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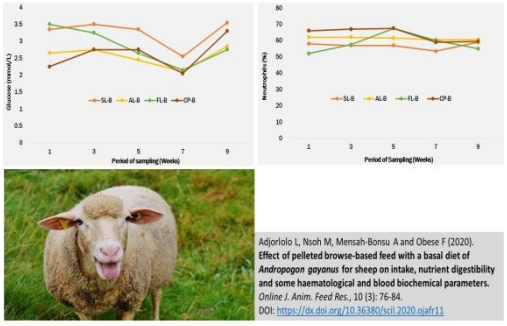
Volume 10 (3); May 27, 2020

Research Paper

Effect of pelleted browse-based feed with a basal diet of *Andropogon gayanus* for sheep on intake, nutrient digestibility and some haematological and blood biochemical parameters.



Adjorlolo L, Nsoh M, Mensah-Bonsu A and Obese F.
Online J. Anim. Feed Res., 10(3): 76-84, 2020; pii: S222877012000011-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr11

Abstract
The study was designed to evaluate the nutritional quality of pelleted diets based on four of the major feed resources fed to small ruminants by farmers in the Accra Plains. Leaves of *Samanea saman, Acacia auriculiformis* and *Ficus exasperata* and cassava peels were dried, mixed with other ingredients and pelleted. A preference trial showed sheep accepted all the four supplements with a marked preference for cassava peels-based (CP-B) and *Samanea saman*-based (SL-B) supplements compared with *Acacia auriculiformis*-based (AL-B) and *Ficus exasperata*-based (FL-B) supplements (P< 0.05). The supplements were subsequently fed to Eight West African Dwarf sheep on a basal diet of *Andropogon gayanus* (Gamba grass) hay in Latin square design. Dry matter intakes (DMI) did not differ by the type of supplement (P> 0.05). However, crude protein intake (CPI) was higher (P< 0.05) in sheep fed AL-B and FL-B than those fed SL-B and CP-B. Dry matter and neutral detergent fibre (NDF) digestibility were lowest (P< 0.05) for sheep fed CP-B. Dietary treatments did not affect haematological parameters, except for neutrophil percentage which was higher (P<0.05) in sheep fed CP-B than those fed SL-B. Animals fed CP-B had the lowest monocyte concentrations (P< 0.05). Furthermore, all the serum biochemical parameters were not affected by dietary treatment except total protein concentration which was highest (P< 0.05) in sheep fed on AL-B. It is concluded that the feed resources available to the small ruminant keepers can be used to prepare pelleted supplements that are acceptable to sheep and help sustain appreciable performance on low quality forages during the dry season without any deleterious effects on intake, digestibility, physiology and health.

Keywords: Acceptability, Browse plants, Dry season, Accra Plains, Blood parameters, Feed intake

[Full text-PDF]

Research Paper

Assessment on defects of wet-blue hide and pickled skin at Modjo Tannery.

Feleke BA and Habtemichael YG.
Online J. Anim. Feed Res., 10(3): 85-92, 2020; pii: S222877012000012-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr12

