many others which are injurious to the animals and could result in death. Detoxification of the seedcakes poses a breakthrough for their use in poultry feeding. However, such techniques should not reduce the rich nutritional property of these seeds. This review focuses on the utilization of croton seedcake as a possible animal feed, documenting breakthroughs and limitations of the practice.

Keywords: Croton, Seed, Seedcake, Nutrient composition, Safety, Detoxification

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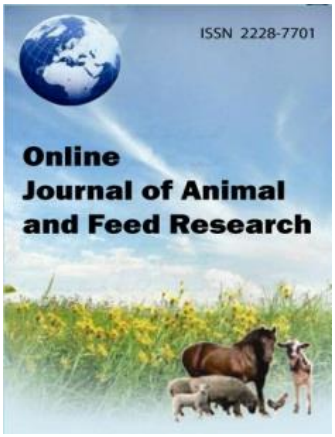

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PREVALENCE AND IDENTIFY ECTO-PARASITES ON SMALL RUMINANTS IN AND AROUND ARSI NEGELLE

Awol KASIM, Mohammed YUSSUF, Muluken YAYEH and Mastewal BIRHAN

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ORIGINAL ARTICLE

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ABSTRACT: A cross sectional study was conducted to determine the prevalence and identify Ecto-parasites on small ruminants. A total of 422 small ruminants; sheep (n=246) and goats (n=176) were included in and around Arsi Negelle. Simple random sampling method was used to determine clinical infestated animal from healthy animals and data was analysed using SPSS 16 software for the description of p-value and chi-square. The result indicated that overall prevalence of ectoparasite in small ruminant was 126 (29.9%). Of which the prevalence of ectoparasite in sheep and goat was about 77 (31%) and 49 (27%), respectively. The most common ectoparasite encountered in order of their predominance were, lice (31.7%), flea (24.6), tick (19%), mixed (12.7%) and mite (12%). The statistical analysis indicated that sex (X^2=13.774, P=0.0001), body condition (X^2=40.463, P=0.0001) and age (X^2=6.129, P=0.013) were some of the important factors showed significant difference to ectoparasite infestation of small ruminants. No statistical significant difference (P>0.05) were found between the species of small ruminants and ectoparasite infestations. The major ectoparasites at genus level identified on sheep and goat, were Ctenocephalides spp (23%), Bovicola (20.6%), Linognathus species (11.9%) Rhipicephaluses (8.7%), Amblyomma (7.1%), sarcoptes (7.1%), Hyalomma (5%) and Demodex (4.8). This study was conducted to identify the major ectoparasite and their prevalence on the small ruminants in study area. The most important ectoparasite identified, were lice, flea, ticks and mite. Therefore based on the findings following recommendations are forwarded: Strategic treatment of small ruminants with insecticides should be practiced in the study area to minimize the impact of ectoparasite on the health of animals; Awareness creation for the local farmers about the control of ectoparasite is essential and Further detailed study on economic loss associated with ectoparasite should be conducted.

Keywords: Ectoparasite, Prevalence, Small Ruminant, Arsi Negelle

INTRODUCTION

Ethiopia has the largest livestock inventories in Africa, including, about 53.99 million cattle, 25.5 million sheep, 24.06 million goats, 1.91 million horses, 6.75 million donkeys, 0.35 million mules, 0.92 million camels and about 50.38 million poultry are estimated to find in the country (CSA, 2013). In addition, wool and manure are also important by products of small ruminants' production (MoARD, 2005). Ectoparasite such as lice, flea, ticks, and mites are widely distributed in all agro–ecological zones in Ethiopia, causing serious economic losses in small holder farms (Kumsa et al., 2012). It was reported that 35% of sheep and 56% of goats skin rejection in Ethiopia are attributed to ectoparasite (Kassa, 2006).

Studies and reports from different parts of the country showed that skin quality deterioration is very evident mainly due to ectoparasite (Tefera, 2004; Numery, 2001; Ermias, 2000). All these established facts imply that ectoparasite pose serious economic losses to the farmers, the tanning industry and the country as a whole (Berhanu et al., 2011). This is because small ruminants' production in Ethiopia is constricted by the compound effects of diseases, poor feeding and poor management (Kassa, 2005; Ayele et al., 2003). Despite the large population of sheep and goats in the region, ectoparasite are also among serious problems in east Arsi zone of Oromia region (Hailu, 2010).

Even though the Oromia Regional State has started control program against ectoparasite in some selected districts of east Arsi zone which is the neighbor of west Arsi. However, there are no any reports presented on prevalence of ectoparasite from Arsi Negelle district. So this paper addresses the current prevalence of ectoparasite in small ruminant in Arsi Negelle, Southeastern Ethiopia, Oromia Regional State. Therefore the objectives of this thesis were: A) To identify common ectoparasite on small ruminant in and around Arsi Negelle district; B) To determine the prevalence of common ectoparasite of small ruminant in and around Arsi Negelle district; C) To determine major risk factors associated to the disease.

Citation: Kasim A, Yussuf M, Yayeh M and Birhan M (2019). Prevalence and identify ecto-parasites on small ruminants in and around Arsi Negelle. *Online J. Anim. Feed Res.*, 9(4): 146-153. www.ojafr.ir

MATERIALLS AND METHODES

Study area and population

The study was conducted from October 29, 2016 to June 16, 2017 in Arsi Negelle districts of West Arsi zone, Oromia regional state, the district capital Arsi Negelle is located 218 km away from Addis Ababa at 7°21°N latitude and 38°42°E longitude. Agricultural production system is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas. In rural areas, mainly local breeds are found, grazing on communal land. The area was selected on the basis of livestock production potential and the presence of different livestock species and breeds. The selected area represents typical crop-livestock production system of the Rift Valley area of Ethiopia. The minimum and maximum annual temperatures are 10 and 25°C, respectively. Generally, the climate of the area is divided into subhumid (32%), semi-arid (42%) and arid (26%) zones with an average annual rainfall ranging from 500 mm to 1150 mm. The target population for this study will be sheep and goat population in Arsi Negelle districts. The study animals include local breeds and including all age groups and both sexes that were selected randomly from the target population (ANAREB, 2016)

Study design and sample size determination

Cross sectional quantitative study design was used from October, 2016 to March, 2017 to determine the prevalence of ectoparasite and its major genus on the small ruminant in ad around Arsi Negelle town. The required sample size for this cross sectional study was estimated by considering 50% of population knowing about prevalence since there is no documentation on ectoparasite of small ruminant in the area before. Thus, the sample size was calculated according to Thursfield (2005) using 95% confidence interval and 0.05 absolute precision. This is calculated by using the following formula: $$n = \frac{1.96^2 x p_{exp}(1 - p_{exp})}{d^2}$$

Where

n = required sample size.

P_{exp}= Expected proportion of population (50%)

d^2= Desired absolute precision (0.05). As a result, 384 study populations were selected.

Total sample size = 422 subjects to increase the precision

Sampling method, data collection tools and procedures

A multi-stage sampling technique was employed for the selection of the sampling units. From the entire Primary sampling unit, i.e. from 3 Kebeles and 5 PA (01, 02, 03, Ali wayo, Mako oda, Sogido ejo, Kersa gara, Edo jigessa), four kebeles were selected by simple random sampling technique (01, 03, Ali wayo and Kersa gara). The numbers of flock to be included in each kebeles were determined by proportional allocation based on the total number of small ruminant found in the field during study period. From the entire tertiary sampling unit, single flocks, in the selected kebeles were selected using a systematic random sampling technique. From each selected flock, further individual sheep or goat was selected by simple random sampling technique and examined.

The sampled animals were clinically inspected for presence of ectoparasites. Ectoparasite encountered either on the skin surface or attached to the hair were sampled or collected in 70% alcohol. For mite diagnosis, from animal showing signs of scales, crusts, alopecia itching, a skin scraping was taken. Scrape the edge of the affected area until blood oozed. Multiple sites were scrapped to increase the likelihood of ectoparasite detection. A few drop of 10% KOH solution were added to the sample, a cover slip applied and cleansing of debris allowed proceeding for 15-30 minutes before microscopic examination (Smith and Sherman, 1994; Bowman, 1999; Wall and Shearer, 2001).

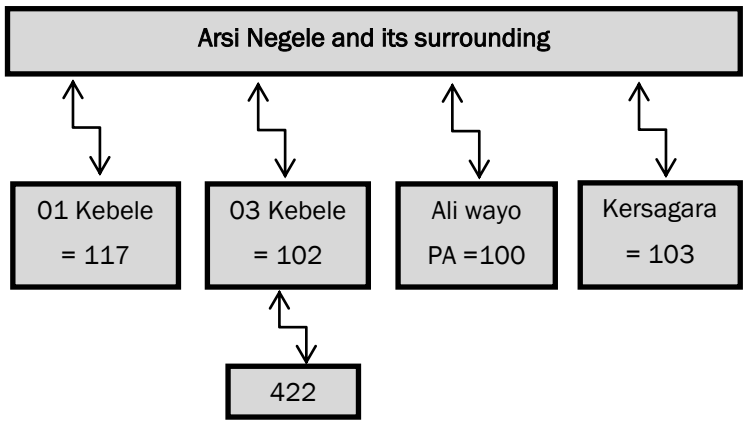

Figure 1 - Multistage sampling procedures, 2017

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Data Management and Analysis

The data collected from the field were cleaned and checked for its completeness. Those incomplete and inconsistent were corrected when possible and removed otherwise. After complete check-up the data were coded and entered to Microsoft Excel and transport to statistical package for social (SPSS) version 16.0 and analysis made. The frequency distribution of both dependent and independent variables were worked out by using descriptive statics techniques (Frequencies and prevalence). Pearson's Chi square used to evaluate Association between independent variables such as species, sex, age, body condition and dependent variables i.e. presence of ectoparasite and genus level of ectoparasite that affects the animals. Body condition was recorded by palpating femoral bone and tail. if femoral bone was prevailed and the muscle of tail become emaciated the animal was considered as poor and otherwise it was considered as good. The age of animal was estimated by asking the owner and if the owner responded greater than three months it was assumed as adult and if it was less it assumed as young.

RESULT

Results of clinical and laboratory examination of sheeps and goats based species exposed for ectoparasite

Out of 246 sheep and 176 goats examined for ectoparasite; 77 (31%) sheep and 49 (27%) goats were infested with one or more ectoparasite (Table 1). The major ectoparasite identified on sheep were *Bovicola, Ctenocephalides spp, Mixed infestation, Sarcoptes, Amblyomma, Demodex, Hyalomma and Rhipicephalus* were 32.5%, 26%, 15.6%, 10.4%, 6.5%, 5.2%, 2.6% and 1.2%, respectively. Also the ectoparasite identified on goats were *Linognathus species*, *Ctenocephalides* spp Rhipicephalus spp, Hyalomma, Amblyomma, Demodex, mixed, and sarcoptes infestations were, 30.6%, 18.4%, 18.4%, 10.2%, 8.2%, 4.1%, 4.1% and 2%, respectively.

The prevalence of ectoparasite in sheep and goat was 31% and 27% respectively. The difference was statistically not significant (X^2=0.586, P=0.444). The small ruminant of both species were highly affected by lice, ctenocephalides spp and tick from highly prevalent to lower prevalent respectively (Figure 2). An overall prevalence of ectoparasite was observed in the two small ruminant species at genera level of ectoparasite. In sheep *Bovicola* (32.3%) was the abundant followed by *ctenocephalides* (26%) and (15.6%). while in goats abundant genera ectoparasite were encountered with the prevalence of 30.6%, 18.4% and 18.4% on *Linognathus*, *ctenocephalides spp* and *Rhipicephalus,* respectively. The frequency of this study animals related with the ectoparasite genus indicate that lice spp were the most abundant which was followed by flea and tick in study area (Table 2).

Table 1 - Prevalence of ectoparasite on sheep and goats in and around Arsi negele, 2016/17. (n=422)

Species	No of examined	No of posative	Prevalence (%)	X^2 (P-value)
Ovine	246	77	31	
Caprine	176	49	27	0.586 (0.444)
Over all	422	126	29.9%	

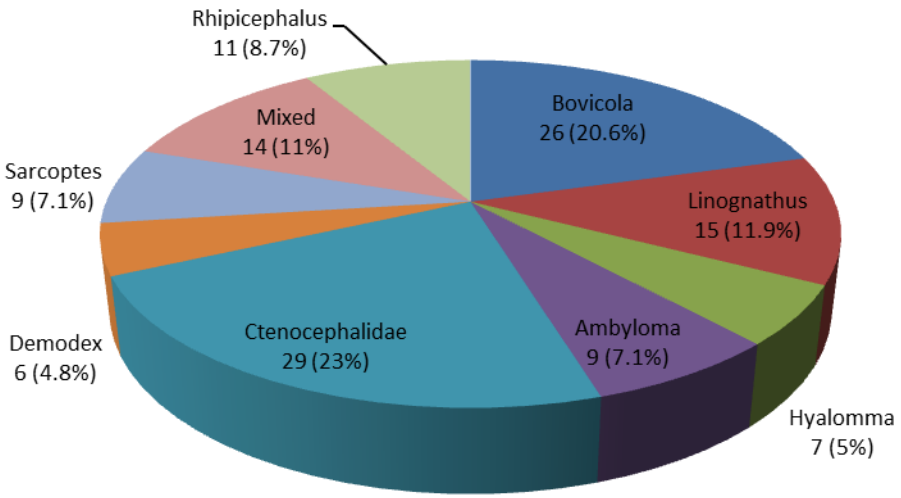

Figure 2 - The percentage of each genus of ectoparasite in small ruminant in and around Arsi nagele, 2016/17 (n= 422).

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Table 2 - The Frequencies and Prevalence of Ectoparasite on the study animals in and around Arsi Nagelle, 2016/17 (n=422)

Ectoparasite	Frequencies	Prevalence %
Lice	40	31.7
Flea	31	24.6
Tick	24	19
Mite	15	12
Mixed	16	12.7
Total	126	100

Results of clinical and laboratory examination of sheep's and goat's sex for ectoparasite

Statistically significant variation in the prevalence of ectoparasite was recorded between male and female of area ($P<0.05$). Hence, the prevalence of ectoparasite was significantly (X^2 =13.774, P=0.000) higher in the female animals than male animals due to one female mounted by money male during estrus, stress of female animal during pregnancy and poor grooming behavior of female animal during pregnancy and lactating period (Table 2).

Results of clinical and laboratory examination of sheep's and goat's age for ectoparasite

With regard to age wise comparison, among the 422 animals examined the high prevalence ectoparasite was recorded 36.8% in adult and 25.5% was recorded in young during study period. Furthermore, the association between the two age categories and ectoparasite were statistically significant (X^2=6.129, P= 0.013) (Table 3).

Results of clinical and laboratory examination of sheep's and goat's body condition for ectoparasite

The overall prevalence of ectoparasite in good and poor body condition were recorded as 17.7% and 46.4% respectively. As a result poor body conditions are more affected by ectoparasite due to weakness of immunity, uncertain grooming behavior of emaciated animal and loss of wool due to malnutrition. The association between the risk factor and ectoparasite is also highly significant (X^2=40.463, P=000).