Abstract
Across-sectional study was conducted from February to June 2015 with the objectives of identifying the major types of hide and skin defects and determining their prevalence in pickled skins of sheep and wet blue goat skins and hides originated from two districts namely Hitosa and Dodota of East Arsi Zone at the Colba and Gelan tanneries in Modjo town. A total of 389 wet blue cattle hides, 385 wet blue goat skin and 399 pickled sheep skin were examined. The study finding showed that there exist various defects responsible for the decline in quality of skin and hide. The major defects at the wet blue hide were flay cut (59.1%), gouge mark (42.2%), and putrefaction (35.2%). In sheep pickled skin higher percentage of cockle (36.9%), gouge mark (28.3%) and scratch (27.0%) were observed. In wet blue goat skin, cockle (48.1%), veininess (44.6%) and crack (41.9%) were the major defects observed. The prevalence of cockle, veininess, scar, corduroying, crack, gouge mark, poor pattern and brand mark were significantly higher (P<0.05) in goat skin at wet blue stage than pickled sheep skin while putrefaction and shoat pox were significantly higher (P<0.05) in sheep skin compared to goat wet blue skin. The major defects that leads to rejection of wet blue hide were flay cut while cockle in sheep and goat skin. In pickled sheep skin, grade of 1-3 accounts 14% and grade 4-7 accounts 86% of the total observation. This study showed large proportion of skin and hides were subjected to rejection because of poor quality and this implies that integrated efforts towards improved livestock husbandry and better health care are vital issues for production of better-quality hide and skin. Furthermore, effective extension system and programs that could raise public awareness on ectoparasites and skin diseases control as well as on methods of flaying, preservation and handling of skins should be implemented.
Keywords: Cockle, Fly cut, Grade, Hide, Quality, Rejection, Skin

[Full text-PDF]

Research Paper

Potentialities of transmission of *Salmonella* Spp from water source to fish in muddy season in River Nile State, Sudan.

Bakhiet HHA and Zaroug M.
Online J. Anim. Feed Res., 10(3): 93-97, 2020; pii: S222877012000013-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr13

Abstract
This study was conducted in river Nile state, north Sudan aimed to give base line information on the potentialities of transmission of *Salmonella* spp from water source to fish in muddy season, in AL-fadlab and Al-akad stations. Twenty samples of water and *Schilbidae* spp fish were taken from the two stations and transferred to the laboratory for physiochemical and microbial analysis of water and studding fish species. Samples were performed using standard bacteriological procedures. Swaps from each fish gill were microbiologically analyzed for *Salmonella* spp and total plate count. Results indicated that studied fish infected by *Salmonella* spp in AL-fadlab station was 44.83±8.6 while in Al–akad station was 9.33±1.4, *Salmonella* spp in water was 5.00±1.0 in AL-fadlab station while it has no growth in Al–akad station. On the other hand, total plate count in fish gills was uncountable in AL-fadlab station and 30.40±7.1 in Al–akad station. Total plate count in water, was 8.13±1.87 for AL-fadlab station and 11.67±2.04 for Al-akad station. Statistical analysis showed significant difference (P< 0.05) in all studied parameters except the total plate count in water. There was also no significant difference in weight and length of studied fish species and also in water turbidity and temperature from both stations, but water pH showed significant difference (P< 0.05, 7.62±0.04 and 9.53±0.08 for Al-fadlab and Al-akad, respectively). *Schilbidae* spp fish infected by *Salmonella* spp in studied stations is an indicator of the contamination by untreated municipal sewage, runoff, and storm-water. Therefore, *Schilbidae* spp fish from studied areas have to be carefully handling and heating before consumption to avoid the pathogenic bacteria risks.
Keywords: Chemical, Foods, Genetically, Health, Organisms, Risk

[Full text-PDF]

Review

Figure 1 - Persisting Kyphoskoliosis in a juvenile Nile crocodile after recovery from osteomalacia. Source: Huchzermeyer, and Van Wyk (2003).

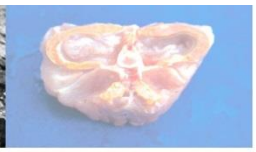


Figure 2 - Fat necrosis: hardened yellow fat between the tail muscles of a Nile crocodile. Source; Huchzermeyer and Van Wyk (2003).

Figure 3 - 'Rubber jaws' and 'glassy teeth' in a Nile crocodile hatchling with osteomalacia. Source; Huchzermeyer and Van Wyk (2003).

Citation: Delene K, Lemma A, Fesseha H (2020). **Major diseases of nile crocodile (*Crocodylus niloticus*) with focus on current status in Arba Minch crocodile ranch, Ethiopia.** *Online J. Anim. Feed Res.,* 10(3): 98-110. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr14

Major diseases of nile crocodile (*Crocodylus niloticus*) with focus on current status in Arba Minch crocodile ranch, Ethiopia.

Delene K, Lemma A, Fesseha H.
Online J. Anim. Feed Res., 10(3): 98-110, 2020; pii: S222877012000014-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr14

Abstract
Crocodylus niloticus is found in 26 African countries including Ethiopia, the largest recorded specimen measuring 17.0 feet Nile crocodile from the Gambela Upeno River in 1969. Its presence and absence also depend on the climatic conditions and the environment (i.e. the landscape for basking and feeding). In Ethiopia, Nile crocodiles have a mating period during September to October, Nesting occurs in the dry season December to January, and hatchling takes place at the onset of the rainy season, i.e. March/April months. Over the period 2007-2016, an average of 201,000. *Crocodylus niloticus* skins were exported globally per year, with an increasing trend over the period 2009-2016. Besides the management problems, at Arba Minch Crocodile Ranch, Nile crocodiles are suffering from nutritional abnormalities and health problems. The diseases of the Nile crocodile are classified as infectious (transmissible) and non-infectious (non-transmissible). A transmissible crocodile disease includes bacterial, viral, fungal, protozoan, and parasitic diseases; non-transmissible crocodile diseases are nutritional, toxic poisonings and metabolic disorders; other diseases like nutritional bone diseases and skin lesions are the major health problems at Arba Minch Crocodile Ranch. The main aim of this review is to highlight the major diseases and management status of *Crocodylus niloticus* in Arba Minch ranches, Ethiopia. In conclusion, the Arba Minch Crocodile Ranch (AMCR) should exercise care and caution when introducing hatchlings from the wild into the Ranch and enhance its husbandry methods to reduce the occurrence of infection and disease. It must also work with professionals and research groups.
Keywords: Arba-Minch, *Crocodylus niloticus*, Diseases, Nile crocodile.

[Full text-PDF]

Research Paper

Evaluation of the chemical composition of Argan (*Argania spinosa* L.) oil according to its extraction method, origin of production and altitude.

Hilali M, El Monfalouti H and Kartah BE (2020). **Evaluation of the chemical composition of Argan (*Argania spinosa* L.) oil according to its extraction method, origin of production and altitude**. *Online J. Anim. Feed Res.*, 10(3): 111--118. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr15

Hilali M, El Monfalouti H and Kartah BE.
Online J. Anim. Feed Res., 10(3): 111-118, 2020; pii: S222877012000015-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr15

Abstract
In this study the chemical composition of Argan (*Argania spinosa* L.) oil was evaluated according to its mode of extraction, origin of production and altitude of the Argan tree. To carry out this work, the physico-chemical characteristics and chemical composition of 5 samples differing by their mode of extraction or coming from different regions was compared. The study of the physicochemical characteristics of the 5 samples showed that the roasting of the almonds of the Argan fruit as a parameter can increase the value of the peroxide index, decrease the percentage of α-tocopherol and the unsaponifiable rates in percentage. Also it found that geographic origin can influence fatty acid values (behenic acid, C22:0). The results of the specific extinction and the refractive index did not give any precise information on the origin, the altitude and the method of extraction of Argan oil. The study of the triglyceride fraction showed that the geographical origin of northeastern Morocco can increase the value of triglyceride. Present study has indicated that the high quality of Argan oil can be extracted by mechanical pressing and hence, the present results may support the commercialization of Argan oil.
Keywords: Argan, Chemical composition, Extraction method, Nutritional value, Sapotaceae.

[Full text-PDF]

Short Communication

Maternal immunoglobulin in the serum of newborn lambs and its relation with neonatal mortality.