Table 3 - Prevalence of ectoparasites with regard to sex of small ruminants in and around Arsi Nagelle,2016/17 (n= 422)

Sex	No of examined	No of posative	Prevalence (%)	X^2 (P-value)
Male	199	42	21	
Female	223	84	37.7	13.774 (0.0001)
Overall	422	126	58.7	

Table 4 - Prevalence of ectoparasite with regard to age in sheeps and goats in and around Arsi Nagelle, 2016/17 (n=422)

Age of animals	No of examined	No of posative	Prevalence (%)	X^2 (P-value)
Adult	163	60	36.8	
Young	259	66	25.5	6.129 (0.013)
Overall	422	126	62.3	

Table 5 - Prevalence of ectoparasite with regard to age in sheeps and goats in and around Arsi Nagelle, 2016/17 (n=422)

Age of animals	No of examined	No of posative	Prevalence (%)	X^2 (P-value)
Good	243	43	17.7	
Poor	179	83	46.4	40.463 (0.0001)
overall	422	126	64.1	

DISCUSSIONS

The moderate prevalence of ectoparasite recorded in sheep (31%) and goats (27%) is suggestive of the importance of these health problems in small ruminants of the study area. Poor management and poor level of awareness of sheep and goat owners on the effect of ectoparasite are believed to have contributed to widespread occurrence of the diseases. Lice infestations were the most prevalent ectoparasite recorded in small ruminant with a prevalence of 31.7% (Table 2).

The current study has shown that 31% of the sheep and 27% of the goats examined were found to be infested by at least single or mixed external parasites. The medium prevalence of ectoparasite in study area could be due to the fact

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that sheep and goats could have frequent exposure to the same communal grazing land that favored the frequent contact and management system of animals, this result is lower than the report of (Tefera, 2004) with a prevalence of 50.5% of sheep and 56.4% of goats. Similarly, (Mulugeta et al., 2010) reported 55.5% and 58% in sheep and goats, respectively in Tigray region and (Sertese et al., 2007) reported about 50.5% and 56.4% prevalence of external parasites, respectively in sheep and goats in different agro climatic zones of eastern Amhara region of Ethiopia.

The overall prevalence of lice obtained in this study area (32.3% in sheep and 30.6% in goats) was higher than observations made in southern range land 0% in sheep and 1.55% in goats (Mohammed, 2001), but lower than the prevalence reported by Tefera (2004) in Amhara region (39.8% in sheep and 29.2% in goats) and (Yisehak, 2000) at Sebata (89.5%) in fresh sheep skin examinations. The genera of lice identified on sheep and goats in the study areas was, *Damalina* which was the highest observed ectoparasite with the prevalence of 32.3% on sheep. *Linognatus* was the second genera of lice identified in the study area with the prevalence of 30.6 on goats.

Flea infestation with *Ctenocephalides spp*. was one of the ectoparasite problem encountered in small ruminants of the study area. It is generally true that ruminants including sheep and goats, horses and pig do not have their own species of fleas (Urquhart et al., 1996). However, most species of flea are not host specific and feed on any available animals, but in many case full fertility is achieved after feeding on specific host. *Ctenocephalides spp*. Occasionally infest sheep and goats and the clinical signs includes; papule, crusts, pruritus and excoriation (Wall and Shearer, 1997). In this study the prevalence of *Ctenocephalides spp*. was found to be 8.3% in sheep and 5% in goats. In goats, the prevalence of *Ctenocephalides spp*. in midlands (23.3%) was significantly higher than the lowland (0.8%) and the highlands (1.2%). This is probably associated with the high humidity, usually above 70% required for ovipositor of their eggs (Wall and Shearer, 1997).

In this study, three genera of ticks (*Rhipicephalus, Ambylomma* and *Hyalomma*) were identified which made a total prevalence of 9% and 34% in sheep and goats, respectively. This was in disagreement with (Teshome. 2002) with the prevalence of 23.8% in sheep and 16% in goats which were reported from the Sidama Zone in Southern Ethiopia. In addition, high prevalence rate of ticks in sheep and low prevalence in goat with current result was reported by Yacob et al. (2008) in and around Wolaita soddo, Southern Ethiopia with the prevalence of (68% in sheep and 19% in goats respectively) and (Abebe et al., 2011) with prevalence of 40% and 58.8% in sheep and goats in selected districts of Tigray region, Ethiopia.

In small ruminants, two mange mite genera were identified. These were *sarcoptes* and *demodex*. The first one is commonly detected in sheep and goats with a prevalence of 3.3% and 0.6%, respectively. This result is closely in agreement with Tefera (2004) with the prevalence of 5.49% and 6.3%. The second is also detected in both species with the prevalence of 1.6% and 1.1% respectively. This result is closely in agreement with (Tadesse et al., 2011) with the prevalence of 6.58% in sheep and 1.51% in goats. The Prevalence of mange mite obtained in this study area were higher than other researches done in different parts of country, 7.4 % by Assegid (2000) and 1.86% by Chalechaw (2001). This increament agreed with Pangui (1994) high temperature, humidity and sunlight favor mange mite infestation.

Several health problems, welfare issues and losses in productivity due to blood loss, pain, lameness, irritation, debilitation, mechanical damage, inflammation and hypersensitivity, secondary complications and transmission of pathogenic agents to small ruminant in the current study areas are possibly associated with the ectoparasite identified, as has been described by Kok and Fourie (1995), Jongejan and Uilenberg (2004) and Mekonnen et al. (2007). For instance, Walker et al., (2003) have described R. (B.) *decoloratus* as a vector of *Borrelia theileri* in ruminants and horses. In addition, Kumsa et al. (2012) recently reported molecular detection of zoonotic bacteria pathogenic to humans from *B. ovis* of sheep and other lice of ruminants in Ethiopia. The other ectoparasite such as ticks is well-known vectors of piroplasmosis and rickettsial diseases of ruminants, zoonotic rickettsial and viral diseases (Kumsa et al. 2012a; Mekonnen et al., 2007; Pegram et al., 1981; Walker et al., 2003). In view of these facts, ectoparasite should play a role in the transmission of pathogenic organisms to small ruminant of the study areas.

CONCLUSION AND RECOMMENATION

This study was conducted to identify the major ectoparasite and their prevalence on the small ruminants in study area. The most important ectoparasite identified were, lice, flea, ticks and mite. Lice were the most abundant ectoparasite in the study area followed by flea, tick, and mite. The infestations of ectoparasite are important affecting the health and productivity of small ruminants in and around Arsi -Negelle. Lack of awareness about the significance of the problems among owners for control schemes have contributed to the wide spread nature of ectoparasite in the area. Species was not found as a risk factor of all ectoparasite infestation in the current study. However, sex, age and body condition were important factors for different ectoparasite infestation in the current study.

In view of the findings of the present study it is possible to conclude that due to absence of control campaign several species of lice, flea, tick and mite represent common health and productivity problems of small ruminant in and around areas of Arsi-Negelle district.

Therefore based on the above points and others the following recommendations are forwarded.

✓ Strategic treatment of small ruminants with insecticides should be practiced in the study area to minimize the impact of ectoparasite on the health of animals.

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✓ Awareness creation for the local farmers about the control of ectoparasite is essential.

✓ Control programs should be designed and implemented with the participation of all stakeholders (farmers, tanners, government and other concerned bodies) and there should be strong coordination between neighboring regions and/or districts with strict follow up and control.

✓ Even though control campaign was implemented the prevalence of ectoparasite is still high hence, the regional government should be find out the causes and set solution.

✓ Further detailed study on economic loss associated with ectoparasite should be conducted.

DECLARATIONS

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Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AM conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. MY, MB and MY conceived the study, coordinated the overall activity, and reviewed the manuscript. 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

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SUPPLEMENTARY DATA

ANNEXES

Annex 1. Laboratory procedures

Mange mite identification

Examination of skin scrapings is essential in the diagnosis of mange. In long standing cases mites are often very few in number and extremely difficult to find and their absence from the skin scraping doesn't negate a diagnosis (Jackson, 1991). Multiple sites should be scrapped to increase the likelihood of ectoparasite detection. Superficial skin scraping (epidermal surface examination) after removing coat hair by gentle clipping can be used to identify surface mites while deep skin scraping (deep epidermal examination) until capillary ooze occurs is useful in the diagnosis of burrowing and follicular mites such as Sarcoptes scabiei and Demodex spp. (Hendrix, 1998; Wall and Shearer, 2001). A few drop of 10% KOH solution or liquid paraffin are added to the sample, a cover slip applied and cleansing of debris allowed proceeding for 15-30 minutes before microscopic examination. Large samples may be processed by boiling 10 minute in 10% KOH solution, centrifuging and performing sugar flotation on the sediment (Smith and Sherman, 1994; Bowman, 1999).

Tick identification

According to (Wall and Sheare 2001) the procedure for identifying ticks is as follows; the preserved specimens of tick collected from the field are poured in to petridish and separated from material such as hair, scale or dry skin. Ticks which are dirty should cleaned their scutum by gently rubbing with cotton. Each tick are placed on clean petridish on the stage of a stereoscopic dissecting microscope and identified. Specimens should not be allowed to dry completely. Low power objective is use to separate genera and sexes of ticks. To identify at the species level medium or high power objective is used. The key morphological structures such as gnathosoma, capitulum, basis capituli, eyes, anal grooves, coxae, festoons ornamentation of the scutum, venteral shield, palpas, chelicaera etc used for identification of ticks.

Lice identification

According to Wall and Sheare (2001) under light microscope structure such as claws per leg, segment of the antennae, eyes, mouth part, stylets of the ventral pouch, thoracic segment, spiracles, head and etc are used to identify the species and genera of the lice.

Fleas Identification

Flea is bilaterally flattened wingless insects with three body parts i.e. head, thorax and abdomen. The thorax has 6 legs arranged in 3 bilateral pairs, and the hind limb are enlarged and specially adapted for jumping. Adult fleas vary in size according to gender, female fleas are larger measuring up to 2.5 mm in length, while male are smaller <1mm. the head often bears genal ctenidium (spines).the two species of small ruminant i.e. c.canis and c.felis are differentiated with this structural part

Annex 2 - Format for field sample collection.

s/n	Species		Sex		Age		Body condition		parasite	Lab identification	Kebeles	date
1	O	C	M	F	A	Y	P	G	L,F,T,M			
2												
3												

Where, Species: O- ovine and C- caprine, Sex: M- male and F- female; Age: A- adult and Y-young; Body condition: P- poor and G- good; Parasite: L-lice,F-flea, T- tick and M- mite

Annexe 3 - body condition scores

Body condition score 1	Whole body-emaciated, bony process can be easily felt Spine-dorsal spinous process are sharp and prominent, easily felt through skin Lones-no fat cover, lone muscle very shallow transverse process- transverse process sharp and easy to pass fingers underneath them
Body condition score 2	Whole body- thin, more difficult to feel between each process Spine-dorsal spines process still prominent but not sharp Loin-loin eye muscle fuller, virtually no fat cover transverse process-transverse process rounded on edge, slight pressure needed to push underneath them
Body condition score 3	Whole body-average Spine-smoother and less process, some pressure required to feel between them Loin- loin muscle full and some of fat cover Transverse process-smooth, firm pressure needed to push fingers between under edge
Body condition score 4	Whole body-fat, fat accumulation over tail head Spine-considerable pressure needed to feel dorsal spinous processes Loin- loin eye muscle with discernible fat cover Transverse process- can't be felt
Body condition score 5	Whole body-obese, fat pad over tail head Spine-dorsal spinous process can't felt, depression often present where they would normally be felt Loin- loin eye muscle very full and thick covering of fat Transverse process-can't be felt

Source: Gatenby, (1991)

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POLYPHENOL OXIDASE CONTAINING EXTRACT: SOURCES AND CONTRIBUTIONS IN ANIMAL NUTRITION

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Supporting Information

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ABSTRACT: Polyphenol oxidase (PPOs) which are found naturally from plants, fruits, and vegetables and agricultural by products are getting considerable attention in food processing industries and in animal nutrition due to their antioxidant activity. Even though synthetic antioxidants have been used widely; they become a major cause for nutritional losses and quality deterioration which subsequently results in health problem in humans. To substitute synthetic antioxidants with natural Polyphenol oxidase (PPOs) containing extracts, many researches are being conducted on characterization of PPO sources and on protection of polyunsaturated oils and protein against ruminal degradation using PPO. Among many sources of PPO, Apple *(malus sylevestris var domestica)*, Banana *(Musa cavendishii*) Mango (*mangifera indicacv.manila*) and Red clover *(Trifolium pretense L.),* Tomato stems *(Solanum lycopersicum L.)*, Potato (*Solanum tuberosum L.*) are mentioned by different researchers from fruit sources and other plant sources, respectively. The contribution of PPO for protein and lipid protection is also being investigated. The main aim of this paper is to document the existing information on different sources and contribution of PPO as a baseline for further investigation. Further laboratory investigation on the role of polyphenol oxidase is crucial to improve the productivity of livestock with minimum feed cost.

Keywords: Polyphenol oxidase, Rumen, Protein degradation, Lipid degradation

INTRODUCTION

Polyphenol oxidase (PPOs) are a group of metalloenzymes which includes: catecholase (EC 1.10.3.2), laccase (EC 1.10.3.1), and cresolase (EC 1.14.18.1) that catalyses' the oxidation of monophenols and o-diphenols to highly reactive o-quinones , which intern interact with proteins and oxygen to form reactive oxygen species (ROS) and typically brown-pigmented complexes (Boeckx et al., 2015; Getachew et al., 2009). It is known for the characteristic post-harvest browning of cut or bruised fruit or damaged plat tissue due to polymerization of PPO derived o-quinones with nucleophilic compounds (Araji et al., 2014). According to the research conducted on the factors affecting post cut browning of eggplant *(Solanum melongena)* in India the extent of browning depends primarily on specific activity of PPO and total soluble phenolics (Mishra et al, 2013). Interest on PPO containing crops and agricultural side stream wastes is growing very rapidly due to the association of browning reaction with reduced protein and oil loss in the silo and rumen (Lee, 2014a) and their role for production of safe human diet, due to their antioxidant properties (Balasundram et al., 2006). There are many sources of PPO and are reported to be available in many organisms including, plants, fungi, animals and bacteria (Bottino et al., 2009).

Potato tuber peels *(Solanum tuberosum L.)* and other related Industrial sidestreams sources of plants such as Apple *(malus sylevestris var domestica),* Pineapple Peels *(Ananas comosus L.) and* Tomato stems and leaves *(Solanum lycopersicum L.)* are among important unutilized sources of PPO (Gadeyne et al., 2016; Zhang, et al., 2009). According to (Hajaji et al., 2011), from the tree Carob, Barks contains the highest amount of polyphenol compounds. It was also indicated that the amount of PPOs were significantly affected by the verities of trees. PPO helps to reduce the extent of lipolysis and proteolysis within red clover feed to ruminates with increase in efficiency of nitrogen utilization and the level of beneficial polyunsaturated fatty acids in meat and milk (Michael and Tweed, 2008; Merry et al., 2006). It was also found that PPO is very important to reduces lipolysis in the silo and in the rumen, when the forage is damaged, it will result in activation of PPO which result in binding of phenols to protein (Lee et al., 2009). This review aims to document different sources of PPO and their contribution in animal nutrition as a baseline for further investigation.