Demis Ch, Aydefruhim D, Wondifra Y, Ayele F, Alemnew E and Asfaw T (2020). **Maternal immunoglobulin in the serum of newborn lambs and its relation with neonatal mortality**. *Online J. Anim. Feed Res.*, 10(3): 119-124. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr16

Demis Ch, Aydefruhim D, Wondifra Y, Ayele F, Alemnew E and Asfaw T.
Online J. Anim. Feed Res., 10(3): 119-124, 2020; pii: S222877012000016-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr16

Abstract
The study was conducted on 153 neonatal lambs of one of the highland breeds of sheep, locally called "Menz sheep" in North-Eastern part of Ethiopia, with the aim of assessing the relationship of total serum immunoglobulin level and neonatal lamb mortality in the first one month of life. The overall mortality in neonates was 8.5%. Surviving lambs (2.43±0.35 kg) were significantly heavier than those that died during the neonatal period (2.21±0.55 kg). Males (2.45±0.31 kg) were significantly heavier than females (2.37±0.43 kg). The lambs that survived the neonatal period had a significantly higher level of immunoglobulin (31.71±12.88 Zinc Sulphate Turbidity units) than those that died (12.77±5.25 Zinc Sulphate Turbidity units). Neonatal lambs with total serum immunoglobulin levels below 12 Zinc Sulphate Turbidity units may be considered as an indication of failure of passive transfer of colostrum immunoglobulins and consequently increased the susceptibility of lambs to diseases and subsequent deaths. The neonatal lambs with lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 Zinc Sulphate Turbidity units) had found dead before the first 30 days of their age. Most deaths of lambs occur in the first few days of birth that are typically associated with lower birth weight which also led to weakness, taking longer time to stand up and reduced chance of survival than lambs of heavier weight. Hence, several works have to be done to further improving the birth weight of newborn lambs as well as the nutritional status of the dam so that lambs may receive sufficient and good quality amount of colostrum from the first few hours of birth.
Keywords: Colostrum, Immunoglobulins, Neonatal lamb, Mortality.

[Full text-PDF]

Research Paper

The effects of broiler feed forms on metabolic and skeletal disorders.


Kuleile N, Ncheche Kh, Kamoho S, Macheli T, Jobo T, Phororo M (2020). **The effects of broiler feed forms on metabolic and skeletal disorders**. *Online J. Anim. Feed Res.*, 10(3): 125-130. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr17

Kuleile N, Ncheche Kh, Kamoho S, Macheli T, Jobo T, Phororo M.
Online J. Anim. Feed Res., 10(3): 125-130, 2020; pii: S222877012000017-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr17

Abstract
A completely randomized study was conducted at the National University of Lesotho farm (altitude 1650 meters) to address the high incidence of

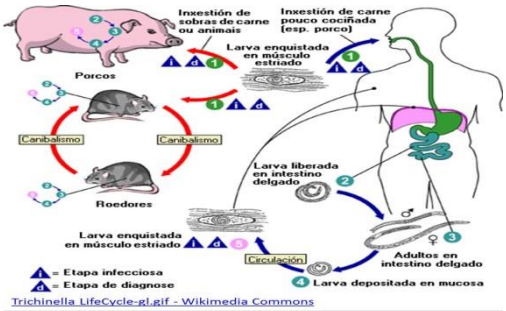
metabolic and skeletal disorders in broiler chickens. The incidence of ascites also increases significantly at altitudes greater than 1300 meters above sea level, presumably because of the low oxygen partial pressure. The ascites incidences are very high in Lesotho during the cold winter months, accounting for more than fifty percent of the total mortality. The main objective of the current study was to assess the effect of different feed forms on the occurrence and control of metabolic disorders in broilers. A total of 200 day-old Ross 308 chicks were randomly distributed into two dietary treatments made up of two broiler feed forms namely mash and pelleted diet replicated four times with twenty-five birds per replicate. The two dietary treatments had similar nutritive value across all feeding phases with exception of feed form. Chicks were housed in a well-ventilated house where treatment diets and water were offered on ad libitum basis. Data collection was done on weekly basis for production parameters such as feed intake, feed conversion ratio, live weight and growth rate while mortality, signs of ascites, lameness and Sudden Death Syndrome (SDS) data were collected daily. All dead birds were examined for the signs of ascites by presence or accumulation of fluids in the abdominal cavity. The findings of the current study indicated that dietary treatment had a significant ($P< 0.05$) influence on all production parameters namely feed intake, live weight, growth rate, feed conversion ratio and mortality rate. The dietary treatment also had a significant effect on incidences of ascites and lameness in broiler chickens whereby birds offered diet in the form of pellets had better production performance and higher incidences of the ascites, lameness and mortality than birds fed diet in mash form. On the other hand the dietary treatments did not have a significant ($P> 0.05$) effect on SDS. However, there were more incidences of SDS in birds offered pelleted diets than mash diet. Birds fed mash diet had fewer incidences because they were experiencing moderate growth rates compared to birds fed pelleted diet with fast growth rates. Birds offered mash spend more time consuming their feed compared to birds fed pellets and therefore, expend more energy in this process resulting in lower feed conversion efficiency. It was evident from the results that diet in mash form can be used to control the incidences of metabolic disorder by reducing growth rates of broilers.
Keywords: Form, Ascites, Mash, Pellets, Growth Mortality

[Full text-PDF]

Review

Epidemiology, diagnosis and public health importance of Trichinellosis.

Yayeh M, Yadesa G , Erara M, Fantahun S, Gebru A and Birhan M.
Online J. Anim. Feed Res., 10(3): 131-139, 2020; pii: S222877012000018-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr18



Trichinella LifeCycle-gl.gif - Wikimedia Commons

Yayeh M, Yadesa G , Erara M, Fantahun S, Gebru A and Birhan M (2020). **Epidemiology, diagnosis and public health importance of Trichinellosis.** *Online J. Anim. Feed Res.*, 10(3): 131-139. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr18

Abstract
Trichinellosis is a parasitic zoonosis caused by Trichinella following ingestion of raw or under cooked meat containing *Trichinella* larvae. Nematode worms of the genus *Trichinella* are one of the most prevalent zoonotic pathogens in the world. The parasite infects domestic and wild animals and has a worldwide distribution. The life cycle of the parasite consists of a domestic cycle in mainly pigs and a sylvatic cycle in a wider range of animals such as bears and wild boar. Humans become infected after eating raw or undercooked meat from domestic pigs, horses or game containing Trichinella larvae. There are twelve genotypes within the genus *Trichinella*, eight of which have been designated as species from which *T.spiralis* is the most pathogenic one. Host animals ingesting even high numbers of *Trichinella* larvae from infectious meat will not develop clinical symptoms. In humans, the clinical picture is usually illustrated by an intestinal stage within the first or second week after infection and later muscular stage with periorbital oedema, myalgia or muscle weakness as the major symptoms. The severity of the clinical course depends firstly on parasitic factors, such as the species implicated and the number of infective larvae ingested, and secondly on host factors, such as sex, age, and immune status. In practice, treatment with anthelmintics and immunosuppressive drugs is used only with human patients, not with animals. *Trichinella* infection in humans is strongly associated with the consumption of raw or undercooked meat; thus, cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products.
Keyword: Human, Parasite, Pig, raw meat, Trichinellosis; Zoonosis

[Full text-PDF]

Short Communication

Participatory evaluation of improved feed technologies to enhance small ruminant fattening on pastoralist research group (PRG) members in Chifra district of Afar national regional state.