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SOURCES AND CONTRIBUTIONS OF POLYPHENOL OXIDASE (PPOS) IN ANIMAL NUTRITION

Sources of Polyphenol oxidase (PPOs)

Differed researchers identified many sources of Phenolic compounds. Among major sources of phenolic compounds in human diets, vegetables, fruits and beverages are found. It is also indicated that food and agricultural products processing industries produces considerable amount of phenolics-rich by- products, which could be valuable natural sources of antioxidants (Balasundram et al., 2006). Despite there is a variation in PPO activity, isoforms and concentration, there are several plant sources of protein which are used for emulsification purpose but, the origin and concentration of PPO extract highly affects the degree of ruminal protection (Gadeyne et al., 2016).

According to Lee, (2014) among the screened forage grasses and legumes which are used for ruminant livestock feed only two, red clover (*Trifolium pratense*) and cocksfoot (*Dactylis glomerata*), have been found to have both a high PPO activity and substrate concentration. Camelina extracts especially camelina cake has also an important antioxidant properties which could be used as an easily accessible and cheap source of natural polyphenols, which could be eye-catching to the food and pharmaceutical industry (Terpinc et al., 2012). Some of sources of PPO which are characterized by different researchers are presented in (Table 1). Methods of characterization and the result found from each sources of PPO could be found from the corresponding references.

Table 1 - Sources of Polyphenol oxidase (PPO)

Sources of PPO	References
Apple *(malus sylevestris var domestica)*	(Holderbaum et al.,2010; Gadeyne et al., 2016; Fievez et al., 2016)
Anamur banana *(Musa cavendishii)*	(Unal, 2007)
Eggplant *(Solanum melongena)*	(Mishra et al., 2013; Gadeyne et al., 2016; Fievez et al., 2016)
Persimmon *(Diospyros kaki L)*	(Navarro et al., 2014)
Pineapple Peels *(Ananas comosus L.)*	(Gadeyne et al., 2016; Fievez et al., 2016)
Mango *(mangifera indicacv.manila)*	(Palma-Orozco et al., 2014)
Red clover *(Trifolium pretense L.)*	(Gadeyne et al., 2015; Gadeyne et al., 2016; Lee, et al., 2009;Yoruk et al., 2016; Webb et al, 2013; Fievez et al., 2016)
Tomato stems and leaves *(Solanum lycopersicum L.)*	(Thipyapong et al., 2004; Gadeyne et al., 2016)
Potato tuber peels *(Solanum tuberosum L.)*	(Ali et al., 2016; Ngadze, et al., 2012; Fievez et al., 2016)
Camelina sativa	(Terpinc et al, 2012)
Names in brackets are scientific names	

CONTRIBUTION OF POLYPHENOL OXIDASE (PPOS) IN ANIMAL NUTRITION

Role polyphenol oxidase (PPOs) for ruminal protection of polyunsaturated oils

Rumen microbes are important to utilize structural carbohydrates and non-protein nitrogen in ruminant nutrition. But they also lead to bio hydrogenation of oils such as polyunsaturated fatty acid (PUFA) and proteins which subsequently results in loss of nutrients for absorption which intern affects the health of animals leads to poor quality animal product (Fievez et al., 2016). Administration of PUFA oils in the diet of animal is stipulated to increase reproductive performance of animal and to increase healthier PUFA rich meat and milk (Gulliver et al., 2012).

Due to the aforementioned consequences polyunsaturated fatty acid (PUFA) degradation in the rumen several technologies have been created protect biohydrogenation of PUFA in the rumen, (Ashes et al, 1984; Sacakli & Tuncer, 2006; Gadeyne et al., 2015; Fievez et al., 2016). According to Lee et al., (2009) positive nutritional effect of red clover was observed with wilted and ensiled clover due to activation of the enzyme PPO during wilting followed by ensiling. In ruminant ration, polyunsaturated fatty acid (PUFA) which are important component of the diet are highly subjected to degradation by rumen microorganisms, which results in the loss of healthy value of PUFA (Gadeyne et al., 2015). Recently, a natural technology has been developed at Ghent University, Bioscience engineering laboratory to create rumen by-pass supplements. This technology relies on a process which is naturally occurring at ambient temperature and makes use of natural resources (Gadeyne et al., 2015). Formation of protein phenol matric is possible through an enzyme PPO extracted from a specific plants and addition of diphenol sources, these emulsion will protect the degradation of lipophilic nutrients by microbes in the rumen which leads to the transfer of non-degraded nutrients to the small intestine (Fievez et al., 2016). According to Gadeyne et al., (2015) ruminal BH is reduced when oils rich in PUFA are first emulsified in PPO extract of red clover with high PPO activity and presence of a diphenolic substrate is important to induce protection. The author suggests that the reason might be due to formation of denser cross linked protein layer in the interference and subsequent improvement in protected emulsions.

Role of polyphenol oxidase (PPOs) for ruminal protein protection

One of the major challenges in ruminant nutrition is improving the efficiency of nitrogen utilization (Dove and Milne, 1994 ; Hart et al., 2016). From the total ingested protein, up to 70 % of nitrogen is excreted which results in loss of

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productivity potential and sever land and water pollution (Hart et al., 2016). It is known that supplying extra quickly rumen-degradable protein (RDP) will not support rumen microbial metabolism and will result in inefficient use of crude protein (CP) by the rumen microflora and, subsequently, the animal rather it will result in formation of urea which in turn promotes the losses of N as urine (Lee, 2014a). For optimal performance in high producing animals there should be enough supply of rumen degradable protein to support microbial growth and bypass protein for normal photolytic digestion and absorption process in small intestine (Dove and Milne, 1994). Therefore, Increasing the rumen undegradable protein content of animal feed is very important to minimize the loss of nitrogen and consequently to improve nitrogen use efficiency and to avoid environmental pollution (Hart et al., 2016).

By-pass protein can be created through technological interventions like treatment with heat, Formaldehyde, acetic acid, tannic acid, lignosulfonate or xylose. The two most commonly used technologies are based on formaldehyde and xylose-heat treatment (Maillard reaction). However, the use of chemicals like formaldehyde is considered unhealthy and there is limited access to purchase. Heat-based treatments show disadvantages as they are energy demanding. Therefore, using PPOs for protecting ruminal protein degradation as a means of increasing undegradable protein (UDP) in the rumen has tremendous role to increase N-use efficiency (NUE) (Lee, 2014b).

The way for PPOs protection of plant protein in the rumen is not due to protease deactivation, rather it is the result of complexing protein. The complexed proteins which are the result of PPOs in the rumen minimizes protein degradability in the rumen which in turn increases undegraded dietary protein flow to the small intestine. However PPOs protection of plant protein and glycerol based PUFA due to deactivation of plant protease and lipase, rather than complexting protein (Kroll and Rawel, 2001; Lee, 2014).

If N intake is balanced in animal diet, PPOs protection of dietary protein could result in increase in N-use efficiency (NUE), but high amount of unbalanced dietary N with high amount of fermentable energy (FME) which could be supplied to maximize microbial protein production in the rumen will result in a loss of N and lower NUE (Lee, 2014b). It is also indicated that strategies of exploiting PPO mediated protein complexing could be very important to prevent the loss of dietary protein and to improve animal health through delivery of essential nutrients (Hart et al., 2016). According to the study conducted on red clover polyphenol oxidase: Activation, activity and efficacy under grazing, when the red clover tissues are damaged to a higher degree, rapid activation of latent PPO occurs with subsequent binding of phenols to protein. It is also indicated that wilting of red clover may assist the positive effect of red clover on plycerol based lipids and protiens in fresh forage and it would enhance the activation of *ortho*-quinone production and formation of protein–phenol complexes (Lee et al., 2009).

CONCLUSION AND RECOMMENDATION

From the document it could be concluded that proper utilization of Polyphenol Oxidase (PPOs) in the area of food processing industries and animal nutrition is vital for safe food production. It could be also drawn that PPO are vital enzymes to improve the productive performance of animals through their role in creation of rumen bypass supplements. To utilize PPO effectively and efficiently, further study on endogenous phenolic substrates available in various sources of the enzyme PPO and amount of diphenol to be added for each sources of enzyme is recommended.

DECLARATIONS

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All authors contributed equally to this work.

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Conflict of interest
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STUDY ON DYSTOCIA WITH HISTORY OF UTERINE INFECTIONS AND CALVING HYGIENE ALONG WITH SUBCLINICAL ENDOMETRITIS IN DAIRY COWS IN AND AROUND GONDAR, NW ETHIOPIA

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Supporting Information

ORIGINAL ARTICLE

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ABSTRACT: The aim of this study was to assess dystocia with history of uterine infections and calving hygiene on the characteristics of the intrauterine environment in dairy cows by cytological tests were performed on intrauterine perfusion fluid along with subclinical endometritis conducted between January 2016 to September 2017. The percentage of polymorphonuclear cells (neutrophil) was calculated. It was found that increase in the number of neutrophils correlated with increase subclinical endometritis. During the study period n=147 apparently healthy 3rd trimester pregnant cows were selected in dairy farms in and around Gondar, North Western Ethiopia. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Abnormal parturition, uterine infections and calving hygiene were statistically significant (P<0.05) for subclinical endometritis. In conclusion, subclinical endometritis in postpartum dairy cows resulting in substantial economic losses due to decreases in both milk production and fertility.

Keywords: Calving hygiene, Cow, Dystocia, Gondar, Subclinical endometritis, Uterine infection

INTRODUCTION

Abnormal parturition technically called dystocia is the main cause for uterine infection. The prevalence of subclinical endometritis depends on the occurrence of early post partum uterine diseases (Sheldon et al., 2006). Difficult calving predisposes to subclinical endometritis for several reasons. Firstly, there is a higher than normal incidence of retained fetal membranes in animals that suffered dystocia. Secondly, there is often damage to maternal tissues causing devitalisation. The vulval seal may be damaged. Thirdly, the obstetrical interventions to correct the dystocia increase the load of pathogens within the uterus (Noakes, 2001).

The two symptoms of dystocia are extended calving periods (over 8 hours) and evidence that the fetus is not oriented properly for a normal birth. Once there is evidence of dystocia, obstetrical assistance is a must to relieve dystocia. If there is reason to suspect a problem, the individual examining the cow should observe strict sanitation practices. These include tying up the tail, thoroughly cleaning the cow's vulva and anal area and the examiner's hands and arms with clean warm water, soap and an antiseptic. A sterile plastic sleeve also should be worn to avoid contamination of the reproductive tract (Noakes et al., 2009). After parturition, bacteria from the animal's environment contaminate the uterine lumen of most cattle. Infection persists in the uterus of many animals for more than three weeks. These animals have lower conception rates, take longer to conceive and are more likely to be culled for infertility than unaffected animals. So, sub clinical endometritis is an expensive condition for vets and farmers to manage (Sheldon et al., 2004).

Many management factors affect the incidence of sub clinical endometritis. Season of the year may also affect the incidence; cows calving during the winter or spring are make prone to sub clinical endometritis than those calving at other times (Noakes, 2001).

A dirty unhygienic calving environment predisposes to the disease. This is probably the explanation for the effect of season of year. Since cows calving in the winter or indoors in the spring are likely to be in a make heavily contaminated environment (Noakes et al., 2009).

A high prevalence of uterine disease such as subclinical endometritis impairs the reproductive performance of high yielding dairy cows due to persistent bacterial infection which leads to inflammation and damage to the endometrium thereby, prolonging uterine involution and impairing fertility (Gilbert et al., 2002; Kasimanickam et al., 2004). Therefore, the present study was designed to assess the effect of dystocia, history of uterine infections and calving hygiene along with subclinical endometritis in dairy cows.

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MATERIALS AND METHODS

Study area

The study was conducted in urban and peri urban areas of Gondar town dairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital city Addis Ababa. It is located at latitude, longitude, altitude of 12.3-13.8°N, 35.3-35.7°E and 2200 above mean sea level respectively. The annual mean minimum and maximum temperature of the area vary between 12-17°C and 22-30 °C, respectively. The area is located under woyna dega, agro-climatic zone and receives a bimodal rainfall the average annual precipitation rate being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June through September (CSA, 2008).

Sample size

A sampling frame i.e. the list of the dairy farms was acquired from the urban agricultural development office at the beginning of the study. Dairy farms / cows were selected from this list using a stratified sampling procedure to ensure the selection of proportional and representative sampling of dairy farms and cows.

Questionnaire survey

A systematic question was designed and instituted to obtain relevant and reliable information about their animals. The questionnaire were checked for clarity of the questions prior the interview, respondents were briefed to the objective of the study. Following that, the actual questionnaires were presented.

Regular follow up: Regular follow up

About 147 pregnant cows were randomly selected in and around Gondar that were expected to give birth within the study period. These cows were subjected to different clinical and gynecological examinations including rectal palpation and findings were recorded once a week.

Data management and statistical methods

Data collected from the longitudinal follow up study were entered in Microsoft excel. For analysis of the data statistical package for social science (SPSS) (version 18) was used. In this chi- square test, confidence interval and logistic regression were calculated. The Generalized Linear Model was utilized to analyse the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analyse the relationship between the amount of neutrophils with parturition, history of uterine infections and calving hygiene. A probability of $P<0.05$ was set as the significance level. The Confidence Interval (CI) was set at 95%. The Receiver Operating Characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

RESULTS

Abnormal parturition, uterine infections and calving hygiene were statistically significant ($P<0.05$) for subclinical endometritis. The last parturition of 36.47% (n=31) of the selected cows was normal and that of 85.48% (n=53) was abnormal. The amount of neutrophils was higher in cows with abnormal parturition (Table 1). Abnormal parturition was associated with an increase in the amount of neutrophils in the uterus ($P=0.001$).

Of the selected cows, 64.44% (n=29) had a history of postpartum uterine infections and 37.25% (n=38) had no previous uterine infections. Metritis, pyometra, and endometritis categorized as uterine infections. The amount of neutrophils was higher in cows with a history of uterine infections than that of cows without previous uterine infections (Table 1). Calving hygiene area 62.69% (n=42) had poor environment and the amount of neutrophils was higher as compared to good calving hygiene.

DISCUSSION

In the present study subclinical endometritis was > or equal to 3 which is in line with the previous report of 4% in Ireland (Barlund et al., 2008). Various percentages of Polymorpho neutrophils (PMNs) in the endometrial cytology samples (>8%, >11%, >14%, >15%, and >18%) were evaluated to determine the most appropriate threshold for the diagnoses of subclinical endometritis in dairy cows between 28 and 41 days postpartum. A threshold of >15% PMNs were found to be the most appropriate. In a related study, Sheldon et al. (2004) showed that a threshold of >18% was the lowest PMN percentage which was significantly associated with an elevation of three endometrial pro-inflammatory cytokines, IL-6, IL-8 and TNF-α in cows sampled between 28 and 41 days postpartum. These threshold PMN percentages are greater than what has been reported previously where the most appropriate threshold was >8% PMNs for defining subclinical endometritis-positive disease status in cows sampled between 28 and 41 Barlund et al. (2008) and 25 Dourey et al. (2011) days postpartum using 150 and 270 day pregnancy status as the outcome, respectively. Kasimanickam *et al.*

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(2005) reported that a threshold of >18% PMNs was the most appropriate for cows examined between 20 and 33 DIM, and that >10% should be used for cows examined between 34 and 47 DIM, <15% did not benefit from intrauterine treatment. Other investigators have reported that cows which were diagnosed as endometritis-negative LeBlanc et al. (2002) or subclinical endometritis-negative Kasimanickam et al. (2005), Barlund et al. (2008) showed that cows with cytological evidence of clinical endometritis (PMNs >8%) became pregnant 24 days later than non-diseased (PMNs<8%) cows, which was much higher than the findings in the current study and the 88-day difference reported by Gilbert *et al.* (2005). Cows that experienced dystocia had a longer interval to first service which is likely due to a delayed resumption of cyclicity post calving. Cows that experienced dystocia also required more inseminations and therefore also had longer calving intervals (Barlund et al., 2008).

Table 1 - Analysis of results of cows' abnormal parturition, history of uterine infections, and calving hygiene examined 40-60 days postpartum for subclinical endometritis by uterine cytology.

Variables	No of examined	Positive for subclinical endometritis	Incidence %	Chi-square	*P*-value
Parturition					
Normal	85	31	36.47	51.97	0.000
Abnormal	62	53	85.48		
History of uterine infections					
Present	45	29	64.44	14.48	0.01
Absent	102	38	37.25		
Calving hygiene					
Poor	79	42	62.69		
Good	51	23	34.33	12.34	0.03
Very good	17	2	2,99		
Over all incidence	147	67	36.47		

CONCLUSION AND RECOMMENDATION

Subclinical endometritis has been implicated as the most common cause of failure of conception and impaired reproductive performance. The owner needs to prepare the calving area which should be clean, dry, quiet and isolated to keep the prepartum cow close for the owner to notice and provide help if the cow shows signs of difficulty during the birth. In general the operation of a dairy farm for maximum profit includes good feeding, breeding, care and management, as well as, good record keeping and a dairy health program.

DECLARATIONS

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Conflict of Interest
The author declares that there is no conflict of interest.

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A CROSS-SECTIONAL STUDY ON PREVALENCE OF *Cryptosporidiosis* AND ITS ASSOCIATED RISK FACTORS IN CALVES IN GONDAR AND ITS SUBURBS, NW ETHIOPIA

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Supporting Information

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ABSTRACT: Cryptosporidiosis is a common gastrointestinal disorder in humans and animals caused by various *Cryptosporidium* species. The present study was carried out to determine the prevalence of *cryptosporidium oocyst* and its potential risk factors in calves less than one year of age in and around Gondar town. For this purpose, 384 fecal samples (n=384 calves) from different dairy farms were collected and screened by using modified Ziehl-Neelsen staining technique. The overall prevalence of *Cryptosporidium* oocysts was 21.4 % (82/384). The association between different risk factors and prevalence of *Cryptosporidium* oocysts was assessed. There were significant associations (P<0.05) between prevalence of *Cryptosporidium* oocysts and age of calves, fecal consistencies, daily cleaning of the farm and water source. On the other hand, there was no significant association between prevalence of *Cryptosporidium* oocysts and sex, breed, and body condition of the calves and also provision of colostrums to the calves (P>0.05). In conclusion, this study demonstrated host factors and management factors greatly affect the prevalence of Cryptosporididosis in calves. Therefore, the current study reported the role of host factors (age and sex) and management factors (water source and daily cleaning of the farm) needed to be clearly recognized by all stakeholders in order to understand their effects on the disease occurrences as well as in control and prevention of in calves.

Keywords: Calves; Cryptosporidiosis; Dairy farm; Oocyst; Ziehl-Neelsen

INTRODUCTION

The productivity of cattle depends largely on their reproductive performance and the survival of calves (Yeshwas et al., 2014). Calve morbidity and mortality are perennial problems for dairy producers worldwide especially the tropics is not an ideal location for calf rearing as the high temperature and humidity introduce many potential disease problems to milk fed calves which impair appropriate heifer replacement (Gebremedhin, 2014). The neonatal calve mortality in the first month of age is more than 80% of the total mortality in calves. Major causes of mortality of neonatal calves are conditions like diarrhea and pneumonia (Khan et al., 2009).

Calf diarrhea (also known as calf scouring) is a commonly reported disease and a major cause of economic loss to cattle producers (Yong-il and Kyoung-Jin, 2014). Diarrhoea is the most important disease in young calves and accounts for approximately75% of the mortality of dairy calves within the first 3 weeks of age (Walter, 2012).

The pathogens most commonly incriminated in neonatal calf scours include viral (rotavirus and coronavirus), protozoal (*Cryptosporidium parvum, coccidia*) and bacterial pathogens (enterotoxigenic *Escherichia coli* K99 and *Salmonella* species). Calves are at greatest risk of developing diarrhea during the first month of life, and the risk then decreases with age (Izzo et al., 2011).

Cryptosporidiosis is an emerging protozoan disease, caused by *Cryptosporidium* species that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide. The infection encountered after ingestion of the microscopic infective oocysts (Dinka and Berhanu, 2015). These protozoan parasites mainly infect the intestinal tract and rarely the respiratory tract of animals and people (Zkan and Yunus, 2001). Clinical cryptosporidiosis is frequently not diagnosed, yet it has been incriminated as an important cause of diarrhea in neonates. Clinically, the disease is characterized by anorexia and diarrhea, often intermittent, which may result in poor growth rate. The severity of clinical disease may be associated with the animals' immune and nutritional status (Olson et al., 1997).

Cryptosporidium are responsible for most cattle infections (*Cryptosporidium parvum, Cryptosporidium bovis* and *Cryptosporidium andersoni* and with *Cryptosporidium* deer-like genotype). *Cryptosporidium parvum* is known to infect humans worldwide and is recognized as the major zoonotic *Cryptosporidium* species, whereas *C. andersoni* has been

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reported in humans only once. The most prevalent species were *C. parvum* in dairy calves. *C. bovis* oocysts are morphologically indistinguishable from *C. parvum* oocysts. *Cryptosporidium bovis* is a highly prevalent species that infects primarily post-weaned calves (Olson et al., 1997). *Cryptosporidium parvum* is the main species in young calves and is a cause of neonatal diarrhoea. *C. bovis, C. ryanae and C. andersoni* are more common in weaned calves and older cattle, with different prevalence and age distribution (Silverla and Blanco, 2008). The prevalence of *C. parvum* infection in animals is high. Dairy and beef calves are generally considered as presenting the highest risk because of their numbers, distribution, high infection occurrence, and high oocyst excretion levels besides be exposed to contamination environment sources, such as soil and water (Almeida et al., 2010).

Taking into account the high prevalence of *Cryptosporidium* infection in calves and the prevalence and its associated risk factors of *Cryptosporidium* infection in calves in the study area is not found. Therefore, the objectives of this experimental trial were to determine the prevalence of cryptosporidium on calves and to know its associated risk factors.

MATERIALS AND METHODS

Study Area Description

The study was conducted in Gondar town at University of Gondar, located 738 km away north-west of Addis Ababa, the capital city of Ethiopia. The total population of Gondar town is estimated to be 206,987 of which 98,085 are males and 108,902 females (CSA 2008). It is situated with a *latitude and longitude* of 2°36' N, and 37° 28'E, respectively. It has an altitude of about 2133 meter above sea level with an average temperature of 20°C and an average annual rainfall of 1800 mm. The livestock population in the area comprises of cattle (8,202), goat (22,590), sheep (2,695), horse (1,065) and donkey (9,001) (CSA, 2008).

Study Population

The sampling units for the study were dairy calves of up to 1 years of age. This was based on the previous reports that indicated higher occurrence of the disease in these age categories. The association of the disease occurrence was seen in relation with different age category with classification of < 3 months, 3-6 months, >6 months and consistency of feces weather it was diarrheic or not. Risk factors for the study animals were considered during data collection and analyze to assess the effect of these risk factors on the prevalence of cryptosporidium oocyt. Calves from dairy farms in and around Gondar town were constituted in the study population. There were few relatively large dairy farms and a lot of small holder dairy farms in the study area.

Study Design

A cross-sectional study supported with close ended questionnaire survey was carried out to determine the prevalence of Cryptosporidiosis in calves and its associated risk factors in calves. Modified Ziehl-Neelsen staining test on feces collected directly from rectum of calves less than 1 year of ages was performed as laboratory technique to detect the oocyst of the parasite.

Sample Size Determination

Simple Random sampling technique was used to select study farms and all calves that are found in the study farms were sampled. The sample size was determined based on the expected prevalence of 13.6% by Dinka Ayana in central Ethiopia and absolute desired the precision of 5% at confidence level of 95% according to formula provided by Thrusfield (1995). The formula is:

$$N = \frac{(1.96)^2 x\ p\ (1-p)}{D^2}$$

Where N=sample size; P=expected prevalence; D= desired absolute precision

Thus, based on the formula the total sample size were 180, to increase, the precision level these number was increased to 384.

Sample Collection and Processing

In this study, fecal samples were collected directly from the rectum of the study animals using disposable gloves, placed in universal bottle and transport to laboratory for processing on the same day. Farm owners and workers were interviewed with questionnaire which focuses on different aspects, associated with management risk factors including, provision of colostrums, daily cleaning of the farm, Age of the calves, Breeds of the calve, housing condition of calf, water source, and also during farm visit the flooring system of the farm was checked. All the fecal samples were tested for the presence of *Cryptosporidium* spp. oocysts in feces. Samples will detect using the modified Ziehl-Neelsen staining technique as described by Clarke and McIntyre (2001).

Data Analysis

The data obtained was entered and managed using Microsoft Excel 2007 excel spread sheet. Then, it were imported to SPSS version-20 and analyzed by chi-square test to determine the significance of the variation in prevalence rates

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between management factors, host factors and consistency of feces. Prevalence was calculated as the number of calves found positive for cryptosporidium parasite per animals examined. A 95% confidence interval and 5% significance level were used to determine whether there was significant difference in the measured parameters.

RESULT

Cryptosporidiosis is an emerging protozoan disease, caused by *Cryptosporidium* species that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide. According to this study, a prevalence of 21.4% (82/384) was recorded among the studied calves for the positivity of *Cryptosporidium* oocysts.

Prevalence of Cryptosporidium Oocyst with Respect to Calve Factors

According to table 2, the highest prevalence among calves aged less than three months with 47.8% (32/67) followed by calves of 3-6 months age group 24.1% (35/145). On the other hand, the lowest prevalence was recorded in older calves (>6 months) 8.7% (15/172). Thus, significant association (P<0.05) between different age groups and the prevalence of *Cryptosporidium oocyst* were recorded. In addition, a result obtained from study to determine association of the parasite infestation with respect to sexes of calves, showed higher rate of infection in females (22.3%) than males (20.5%). There was no significant association (P>0.05) between both sexes in shedding of *Cryptosporidium* oocyst. As reported in Table 2, no significant association between prevalence of *cryptosporidium* oocyst and body condition (P>0.05) was observed. The study result showed high prevalence of *cryptosporidium* oocyst in calves that had poor body condition (55.3%) followed by medium (20.5%) and the lowest prevalence was documented in calves that had good body condition (6.8%).

Furthermore, prevalence of *Cryptosporidium* in calves were breed considered as risk factor, the result in table 2 showed a relatively lower prevalence rate of (19.8%) in local breed calves than in cross breed calves (22%). Thus, prevalence rate among local and cross breed calves were not significant statistically (P>0.05). The prevalence of *Cryptosporidium* was studied based on fecal consistency and out of 384 calves sampled, almost all (98.4%) was non diarrheic while about 1.56 % of them were diarrheic. The prevalence rates of *Cryptosporidium* were 20.6% and 66.7% in non-diarrheic and diarrheic calves, respectively. Hence, consistency of feces has a direct link with prevalence of cryptosporidium (P<0.05).

Table 2 - Prevalence of *Cryptosporidium oocyst* with respect to calve factors

Calf factors	Categories	Total calves Screened (N)	*Cryptosporidium* Positive calves (%)	P-value
Age	< 3 months	67	32(47.8)	0
	3-6 month	145	35(24.1)	
	>6month	172	15(8.7)	
Breed	Local	114	22(19.3)	0.52
	Cross	270	60(22.2)	
Sex	Female	184	41(22.3)	0.67
	Male	200	41(20.5)	
Body condition	Poor	38	21(55.3)	0.062
	Medium	273	56(20.5)	
	Good	73	5(6.8)	
Consistency of Feces	Diarrheic	6	4(66.7)	0.006
	Non Diarrheic	278	78(20.6)	

Prevalence of Cryptosporidium Oocyst with Respect to Management Habits

As indicated in table 3, no significant association (P>0.05) was found between provision of colostrums to calves and the prevalence of *Cryptosporidium* oocyst. Although, the prevalence was high (26.8%) in calves that denied provision of colostrum than calves supplied a colostrums (19.9%). As per the exploration carried out to see association between prevalence of *cryptosporidium* oocyst and number of daily cleaning of the farm, it showed significant association (P<0.05). In farms experienced a single barn cleaning habit recorded highest prevalence (32.5%) compared to farms cleaning calve barns twice a day (25%) and three times per day (15.1%).

Moreover, water source showed its significance as a potential risk factor for the occurrence of the disease. Thus, higher prevalence (28.6%) of *cryptosporidium* oocyst was recorded in calves used to drink water from river than calves getting drink tap water (18.9%). Hence, source of water for calves to drink and the prevalence of cryptosporidiosis was found to be statistically significant (P<0.05).

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Table 3 - Association of *Cryptosporidium* oocyst with management habits

Management factors	Categories	Total calves N	*Cryptosporidium* positive calves (%)	p-value
Colostrums	Yes	302	60 (19.9)	0.173
	No	82	22 (26.8)	
Daily cleaning of the farm	≤ 1 times/day	77	25(32.5)	0.04
	2 times/day	108	27(25)	
	≥ 3 times/day	199	30(15.1)	
Water source	River	98	28 (28.6)	0.043
	Tap water	286	54(18.9)	

DISCUSSION

The overall prevalence of *Cryptosporidium* in calves was relatively higher (21.4%) than that reported from eastern Ethiopia 17.6% by Rhmato et al. (2007) and from central Ethiopia 13.6% by Ayana and Alemu (2015). On the other hand, the current finding is relatively lower than reports from central Ethiopia 27.8% by Regassa et al. (2013). However, there were close similar result that reported from other countries on the prevalence of Cryptosporidosis in calves with the present study like a prevalence of 21.65% in Iran (Radfar et al., 2006), 21.9% in Brazil (Melissa et al., 2015) and 20% in Malaysia (Nur Hazirah et al., 2016). Furthermore, there were also a variety of results from various studies concerning the prevalence of *Cryptosporidium* in calves worldwide, 86.7% in Tunisia (Soltane et al., 2007) and 83.3% in chain on calves less than one month of age (Zhaohui et al., 2014) and 3.9% in Turkey (Esin et al., 2013). The difference in the prevalence of the disease might be attributed to variation in the season of study time or it could be due to the age of examined animals. It might be also due to hygiene of the farm and sensitivity of the test.

In the current study animal age played a great role for the prevalence of *Cryptosporidium* oocyst. It has been observed that the high prevalence (47.8%) of *Cryptosporidium* oocyst in this study was observed in calves < 3 months followed by calves aged 3-6 months (24.1%) while calves aged greater than 6 months had the lowest prevalence of *Cryptosporidium* oocyst (8.7%). The findings are in agreement with findings obtained by Regassa et al. (2013), who found calves less than 3 months are at higher risk of infection compared to the older ones and studies which stated that there was significant association between the prevalence of *cryptosporidium* and age, by Ayinmode and Fagbemi (2011), Bawm et al. (2014) and Akinkuotu et al. (2014). On the other hand, it was in contrast to the findings of Rhmato Abebe et al. (2007), who stated that, there were no significant association between the prevalence of *cryptosporidium* and age. The contradiction between reports of the studies might be due to different grouping of ages of the calves since different authors used different categories of age. For instance, Rhmato Abebe et al. (2007) grouped calves age <6 monthes and > 6monthes. In addition, this study and Regassa et al. (2013) grouped the age of calves into <3 months, 3-6 months and >6 months. Moreover, it could be due to species of *cryptosporidium oocyst* that found in the study area. The higher prevalence in calves less than 3 months can be attributed to the fact that these age groups are highly susceptible to the disease because of the immature immune system of the animal at this age (Regassa et al., 2013).

The current study reported that there was no significant difference between the prevalence of *Cryptosporidium* oocyst in female and male calves, although the result showed high prevalence (22.3%) in female than (20.5%) male calves. This report was supported by Emanuel and Luuk, (2010), Regassa et al. (2013), and Akinkuotu et al. (2014). There was no significant difference in *Cryptosporidium* infection ratio between males and females when they are breed in the same place and expose to similar condition, because neither males nor females have factors facilitate or impede infection while the other gender lacks them (Al- zubaidi, 2012). In contrast the study disagrees with the report of Ayinmode and Fagbemi (2010) who reported that there is a significant difference existed between infection rates in females and males calves.

The present study also showed breed of the calf was not statistically significant with *oocyst* shedding of *Cryptosporidium,* however, the result indicated that higher prevalence (22.2%) in cross breed than (19.3%) local breed calves. This was in agreement with the report of Nasir et al. (2009). This could be due to cross breed calves had lower immunity than local breeds.

The result of the present study revealed that the consistency of feces had a significant effect on the prevalence of *cryptosporidium* oocyst. This report agreed with the report of Nasir et al. (2009), Sharma and Busang (2012), Esin Guven et al. (2013), Samir et al. (2014) and Danlmadi and Ugbomoiko (2015). On the other hand, the present study contradicted with the work of Melissa Carvalho et al. (2015) who reported that the absence of correlation between the occurrence of diarrhea and positivity for *Cryptosporidium* species can be related to other factors inherent to the animals such as the presence of other gastrointestinal parasites and/or concomitant infections. The difference between this reports might be due age of target population since older calves did not show sever clinical sign even if they were heavily infected.

According to the findings of current study, it was observed that the prevalence of *cryptosporidium* oocyst in calves were associated with daily cleaning of calves' house. The risk for being shedder of oocyt was significantly higher in calves

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that used to clean their calves barns < ones day followed by two times per day, while farms that cleaned their calves house ≥ three times per day showed the lowest prevalence. This observation was in agreement with Rhmato Abebe et al. (2007) and Emanuel and Luuk (2010) who reported that Calves were more likely to shed *Cryptosporidium* species positive oocysts if they were raised at dirty floor houses most likely due to the increased microenvironment for *Cryptosporidium* species. The findings of this study disagreed with the report of Nasir et al. (2009) and Almeida et al. (2010) who indicated that there was no significant association between the prevalence of *cryptosporidium* with hygiene of the house.

In the present study, a significant association was found between water sources and shedding of *cryptosporidium* oocyst. Thus, higher prevalence was observed on calves that drunk water from the river than tap water. This report was supported by Bawm et al. (2014) who stated that there was high significant association between water source and prevalence of *cryptosporidium* oocyst. The higher prevalence on calves that drunk water from the river could be due to higher contamination of river by feces of animas and survival of oocyst for long time in the water.

There was no significant association between prevalence of cryptosporidium oocyst and Colostrums in the study. But the present study showed higher prevalence in calves that had not chance to feed colostrums than that had chance to feed colostrums. This might be due to ingestion of protective factors in the colostrums that can reduce shedding of cryptosporidium oocyst.

CONCLUSION AND RECOMMENDATIONS

Cryptosporidiosis is a significant disease in livestock, affecting mostly neonates. From the present study, it can be concluded that *cryptosporidium* was prevalent in Gondar and its suburbs and its present prevalence was comparable to other parts of Ethiopia. The result showed some of the calves and management factors greatly affect its prevalence. Calves related possible risk factors significantly affect the prevalence *cryptosporidium* oocyst. Likewise, from management habits barn cleaning and water source supply strongly contribute to the high prevalence of *cryptosporidium* oocyst. Based on major findings and the above conclusion the following recommendations were forwarded

- Awareness creation for the farm owners about the potential risks of the Cryptosporidiosis on their farm and possible control programme of the disease should be undertaken.
- A further study prospective in nature, capturing seasonal variations to elucidate the magnitude of the disease, is desirable.
- Further study using molecular technique to identify the species of the parasite must be conducted in order to implement successful control and prevention programmes.

DECLARATIONS

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Andargachew Misganaw (AM) conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. Mastewal Birhan (MB) participated in drafting and reviewing the manuscript and conceived the study, coordinated the overall activity, and reviewed the manuscript. Muluken Yayeh (MY), Amebaye Kinubeh (AK) and Tilahun Gasses (TG) participated in drafting and reviewing the manuscript. Participated in the design of the study, and reviewed the manuscript. 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

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CYTOLOGICAL STUDY OF SUBCLINICAL ENDOMETRITIS WITH RESPECT TO AGE, PARITY, FARM SCALE AND BODY CONDITION SCORE IN DAIRY COWS

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Supporting Information

ABSTRACT: The objective of this study was to investigate the incidence of subclinical endometritis with respect of age, parity, farm scale and body condition score in dairy cows. A total n= 147 of apparently healthy 3rd trimester pregnant cows were selected with no signs of clinical endometritis were examined from January 2016 to September 2017. Questionnaire survey and regular follow up were conducted to determine subclinical endometritis in dairy cows. Age and parity were statistically significant (P<0.05) for subclinical endometritis. Older cows greater than 6 years were more affected sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) (χ^2=51.97; P<0.05), the difference was statistically significant. The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) (χ^2=14.48; P<0.05), the difference is statistically significant. However, body condition score and farm scale showed no significant variation with regard to subclinical endometritis.

Keywords: Age, Body condition score, Cytology, Gondar, Parity, Subclinical endometritis

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INTRODUCTION

A high level of reproductive efficiency requires each cow to be bred successfully, and calve with a calving interval that maximizes the output of milk within the herd. Under normal circumstances, however, microbial contamination of the uterus is a frequent finding in postpartum dairy cows (Groenendaal et al., 2004).

Interestingly, multiparous cows have increased bacterial contamination ~50 days after calving compared to primiparous cows (Galvão et al., 2009a). Milk production has a detrimental effect on leukocyte function; therefore, leukocytes from multiparous cows are expected to be more severely affected because of greater milk yields (Sheldon et al., 2009). In fact, phagocytic activity of neutrophils in older cows is more markedly reduced after calving compared to younger cows. Therefore, increased levels of pro-inflammatory cytokine production in the uterine endometrium might help to prevent subclinical endometritis; however, because ultiparous cows have greater demands for milk yield, they might be less able to clear an infection completely and, therefore, might be more likely to have subclinical endometritis. Another important factor that might be involved in the susceptibility to subclinical endometritis is the circulating levels of immunoglobulins. Immunoglobulins work as opsonins, which greatly enhance phagocytic capacity. Primiparous cows have lower immunoglobulin content in colostrums, which indicates lower circulating immunoglobulin levels; therefore, phagocytosis might not be optimal in early lactation in primiparous cows (Sheldon et al., 2009).

New techniques have been described for the diagnosis of subclinical endometritis. The inflammation of the endometrium is characterized by the proportion of polymorphonuclear (PMN) cells in a cytological sample taken from clinically healthy cows. Cytological samples obtained by flushing the uterine lumen (Gilbert et al., 2005; Mateus et al., 2002; Sheldon et al., 2006).

Cows with a higher condition score at calving were less prone to subclinical endometritis and conceived more successfully to first service. Cows calving in a higher body condition score produced more milk, fat and protein in the first 90 days of lactation. Body condition score represents a subjective assessment of the tissue reserves of dry and lactating cows (Sheldon et al., 2009). Therefore, the objective of the study was to identify the effect of age, parity, farm scale and body condition score in dairy cows in and around Gondar.

MATERIALS AND METHOD

Study area

The study was conducted in urban and peri urban areas of Gondar town dairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital city Addis Ababa. It is located at latitude, longitude, altitude of 12.3-13.8°N, 35.3-35.7°E and 2200 meters above sea level, respectively. The

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annual mean minimum and maximum temperature of the area vary between 12-17°C and 22-30 °C, respectively. The area is located under woyna dega, agro-climatic zone and receives a bimodal rainfall the average annual precipitation rate being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June through September (CSA, 2008).

Sample size

A sampling frame i.e. the list of the dairy farms was acquired from the urban agricultural development office at the beginning of the study. Dairy farms / cows were selected from this list using a stratified sampling procedure to ensure the selection of proportional and representative sampling of dairy farms and cows.

Questionnaire survey

A systematic question was designed and instituted to obtain relevant and reliable information about their animals. The questionnaire were checked for clarity of the questions prior the interview, respondents were briefed to the objective of the study. Following that, the actual questionnaires were presented.

Regular follow up

About 147 pregnant cows were randomly selected in and around Gondar that were expected to give birth within the study period. These cows were subjected to different clinical and gynecological examinations including rectal palpation and findings were recorded once a week.

Body condition scoring

The Body Condition Scoring (BCS) was determined according to Richard (Barlund et al., 2008; Richard, 1993). For all cows under the study their body condition were grouped from 0-5. Body condition score 0 stands for cows with the poorest body condition while score 5 for cows with the best condition.

Data management and statistical methods

Data collected from the longitudinal follow up study were entered in Microsoft excel. For analysis of the data statistical package for social science (SPSS) (version 18) was used. In this chi- square test, confidence interval and logistic regression were calculated. The Generalized Linear Model was utilized to analyse the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analyse the relationship between the amount of neutrophils with age, parity and body condition score. A probability of $P < 0.05$ was set as the significance level. The Confidence Interval (CI) was set at 95%. The Receiver Operating Characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

RESULTS

In the present study endometrial cytology revealed that the polymorpho neutrophils count of 3% and above was suggestive of subclinical endometritis. The samples which ranged from 3% to 15% of PMN cells could be correlated with subclinical cases of endometritis. Hence, the endometrial samples which contain PMN cells of 3% and above were considered as positive for subclinical endometritis.

Age and parity were statistically significant ($P<0.05$) for subclinical endometritis. Older cows greater than 6 years were more affected by sub clinical endometritis 38 (71.70%) than younger cows 13 (29.55%) (χ^2=51.97; $P<0.05$), the difference was statistically significant (Table 1). The incidence of sub clinical endometritis in cows primiparous was 21 (40.38%) and multiparous was 46 (48.42%) (χ^2=14.48; $P<0.05$), the difference is statistically significant. Body condition score was also statistically significant (Table 1). However, farm scale showed no significant variation with regard to subclinical endometritis (Table 1).

DISCUSSION

In the present study, multiparous cows (48.42%), emaciated cows (40.40%), and cows above 6 years of age (71.70%) were the most affected by subclinical endometritis which is higher than the previous finding (19.23%) by Takele et al. (2005). This could be due to the repeated exposure of the genital tract of multiparous cows to environmental pathogenic microorganisms causing gradual decrease in the efficiency of immune mechanism.

The current study revealed an overall incidence of 46% (67/147) of subclinical endometritis infection in dairy cows with a Polymorph nuclear cells (PMN) level of ≥3 neutrophils in the uterine sample set as a threshold value indicative for Subclinical endometritis (SCE). This incidence of Subclinical endometritis (SCE) was similar with Belachew and Fekadu (2009) in Debre zeit which was 47.5% and 30.5%, respectively. However, in other studies samples were taken at 4 and 8 weeks postpartum period separately, whereas samples in this study were taken 4 to 8 weeks postpartum together. An explanation for the higher prevalence of SCE in this study could be the difference in the time arrangement. Gilbert (2005) in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Dubuc et al. (2010) in

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Québec which was the prevalence of subclinical endometritis of 56%. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk (DIM) 45% for cows between 34 and 47 DIM (Gilbert, 2006). The variation of subclinical endometritis in the current study as compared to the other cited studies could be due to the difference in the management system of dairy cows.

The incidence of SCE in this study was 46% which was higher than the incidence of 13.4% as reported by Singh et al. (2008) in Germany. However, samples in this study were taken between 4 to 8 weeks postpartum period, whereas samples in the cited study were taken four hours after breeding. The higher incidence of SCE in this study as compared to the above cited study could be the difference in the time of sampling.

The incidence of subclinical endometritis in cows having body condition score 1 and 2 was 40.40% which is lower than cows having body condition score 3 and 4 (56.25%) significant (P=0.03) difference in the body condition score was also recorded. The prevalence of subclinical endometritis in cows with body condition score (BCS)-2 was 73% which was not statistically significant (P=0.554) and was higher than cows with BCS-3 of 62.6% which is in agreement with Belachew and Fekadu (2009).

Table 1 - Analysis of results of cow's age, parity, farm scale, body condition score and pregnancy status examined 40-60 days postpartum for subclinical endometritis by uterine cytology.

Variables	No of examined	Positive for subclinical endometritis	Incidence %	Chi-square	p-value
Age					
2-3	44	13	29.55		
4-6	50	16	32.00	51.97	0.000
>6	53	38	71.70		
Parity					
Primiparous	52	21	40.38	14.48	0.01
Multiparous	95	46	48.42		
Farm scale					
Small	54	25	46.30		
Medium	66	29	43.94	0.30	0.85
Large	27	13	48.15		
Body condition score					
1&2	99	40	40.40	10.24	0.03
3&4	48	27	56.25		
Pregnancy status					
Pregnant	72	7	9.72	70.63	0.000
non pregnant	75	60	80.00		

CONCLUSION and RECOMMENDATION

A herd health program is critical in maintaining uterine health and identifying potential problem areas. Routine postpartum examinations will help to identify problems early so that effective therapy can be administered in problem situations. The study showed that direct association of subclinical endometritis with age and parity. Therefore, proper herd health management and proper feeding is very important and also detailed studies should be conducted to identify their etiology, distribution and prevalence.

DECLARATIONS

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Conflict of interest
The author declares that there is no conflict of interest.

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URBAN AND PERI-URBAN DAIRY CATTLE PRODUCTION IN ETHIOPIA: A REVIEW

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Supporting Information

REVIEW ARTICLE

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ABSTRACT: Dairy production is among the developing agricultural sector in the urban and peri-urban areas of Ethiopia. This paper reviews the status of the current urban and peri-urban dairy cattle production systems and indicates possible recommendations. Hence management practices which includes feeds and feeding, breeding, housing and cattle holding are assessed based on different findings. Besides milk production potential, change drivers of the sector and major challenges are reviewed. The major feeds available for cattle are hay, crop residue and agro-industrial byproducts with stall feeding being the dominant system. In all reviewed findings both natural mating and AI is used though the preference is mostly affected by accessibility. Cities with highest population and better market and input access hold higher number of cattle per house hold than with lower population and market access. The average daily milk yield is higher in and around Addis Ababa followed by other big regional cities. High rate of urbanization, population growth, change in life style together with better access to inputs are behind the expansion of the sector. But shortage and cost of feed, shortage of land and waste disposal are the major challenges. Therefore, there is a need to organize the current status of the sector and look for solutions to cope up with the growing need of milk and milk products around the cities.

Keywords: Cattle Feeding, Dairy Breeding, Milk Production, Peri-Urban Dairy, Urban Dairy

INTRODUCTION

With a cattle population of 59.5 million heads (CSA, 2016/17) Ethiopia stands at the top in Africa. They play important role in different agricultural activities and socio-economic aspect of people. Power, meat, milk and cash income are among the major roles. Dairy production is practiced almost all over Ethiopia involving a vast number of small subsistence farms are one of the major economic development contributor (Ayalew and Abateneh, 2018; Belay, 2014). They contribute enormously for agricultural and total GDP (Tegegne et al., 2013).

According to Land O'Lakes (2010) the dairy production systems can be divided into four main systems: rural dairy smallholder, peri-urban and urban, commercial, and pastoral and agro-pastoral. The largest cattle population, 75% of the total is found in rural dairy smallholders where they are used mainly for traction followed by milk and meat production.

Ethiopia is the second most populous country in Africa next to Nigeria with a population estimated at 99.39 million out of which over 19.4% live in urban and peri-urban areas (SADC, 2017). The need for milk and its products majorly come from the urban and peri urban dairy production systems. The systems involve production, processing and marketing of milk and milk products that are channeled to urban centers (Eyassu and Asaminew, 2014; Tegegne et al., 2013). This system is contributing immensely towards filling in the large demand-supply gap for milk and milk products in urban centers, where consumption of milk and milk products are remarkably high. Dairy farmers and cooperatives involved in milk production in the peri-urban and urban areas are selling milk to consumer in the nearby town and city (Metekia and Nezif, 2017; Gebremichael et al., 2014). Producers have a better understanding of dairy management, processing facilities, better genetics (50 – 62.5% crosses) with experience of receiving AI services (Land O'Lakes, 2010).

Due to 3% population growth, 4.8% growth per year urbanization (Ulfina et al., 2013) together with change in life style, this production system is expanding and will have a bright future ahead. The system is practiced in and around major cities due to market and relatively better input access than the rural dairy small holders. The feeds and feeding system, breeding, housing, cow performance, and challenges of the sector have been studied in most of the cities and towns by different researchers. But the differences and similarities in management and constraints are not well organized and reviewed to show the comparative variance in between the locations.

So, the objective of this paper is to review the management, milk production performance,

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MANAGEMENT OF DAIRY CATTLE

Feeds and feeding

The dominant feeding systems for keeping dairy cattle in Jimma, Badalle, Ambo, Gimbi and Naqamte towns were grazing and grazing with some stall-feeding (Ulfina et al., 2013). In Adet and Merawi towns stall-feeding followed by seasonal grazing is mostly practiced whereas in Ejere and Hinchini towns, mixture of concentrate with some forage (in stall feeding) is dominant (Gizaw et al., 2016) though there is some feeding system variation between local and crossbreed cows. This can be due to the fact that crossbreed cows require more quality and quantity feed than local ones to produce higher amount of milk.

In Adwa and Axum, hay, crop residue, grazing, crop after math and non-conventional feed like *Atella* (by-product from the production of local beer) were identified as major sources of feeds (Gebrekidan and Gangwar, 2015). Whereas in Bishoftu the principal feed available were concentrates (noug cake and wheat bran), crop residues, stored hay, some forage legumes, vegetable and fruit wastes (Mulisa et al., 2011). The proportion of concentrate in the stall-fed diet was high in Gondar as compared to rural dairy cattle production (Addis et. al., 2011) and the feeds were of natural pasture, purchased concentrates, beer brewery and roughages (Moges, 2015). Hay and wheat bran were the main feed type used in Adis Ababa (Awoke and Mekibib, 2017).

The main feed resources available in the farm for cows in Hawassa city were crop residues, including stover (especially maize), grass hay, industrial byproducts and to some extent *Attela* (Haile et al., 2012). Concentrate feed composed of wheat bran and noug cake and salt additionally during the dry season is provided mainly to cows and calves at Shambo, Fincha and Kombolcha (Demissu, 2014). According to Hulagersh et al. (2017) the commonly available and frequently applied feed stuffs in Mekele dairy farms include straw, grass hay, concentrate “Frusca”, and other green grasses (Alfalfa and Elephant grass). A study in Jimma town revealed that majority of farmers use green feeds as the main basal diet, and wheat bran, commercial concentrate mix and noug cake were the most important concentrate supplements (Duguma and Janssens, 2016). Though crop residue and hay are most common feeds in the system, the difference in the kind of concentrate feed is due to the type and availability of agro-industries in the area.

Housing

According to Gizaw et al. (2016) almost all farmers in urban areas keep their cows in a separate improved housing, whereas only about half farmers do so in peri-urban areas of West Gojam and West Shoa zones with 40.0 % and 81.0% of the farmers in peri-urban and urban areas provide feeding and watering troughs in the barns, respectively. In Nekemte, closed barn housing system is mainly used for urban dairy housing and traditional housing system (open crush barns) is mainly used in peri-urban area (Misgana et al., 2015). Different from most areas, 71.6% of the households in Boditti town used cooking places (kitchen) for their animals (Asrat et al., 2013).

On the other hand housing in the peri-urban dairy production system of Shambo and Kombolcha was mainly roofed housing where 65.6% of cows living under shade while the remaining 34.4% passed the night in open backyard and/or traditional barn (Demissu, 2014). In Bishoftu town most farms have permanent house for their cattle and 83 % of the house of dairy farms is roofed while the remainder is not roofed (Mulisa et al., 2011).

Mating system

The most common performance trait mentioned in different studies for selection of superior bull is high daily milk yield followed by high fat content and shorter age at first calving. With regard to mating system in Addis Ababa for instance 77.4 % use artificial insemination (due to its accessibility), 3 % use only natural mating and 19.4 % use both Artificial Insemination (AI) and Natural mating alternatively (Awoke and Mekibib, 2017). In Shashemene and Dilla only 50% use AI as sole source of genetic improvement and the rest use a combination of both AI and natural mating (Sintayehu et al., 2008). On the contrary in Gondar 57% use natural mating, 20% AI and 22.5% use both AI and natural mating (Chanie et al., 2018). In Boditi 48.4 % used AI while 51.7% used natural mating with local bull where access and cost of AI is the determining factor (Asrat, 2013). In Bishoftu 40% use only natural mating, 22.5% use only AI and 37.5 use both natural mating and AI (Mulisa et al., 2011) depending on the accessibility at the time when cows are in estrus. On the other hand, according to Yayeh et al. (2017) natural mating was the only method used for dairy cattle breeding in and around Debre Markos town. The variation in the use of AI and bull is due to the access and preference by farmers to insure conception.

Dairy cattle holding

The average cattle herd size per household in peri-urban areas of greater Addis milk shed was 11.8 TLU (Tropical Livestock Unit) (Fekede et al., 2013). In Debre Markos 7.35 cattle her size is reported by Yayeh et al (2017). This result is higher than the finding of Yitaye et al. (2009) at Bahir Dar and Gondar peri-urban milk shed areas which is 6.5. The average number of cattle from the total herd in urban and peri-urban areas of Adwa and Axum is 6.78 and 4.83, respectively (Gebrekidan and Gandwar, 2015). In Boditti town the average number of cattle per house hold was 3.9 (Asrat et al., 2013) while in Hawassa city was 3.15 cow per house hold (Haile et al., 2012) in Shashemene 3.34 and in Dilla 1.51

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(Sintayehu et al., 2008). From this we can understand that the bigger the city the larger the number of cattle per house hold. This could be due to the fact that bigger cities have better access to AI, concentrate feed and market.

MILK PRODUCTION POTENTIAL

Daily milk yield

A daily milk yield (DMY) of 11.6 and 10.8 liters were recorded in Bishoftu and Akaki towns, respectively, for crossbred cows (Dessalegn et al., 2016) and in Adama milk shed was 11.3 liters (Nigusu and Yoseph, 2014). In Hawassa a DMY of 10.32 liters for cross bred cows is recorded (Haile et. al., 2012). This result is higher than the finding of Yitaye et al. (2009) who reported 7.8 liter at Bahir Dar and Gondar peri-urban milk sheds and Yayeh et al (2017) who reported 7.3 liter in Debre Markos town. On the other hand Asaminew and Eyasu (2009) reported 5.2 liter of DMY in Bahir Dar Zuria. Demissu (2014) reported the average daily milk yield in three towns of Horo Guduru Wollega zone was 7.21 liters. Those findings revealed that the bigger the cities the dairy production is found the higher the daily milk yield is recorded. This could be due to the fact that bigger cities have better access to inputs like concentrate feeds, AI, veterinary service and market.

Lactation length

Lactation length is the time of period from when a cow starts to secrete milk after parturition to the time of drying off. A lactation period of 10 moths is recommended to take advantage of 2 months dry period. Zewdie (2010) reported that the average lactation period of crossbreed dairy cows in Debre-Birhan and Sebeta were 9.7 and 10 months, respectively. Similarly in Shambu and Kombolcha towns of Oromia region 10.53 and 12.0 months of lactation length is reported, respectively (Demissu, 2014). While in Melkasa and Welenchiti was 10.8 and 11.4 months, respectively (Nigusu and Yoshep, 2014). Those findings are larger than 9.17 months in Gondar town (Kumar et al, 2014) and 8.7 months in Debre Markos (Yayeh et al., 2017). While Assaminew and Eyasu (2009) reported 10 months of lactation length in Bahir Dar Zuria. In Bishoftu and Akaki a lactation length of 9.22 and 9.36 months was reported by Dessalegn et al. (2016) at Bishoftu and Akaki towns, respectively. While in Jimma town a lactation length of 10 months was recorded (Ulfina et al., 2013). So lactation length is not a problem in the production system except in Debre Markos town.

Change drivers of the sector

Eastern Africa is the leading first milk-producing region in Africa, representing 68% of the continent's milk output. In Ethiopia, according to FAOSTAT (2014) milk production in 2011 was 4.4 million tons with 14.2 % growth rate between 2000 and 2011. Most Ethiopians are important consumers of milk and dairy products. Generally, milk consumption is rising although there are disparities among different cities and towns. Population growth, urbanization, rising incomes and change in lifestyles are the main drivers of this trend. Currently Ethiopia's milk consumption is only 19 liters per person– but urbanization is driving up consumption and per capita consumption in Addis Ababa is 52 liters per person (ATA, 2016). According to different authors, the majority of dairy farms in and around most of the cities were established in the past 20 years in response to the growing market demand for fresh and processed milk (Adiss et al., 2011; Zelalem et al., 2011) and contributing immensely toward filling the larger gap for milk and milk product supplement (Awoke and Mekibib, 2017). Access to different inputs, though a lot of work is needed, is another driver of the change in the sector. The accessibility to AI, concentrated animal feeds like agro-industrial by products and better veterinary services are additional opportunities for owners to rear dairy cattle in and around cities and towns.

Challenges of the sector

Shortage of animal feeds is the most important limiting factor of dairy production followed by limited space for proper housing, milking, waste disposal, and expansion, and animal disease incidence in Hawassa town (Haile et al., 2012). Availability and costs of feeds, land shortage, and problems related to waste disposal are the main challenges in Boditti town (Asrat et al., 2013). The same result was observed in Shashemene and Dilla towns (Sintayehu et al., 2008). Lack of market in the fasting season and poor genetic potential of local cows are main constraints in Debre Markos town (Yayeh et al., 2017). The main challenges mentioned in Mekele town were feed shortage, disease problems, and market problems (Huluageresh et al., 2017). This finding agrees with the report of Zekarias and Shiferaw (2012) in Jimma town where shortage of feed, disease and large number of local animals are the major bottle neck. The major constraints for dairy development in Bahir Dar and Gondar peri-urban milk shed areas include little availability and high costs of feeds in connection with shortage of farm land, poor access to waste disposal, poor market infrastructure and marketing systems (Yitaye et al., 2009). So in urban areas shortage and cost of feed and land are the major challenges while in peri-urban areas large number of local cattle and disease are additional problems.

Conclusions and Recommendations

Hay, straw and concentrates are main source of feed and free grazing with some stall feeding is a common feeding system. All in urban area and most dairy cattle owners in peri urban area keep their animals in a separate improved housing. Natural is still the major mating system due to less AI access, cost of AI and farmers preference to ensure

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conception. The average dairy cattle holding is negatively correlated with population size and size of towns. Though there is a variation in daily milk yield in between crossbreed and local cows, the average is still low. In all reviewed papers the lactation length is almost up to standard which is around 10 months. Population growth, urbanization, rising incomes and change in lifestyles are the main drivers of the sector. Shortage and cost of feed, shortage of land and waste disposal are major challenges reviewed in this production system.

From this review the following recommendations are made:

- Formulated feed mixers (to supplement quality feed) should invest to support the sector and chemical treatment improve the quality of hay should be implemented.
- The government and nongovernment organizations should strive to give access of AI for cattle owners.
- To improve the conception rate of AI, the technicians should improve their efficiency through training and experience.
- Waste disposal is an emerging challenge in the sector. So, using manure to biogas and compost should get attention.

DECLARATIONS

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UTILIZATION OF CROTON SEED AS A POSSIBLE ANIMAL FEED: A REVIEW

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REVIEW ARTICLE

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ABSTRACT: Croton is one of the largest genera in the Family Euphorbiaceae. Its species are distributed in a wide range of environmental and climatic conditions. The plants have for a long time been exploited for medicinal purposes but still hold the potential for nutritional purposes. The seeds of the plant have been exploited in oil extraction to produce biofuel but the residual seedcake has been less utilized. The use of croton seed and seedcake as feed has been in practice in Kenya and other countries but questions on its safety have been raised. Croton seeds are known to contain various phytochemicals and toxins such as crotin I, crotonic acid and tiglic acid that have deleterious health effects on animals. Notwithstanding the rich nutritional composition of the croton seeds, its safety concerns have limited their utilization as feeds. The croton seeds are rich in both essential fatty acids and protein whereas the residual seedcake is only rich in protein. However, the seedcake has toxic phytochemicals that include cardiac glycocides, alkaloids, phorbal esters and many others which are injurious to the animals and could result in death. Detoxification of the seedcakes poses a breakthrough for their use in poultry feeding. However, such techniques should not reduce the rich nutritional property of these seeds. This review focuses on the utilization of croton seedcake as a possible animal feed, documenting breakthroughs and limitations of the practice.

Keywords: Croton, Seed, Seedcake, Nutrient composition, Safety, Detoxification

INTRODUCTION

The Genus Croton is the second largest under the *Euphorbiaceae* Family and has approximately 1300 species which are largely trees, herbs and shrubs (Torres et al., 2008). The tree grows in the tropical and sub-tropical regions under varied environmental conditions. The stem, roots, leaves, seeds and the juice from the bark have been used for medicinal and nutritional purposes with varied utilization among different communities globally. Its traditional use as a medicinal plant has principally been exploited in Africa, Asia and Latin America (Sai Prassana and Karpaga, 2015). Croton *spp* have been utilized to treat gastrointestinal problems especially among the Asian communities (Ojo et al., 2017). In East Africa, Croton trees are mainly found in the mountainous regions (Aliyu, Agnew and Douglas, 2010). The species under this genus have heavily been exploited for their medicinal and nutritional property in Tanzania, Kenya and Uganda (Filho et al., 2017). Some of the species that have been used for either nutritional or medicinal purposes include *Croton heliotropiifolius* (Silva et al., 2017), *Croton tiglium* L (Ahmed et al., 2007), *Croton megalocarpus* (Wu et al., 2013), *Croton megalobotrys* (Maroyi, 2017b), *Croton bonplandianus* (Dutta, Dey and Chaudhuri, 2014) and *Croton macrostachyus* (Abebayehu et al., 2016) among others. *Croton megalocarpus* is the widely distributed species of the genera across African continent (Kivevele and Mbarawa, 2010).

Some species of croton has been utilized mainly for its oil and as animal feed in Kenya (Leakey et al., 1996). The Croton megalocarpus plant is locally known as *Mukinduri* (Kikuyu) or *Msuduzi* (Swahili) (Maroyi, 2017a). *C. megarlocapus is* the most widely distributed croton species in Kenya (Jacobson et al., 2018). Croton mainly grows in the areas with bimodal rainfall pattern including Central and Western regions in the country. In the Mount Kenya region, approximately 40% of the farms have croton though it is largely undomesticated (Jacobson et al., 2018). The plant is not a preferred browse by most of the animals for its seeds, bark and leaves have skin and mucosa irritating property that keeps most of the animals away (Diaz, 2011). The seeds of croton *spp.* have been exploited in oil extraction and the residual seedcake is exploited as feeds (Jacobson et al., 2018). However, toxicity concerns have been raised over the use of the seedcake as feed. This review focuses on the utilization of croton seedcake as animal feed and possible ways of reducing its toxic components while improving its nutritional benefits.

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METHODOLOGY OF REVIEW

A literature search for the keyword croton and its various species was conducted in major literature databases that included Google scholar, Springerlink, PubMed, Science Direct, BioMed Central, Elsevier, Hindawi publishers, Academic journal, Europe PMC and John Wiley and Sons that documented the historical uses, medicinal uses, nutritional composition, safety and toxins, oil extraction and uses of the seedcake. Additional literature was obtained from documented reports by government agencies in Kenya and other international bodies like FAO and EFSA and thesis that had been submitted to the University of Nairobi. Mendeley referencing software was used to collate relevant literature that had been selected and used in the review.

Varieties of croton

There is a diversity of species of croton distributed in different environmental and climatic conditions globally. Since the inception of the name of the genus Croton by Linnaues in 1753, 1250 species have been classified under it (Van Ee and Berry, 2010). Some of the species of croton that have been identified include *C. cajucara*, *C. celtidifolius* Baill., *C. eluteria* Bennett., *C. palanostigma*, *C. lechleri*, *C. urucurana*, *C. draconoides*, *C. malambo, C. nepetaefolius* Baill., *C. palanostigma* Klotzsch, *C. schiedeanus* Schlecht. and *C. zehntneri* Pax. majorly in South America; *C. arboreous* Millsp., *C. californicus* Mull. and *C. draco* Cham. & Schltdl. in the North and Central America; *C. macrostachys* Hochst., *C. megalocarpus* and *C. zambesicus* Mull. in Africa; *C. kongensis* Gagnep., *C. oblongifolius* Roxb., *C. sublyratus* Kurz, *C. tiglium* L. and *C. tonkinensis* Gagnep. in Asia (Salatino, Salatino and Negri, 2007). All these species are known for their medicinal values to various communities. *C. megalocarpus* is the most common indeginous species to East Africa and can produce seeds up to 50kg per tree (Atabani et al., 2013).

Cultivation of croton *spp.*

Croton *spp.* often grows in areas with volcanic soils at an altitude of 200-1500m above sea level and mean annual rainfall of 150-1200mm (Maroyi, 2017a). The plant majorly grows as secondary forests along the edges of forests, rivers and lakes. They grow optimally under rainfall of 800-1900mm, temperature of 11-38^{o}C, elevation of 1200-2450m and light deep well drained soil (Tenaw, Mitiku and Tamene, 2017). The tree grows to a height of 35m with a great variation among different species (Gichui, 2016). Dey et al. (2015) reported a height of 4-6m for *C. tiglium* whereas Nagireddy et al. (2017), Berry and Galdames (2013) and dos Santos et al. (2016) reported that *C. scabiosus* species had a height of 3-5m, *C. cerroazulensis* had a height of 7-7.5m and *C. sapiifolius* had a height of 14m respectively. Flowering on the tree starts after 3-4years of their growth with the fruits ripening at around 5 months after flowering (Jacobson et al., 2018). The plant can be propagated using seeds, cuttings, grafting or air layering. In East Africa, the trees are known to fruit twice a year; about five months after the rainy season. The seed yields of the tree average 25-40kg annually with a great variance on the weight of seeds from tree to tree (Aliyu et al., 2010), thus proper selection when breeding is needed. The plant largely grows in the mountainous regions as shrubs in East and South Africa (Sarin, 2012). In Tanzania, there are large scale plantations of *Croton spp.* that are utilized in biofuel production by a privately-owned company; whereas in Kenya, monoculture of croton trees is very minimal and just in small farms (Endelevu Energy, 2009).

Utilization of croton seeds

Croton has been exploited for various uses especially its nuts, much of which are non-nutritional as the seeds are inedible (Sharma et al., 2016). Croton seeds have been exploited for oil extraction which has multiple uses. Kim et al. (2014) in their study on croton oil, observed that it possesses a possible lipolytic property. In China, the oil has over time been exploited for its medicinal properties which include antimicrobial, insecticidal, purgative and analgesic (Dey et al., 2014).

The residual seedcake after oil extraction from croton seeds has been sold locally in Kenya as a poultry feed (Jacobson et al., 2018). Currently in Kenya, the croton seedcake are sold as ingredient of poultry feed in a process aimed at extending the value chain and importance of *C. megalocarpus* (Sharma et al. 2016). Croton seeds have also been exploited for their medicinal property just like the bark, leaves and its roots. Traditional health practioners from the Lower Eastern Region of Kenya reported the use of croton in the management of diabetes mellitus (Keter and Mutiso, 2012). Obey et al. (2018) reported an antimicrobial activity of up to 100% suppressive effect on plasmodium parasites by extracts of the bark of *C. macrostachycus.* There is evidence of use *C. tiglium* seeds for medicinal purposes as early as 450 BC in India (Dey et al., 2014). The seeds have been used as purgative and in wound healing, constipation and traditional dysentery and dyspepsia (Oyesola, Oluwole and Oyesola, 2009; Dey et al., 2014).

Croton has also been used in the production of biofuel with its commercial production currently being done in Kenya and Tanzania (Endelevu Energy, 2009; Jacobson et al., 2018). Seeds of croton *spp.* including *C. tiglium* have been recommended as possible sources of alternative fuel (Ariharan et al., 2015). Croton has been touted as an alternative fuel that is environmental friendly. In a study that evaluated a composite biofuel incorporating *C. megalocarpus* as a substitute to fossil fuel, Ruhul et al. (2016) reported a reduction of 5.21%, 8.38% and 20.71% in the carbon monoxide, hydrocarbon and smoke emission, respectively.

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Extraction of croton oil from croton seeds

Croton is renowned for the oil extracted from its seeds for use as biodiesel. The oil in croton seed can either be extracted mechanically (pressing) or solvent (chemical) methods (Jiyane, Tumba and Musonge, 2018). The chemical extraction mainly explores the use of solvent, thus is also known as solvent extraction (Bhargavi, Nageswara Rao and Renganathan, 2018). The solvent *n*-hexane has been proven to be effective in the soxhlet extraction of croton seed oils (Ojo et al., 2017). Saputera and Atikah (2014) recommended a raw material/solvent ratio of 1:5.18g ml^{-1} with an optimal maceration time 6.22 days for optimal extraction of croton oil. The mechanical extraction is often viewed as a cheaper technique and more convenient where mechanical pressers are often used. Osawa et al. (2014) obtained a yield of 38.42% (v/w) of croton oil using mechanical pressers. However, the solvent extraction has higher yields of up to 41% and consistent performance as noted in the extraction of oil from jatropha seeds (Atabani et al., 2013; Gonfa Keneni and Mario Marchetti, 2017). Solvent extraction has a reported higher efficiency of only 1% (w/w) residual fat in the cake (Kibazohi and Damson, 2013). Processing of the seeds has an impact on the nutritional composition of the croton oil. Ojo et al. (2017) reported a higher saturated to unsaturated fatty acid ratio in the dehulled seed oils as compared to the whole seed oils.

Nutritional Composition of Croton *spp* seeds

The proximate composition of seeds of various croton *spp.* are as shown in Table 1. The seeds of *Croton megalocarpus* have protein and oils contents of up to 50% and 30-32% respectively (Wu et al., 2013). However, some varieties of croton *spp.* seeds have lower oil and protein contents. A study by Bello et al. (2014) on *C. zambesicus* seeds found a lower crude protein and fat of 9.64% and 4.15% respectively as compared to other species. Variation in terms of nutritional composition of the seeds based on species calls for careful selection of species cultivated for a given purpose. The oil from croton seeds is largely inedible. A study by Adeyinka et al. (2013) reported high values of 4.10mg and 2.42 mg KOH/g for saponification values of *C. penduliflorus* seeds and seeds without seed coat respectively. The values are higher than those of known edible oils such as coconut oil (0.6 mg KOH/g) and cotton seed oil (0.6 mg KOH/g); proving they are inedible.

The croton seed oil is rich in saturated, monounsaturated and polyunsaturated fatty acids (Maroyi, 2017a). In his study, Bello et al. (2014) on *C. zambesicus* concluded that the seeds of this plant are a rich source of essential fatty acids beneficial to both animals and humans. Croton seed has a richer fatty acid profile as compared to other greatly utilized oilseed crops (Table 2), however concerns of its toxicity has limited its utilization for nutritional purposes. Ahmadi et al. (2017) reported linoleic and oleic acids as the major fatty acids in *C. tiglium* oil.

Table 1 - Proximate Composition of *Croton spp*. seeds

Proximate composition (% fresh weight)	*C. zambesicus*[a]	*C. penduliflorus*[b]	*C. tiglium*[c]
Moisture	7.94±0.02	6.12±0.44	6.2
Protein	4.15±0.01	0.06±0.01	26.69
Fat	9.64±0.14	34.01±0.14	40.01
Ash	15.45±0.21	3.26±0.43	3.14
Fibre	26.73±0.32	38.50±1.27	8.45
Carbohydrate	67.09±2.28	18.03±0.54	15.51

Adapted from [a]-Bello et al. (2014a), [b]-Adeyinka et al. (2013) and [c]-Saputera et al. (2006)

Table 2 - Fatty Acid composition of croton oil in comparison with other vegetable oils (wt %)

Fatty acid	Croton	Sunflower	Rapeseed
Lauric acid	0.11	0.11	0.04
Myristic acid	0.04	0.16	0.04
Palmitic acid	6.23	6.47	4.96
Palmitoleic acid	0.11	0.10	0.32
Stearic acid	4.37	4.34	1.73
Oleic acid	9.95	24.59	62.07
Linoleic acid	74.31	62.68	19.16
Linolenic acid	3.62	0.44	9.63
Arachidic acid	0.92	0.40	1.49
Erucic acid	0.33	0.71	0.53

Adopted from Wu et al. (2013)

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The seeds of croton species are not only rich in macronutrients but also the micronutrients. Bello et al. (2014a) reported that the seed of *C. zambesicus* are richer in strontium (869.27 mg/100g), potassium (2.2 g/100g) and iron (467.53 mg/100g) than its leaves. Another study by Adeyinka et al. (2013) on seeds of *C. penduliflorus* found that they are rich in magnesium and calcium which are important micronutrients. Removal of the seed coats significantly reduces the mineral content by 8.1% while increasing the fat content by 16.02% (Adeyinka et al., 2013).

Extraction of oil from the seeds leaves a protein rich seedcake, whose utilization in animal feeding is highly recommended. A study on *Jatropha curcus* seedcake, a tree from the same sub-family with croton with similar toxicity concerns, reported a protein content of 43.48% (Sánchez-Arreola et al., 2015). Oil extraction bears the benefit of reducing the phorbol esters, but concerns of toxicity still remain with the presence of toxic proteins (Rajput and Gaur, 2015). Evaluation of amino acid profile of croton seedcakes in comparison with common animal feed seedcakes by Peoples et al. (1994), showed that seedcakes of *C. capitatus* are richer in essential amino acids as compared to the others (Table 3).

Table 3-Essential amino acid profile of *Croton capitatus* seedcakes in comparison with other common animal feed seeds (% DM)

Essential amino acid	Sunflower	Croton (*C. capitatus*)	Redwood amaranth
Arginine	0.61	1.29	0.53
Glycine (plus Serine)	0.96	1.01	0.73
Histidine	0.11	0.24	0.12
Isoleucine	0.31	0.42	0.23
Leucine	0.51	0.63	0.38
Lysine	0.47	0.61	0.41
Methionine (plus cysteine)	0.11	0.16	0.08
Phenylalanine	0.36	0.50	0.25
Phenylalanine (plus tyrosine)	0.55	0.80	0.45
Threonine	0.31	0.40	0.22
Valine	0.36	0.57	0.29

Adapted from Peoples et al. (1994).

Phyto-chemicals and antinutrients in Croton *spp.* seeds

Phytochemicals in croton seeds range from health promoting to compounds with deleterious effects to health. The phytochemicals that have been found in the seeds of different croton *spp.* include diterpines, phorbol ester, alkaloids, terpenoid, flavonoids, tannins, cardenolides and many others as shown in Table 4 (Parameswararao et al., 2016). The major phytochemical of concern in croton *spp.* seeds are the phorbol esters. This is majorly in the seed oil, with the most active phorbol ester being 12-O-tetradecanoylphorbol-13-acetate (TPA) (Nath et al., 2013). These compounds have tumour promoting properties. Seeds of similar species of croton from different regions also differ in their phyto-chemical composition. The proanthocyanodin and alkaloid property of croton induces a red sap property in croton seeds (Prassana and Karpaga, 2015).

Table 4 - Phyto-chemicals in different species of croton seeds

Croton *Spp*	Country of origin	Phyto-chemicals	Reference
C. tiglium	Far East	Alkaloids, flavonoids, terpenoids and phorbol esters	(Dey et al., 2014)
Croton bonplandianum Baill	India	Squalene, (9Z, 12Z)-octadeca-9, 12-dienoic acid, methyl 12-oxo-octadec-9-enoate, alkaloids, terpenoid, flavonoids, tannins, cardenolides and phytol.	(Parameswararao et al., 2016)
C. tiglium Linn.	India	Resin, resin, steroids, sugars, saponin	(Kishore et al., 2013)
C. tiglium	Pakistan	Alkaloids, steroids, terpenoids, glycosides and saponins.	(Abbas et al., 2011)
C. bonplandianum	India	Resins, alkaloids, saponins , phenols, flavonoids and steroids.	(Jeeshna et al., 2011)
C. megalocarpus	Kenya	Saponins, flavones, alkaloids, glycosides, terpenoids, steroids and flavonoids	(Waiganjo et al., 2013)

Safety of croton oil and seedcake

The croton oil is toxic to all living organisms, ranging from simple single cell organisms like bacteria to complex organisms such as the vertebrates (Pagani et al., 2017). The compounds that are known to be responsible for this toxic property include cardiac and cyanogenic glycosides, lectin, phorbol esters and alkaloids (Yumnamcha et al., 2014). Yumnamcha et al. (2014) in their study on *C. tiglium* found saponins and alkaloids in croton oils, which are known to possess DNA damaging property thus are genotoxic. Similar findings were reported by Aylate et al. (2017) in their study on the extracts from *C. macrostachycus*. Phorbol esters in the croton oil and seedcake has been associated with tumour-

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enhancing property (Dey et al., 2014). Phorbol esters occur to the tune of 3-5% in the fatty acids in croton seeds (Jain, Mangal and Kushwaha, 2015). Sharma et al. (2016) reported a skin-cancer inducing property in the croton seed oil. The most studied mechanism by which phobol esters causes cancer is its role in binding and activating protein kinase C (PKC) that plays a role in signal transduction (Goel et al., 2007). They hyperactivate the PKC thus causes a proliferation of cells thereby amplifying the efficacy of carcinogens in the body of an animal. These phobol-12,13-diesters are also responsible for the purgative and irritant property of ingested seeds. As it had been indicated earlier TPA is the most renowned toxic phobol-ester in croton seeds is the (EFSA CONTAM Panel, 2015).

Concerns have been raised with regard to the safety of the croton oil and seedcake as animal feed. Apart from croton oil inducing gastrointentinal discomfort, the proteins in the seedcake have been shown to possess proinflammatory effects on the gastrointestinal system in clinical trials (Liu et al., 2017). EFSA has raised concerns on the safety of *C. tiglium* or its seed cake as an animal feed as it contains crotin I which is a ribosome inactivating protein (RIP II) thus causes acute death of animal (Alexander et al., 2008). The compound has a LD_{50} of 20mg/kg body weight in mice.

Other health concerns have also been raised on oils from some species of croton. Ojokuku, et al. (2011) posits that in as much as *C. penduliflorus* seed oil has the positive hypocholesteremic health effect, it also poses the risk of inducing anemia. Crotonic acid, a compound in croton *spp.* seed oil, induces hemagluttination and hemolytic activity in animals (El-Kamali et al., 2015). *Croton tiglium* L. seed oils also possess piscicidal properties that some communities have exploited in fishing especially in India (Rajput and Gaur, 2015; Saha et al., 2015). Acute toxicity studies on the *C. penduliflorus* seed oils also showed deleterious effects on some internal organs at doses of 200, 600 and 800 mg/kg of feed in mice (Ashafa et al., 2012). *C. penduliflorus* seed oils have been shown to cause adverse effects on the kidney and nervous systems of animals (Ojokuku et al., 2011). Extracting oil is not an automatic procedure for removal of toxins from the seedcake in as much as the most toxic compounds, phorbol esters, are in the oil (Sadubthummarak et al., 2013).

CROTON SEEDCAKE AS ANIMAL FEED

There are only few documented studies of the exploitation of croton seeds and seedcakes as animal feed. Most documented feeding trials have exploited whole seeds of croton *spp* with varied efficacy as shown in Table 5. However, care is advised as these seeds have toxins that have deleterious effects on the health of the animal. Feeding trials by Gadir et al. (2003) on goats using *C. macrostachycus* whole seeds at 1g/kg and 0.25g/kg showed 100% mortality for all animals and weight loss of 21.6% and 26.3% respectively within 21 days. On the other hand, Thijssen (1998) reported satisfactory feeding efficiency, weight gain, feed intake and growth rate of the chicks fed on feeds with *C. megalocarpus* seedcake incorporated at the proportions of 10-25%. *C. macrostachycus* based feeds were found to be some of the high proteins feeds suitable for use in aquaculture (Kassahun et al., 2012). Feeding trials for croton have also been done on fishes. Dada and Adeparusi (2012) reported an increased fecundity index of 21,987.16 for catfish that were fed on 200g/kg body weight of *C. zambesiscus* seeds. A study by El-Kamali et al. (2015) established that croton seeds incorporated into feed for rats in experimental diets induced no deleterious hematological effects.

Table 5 - Performance of animals in feeding trials utilizing croton seeds

Croton *spp.*	Test animal	Type of seed	Amount incorporated	Performance of the animal
C. macrostachys	7 month-old Nubian kids	Whole	1 g/kg body weight	Mortality within 7-21 days
C. macrostachys	6 month-old Nubian kids	Whole	0.25 g/kg body weight	Mortality within 7-21 days
C. zambesiscus	Catfish (*Clarias gariepinus*)	Powdered seed	50 g/kg body weight	Increased fish weight and increased fecundity
C. zambesiscus	Catfish (*Clarias gariepinus*)	Powdered seed	100 g/kg body weight	Increased fish weight and increased fecundity
C. zambesiscus	Catfish (*Clarias gariepinus*)	Powdered seed	200 g/kg body weight	Increased fish weight and increased fecundity
C. zambesicus	Wistar rats	Aqueous extract of powdered seed	75 mg/kg body weight	0.07 g/day weight gain
C. zambesicus	Wistar rats	Aqueous extract of powdered seed	300 mg/kg body weight	0.68 g/day weight loss
C. zambesicus	Wistar rats	Methanol extract of powdered seed	75 mg/kg body weight	0.71 g/day weight loss
C. zambesicus	Wistar rats	Methanol extract of powdered seed	300 mg/kg body weight	0.28 g/day weight loss
C. tiglium	One-week old Chicks	Seedcake	10-25 % incorporated in commercial chick mash	Increased growth rate of up to 117.5 g

There are other plants that are similar to Croton *spp.* that have had their seedcakes exploited as animal feeds. *Jatropha curcas* (belongs to the same sub-Family with croton) seedcake were incorporated into soybean meal at 25% both as raw Jatropha seedcake and detoxified Jatropha seedcake, where 33.3% mortalities was noted in each case

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(Elangovan et al., 2013). Detoxification in this study employed addition of 3% sodium bicarbonate as similar to croton *spp.* seeds, toxicity concerns have been raised for Jatropha seeds. Another study by Barros et al. (2015) noted more deleterious effects as increasing the Jatropha seedcake in the diet of broiler chicken, resulted into decreased body weight and diminishing size of spleen and kidneys; chicken fed on 100g jatropha seedcake per kg of diet lost up to 21.6g/day. This was attributed to the phorbol esters in the seedcake. Thus, detoxification of these seedcakes is necessary before their utilization as feeds.

Research has delved further into making croton seeds safe for use as animal feeds. Solvent extraction accompanied with heat treatment of the seed cakes has successfully been employed as a way of producing feeds free of toxic levels of phorbol esters (Goel et al., 2007). Phorbol esters are mainly in the croton oil rather than the seedcake (Pagani et al., 2017). However, oil extraction is not an assurance of total removal of the phorbol esters from the seedcake (Sadubthummarak et al., 2013), thus other efficient detoxification methods are necessary.

In India, milk is employed as a detoxicant of croton seeds but this has only been evaluated in its medicinal use but not as a feed (Maurya et al., 2015). A study by Pal et al. (2014) found that the use of milk in detoxifying *C. tiglium* seeds reduced the phorbol esters and crotonic acids by 65.4% and to undetectable levels respectively. Kishore et al. (2013) reported that the method has no significant impact on the phytochemical components such as alkaloids, saponins, resin and saponins. However, it reduces the fat content thus would reduce the phorbol esters (Jain et al., 2015). The effect of such treatment on the nutritional composition of these seedcakes has also not been studied. Phorbol esters that have been traced in seedcakes in plants with the same toxicity as croton have been shown to degrade after 21 days of storage at moisture content of 130g/kg (Fujiki et al., 2017). This, points to a possible way of detoxification of the seedcakes to free them of these tumour-enhancing compounds thus making them safe as feeds. In the detoxification procedure, it is necessary not to induce any deleterious effects in the nutritional composition. Another study on Jatropha seedcake found that thermal treatment at 120°C and 220°C, followed by addition of 10% adsorbing bentonites (nanoparticle of zinc oxide) and 4% $NaHCO_3$ and incubation for four weeks, reduced phorbol esters to non-toxic levels of 0.04-0.05 mg/g with no cytotoxicity while on other hand not affecting the proximate composition (Sadubthummarak et al., 2013). Absence of cytotoxicity is an indication of absence of the toxic lectin proteins.

In croton *spp.* seedcakes, the most injurious proteinous compounds are the crotins, a lectin class of proteins (Vasconcelos and Oliveira, 2004). Crotins are hemolytic proteins that exhibit hematological adverse effects in animals. Crotin I and II exhibited toxicity in mice at acute LD_{50} of 1.33 and 4.38 mg/mouse respectively (at 72 hours), and delayed LD_{50} of 0.92 and 1.68 mg/mouse respectively (at 7 days) (Stirpe et al., 1976). The *C. tiglium* seeds have an acute LD_{50} of 2000mg/kg body weight (Harshavardhan et al., 2016). For these seedcakes to be exploited for feeds, it would be necessary to detoxify them of these proteins. In the detoxification of jatropha seeds, it was found that samples that were deshelled followed by defatting, enzyme hydrolysis (pectinase and cellulase) and treatment with 60% methanol and 65% ethanol; lowered toxic lectin proteins by 52.8% and other antinutrients such as saponins, phytic acid, total phenolics and trypsin inhibitor activity by 65.1%, 31.8%, 71.9% and 21.5% respectively (Xiao et al., 2011). The treatment also improved the protein digestibility corrected amino acid scores by 47.1% and crude protein content by to 15.22%.

CONCLUSION

There is increasing utilization of seeds croton *spp.* for oil extraction expanding the use of croton, but the possibility of extending the value chain by using the seedcake as a feed remains less explored. The nutritional composition of the croton seedcakes make them viable for possible exploitation as poultry feed. However, the toxicity of these seeds induced by various phytochemicals still remains the greatest challenge to this venture. Croton has been shown to pose various toxic effects to various animals ranging from simple organisms to complex ones including poultry. A possible way for detoxification of these seeds serves to ameliorate their quality as feeds. Practical ways for detoxification have been tried but not possibly for its utilization for feed for poultry or any other animal. The effect of such technique to the nutritional composition has also not been explained. For its possible use as a feed, it would be important for the safety of croton seedcakes to be evaluated to provide scientific justification for its use. Future research should evaluate the use of these identified detoxification technique and their influence on nutritional composition of the croton seeds.

DECLARATION

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

Availability of data
The data can be availed to the journal upon request.

Consent to publish
Not applicable

Conflict of Interest

The authors declare they have no competing of interests.

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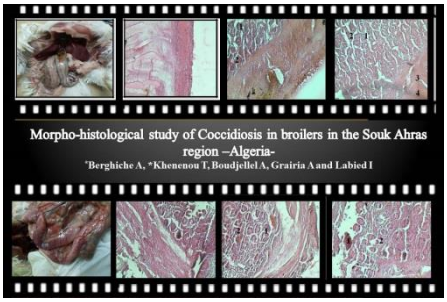

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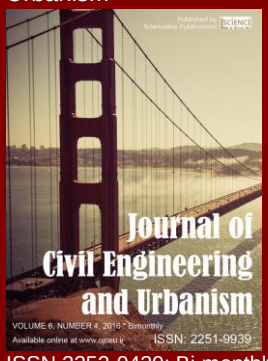

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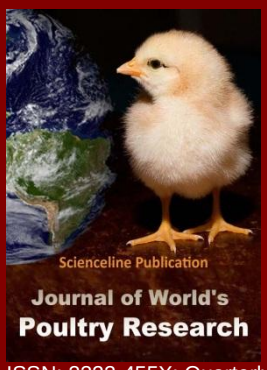

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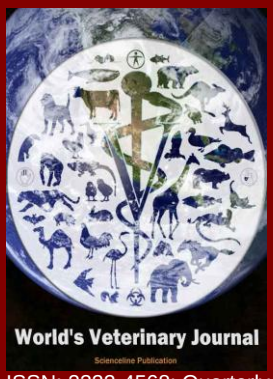

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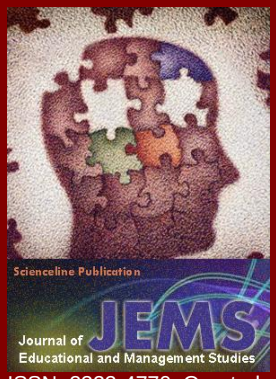

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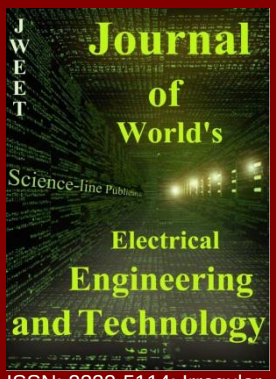

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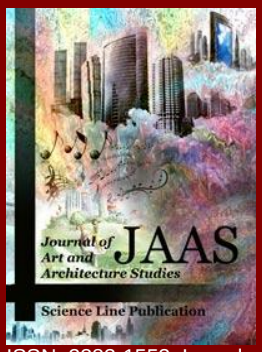

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