Nuru M and Yasin M.
Online J. Anim. Feed Res., 10(3): 140-143, 2020; pii: S222877012000019-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr19

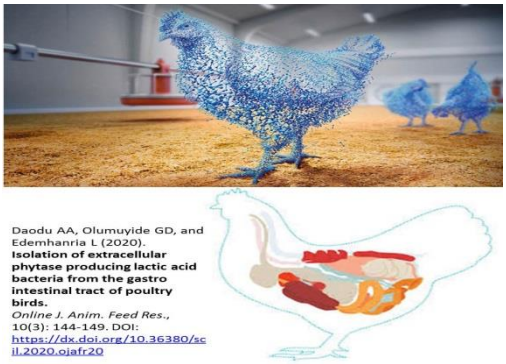
Nuru M and Yasin M, (2020). **Participatory Evaluation of Improved Feed Technologies to Enhance Small Ruminant Fattening on Pastoralist Research Group (PRG) members in Chifra District of Afar National Regional State.** *Online J. Anim. Feed Res.*, 10(3): 140-143. DOI: https://dx.doi.org/10.36380/scil.2020.ojafr19

Abstract
The causes for low productivity of sheep and goat include poor veterinary services, inadequate quantity and quality of feed. Among these limiting factors, poor feed supply and feeding system is the most important. The objectives of this

project were to demonstrate and evaluate Urea Molasses Multi-Nutrient Blocks (UMMNB) and concentrates mix feed technologies in participatory manner through Pastoralist Research Group (PRG) approach and look in to the perception and opinions of agro-pastoralist to the new feed and feeding techniques. The PRG has 25 members and was established a year ago. Among the PRG members, 6 trial agro-pastoralists were selected by the PRG members purposefully to implement the experiment. A total of 36 small ruminants (sheep and goat) were used for the trial. Training on UMMB preparation, formulation of concentrate feed, animal management and data collection were given for PRG members and Development Agents (DAs). The demonstration and evaluation trial were lasts for 4 months data collection period. Data were collected by the trial PRG agro-pastoralist throughout the trial period with close follow up of DAs and woreda experts. For data analysis purpose the researchers used descriptive statistics. The results showed that the final body weight and daily body weight gain was higher in grazing when supplemented with concentrates mix (Treatment 3) in compared to grazing + urea molasses block supplementation (T2) and control one/free grazing (T1). The partial budget analysis also indicated that small ruminant daily weight gain for 4 months with supplementation of concentrate mix supplemented groups (T3) was found to give on average economic return of 250 Ethiopian Birr (ETB) or 6.70 Euro per head. From this study, it can be concluded that the supplementation concentrate mix for small ruminants (sheep and goat) has better weight gain and economically feasible for the chifera district PRG established in 2017. It was also economical at PRG level, with average gross profit of 250 ETB per head in chifera area. Because of this, so money agro-pastoralists has been attracted by the business and engaged in small ruminant fattening practices by purchasing animals from market place without external advice and support.
Keywords: Small ruminant, Urea molasses block, Feed technology, Pastoralist research group

[Full text-PDF]

Research Paper



Isolation of extracellular phytase producing lactic acid bacteria from the gastro intestinal tract of poultry birds.

Daodu AA, Olumuyide GD, and Edemhanria L.
Online J. Anim. Feed Res., 10(3): 144-149, 2020; pii: S222877012000020-10
DOI: https://dx.doi.org/10.36380/scil.2020.ojafr20

Abstract
Bacterial phytases and phytase-producing bacteria are of great industrial significance in the poultry industry and also in phosphorus pollution management. This study was designed to isolate and screen for phytase producing lactic acid bacteria from the duodenum, ileum and cecum of eight healthy cockerel samples. Standard microbiological procedures were followed to isolate phytase producing lactic acid bacteria using de Man Rogosa and Sharp (MRS) agar while extracellular phytase screening was done using phytase specific medium. The range of total microbial count obtain was highest at the cecum (2.85±0.11 to 4.34±0.12 $\log_{10}$ cfu/ml), lower at the duodenum (2.02±0.11 to 4.27±0.20 $\log_{10}$ cfu/ml) and lowest at the ileum (2.00±0.21 to 4.19±0.25 $\log_{10}$ cfu/ml). Nineteen bacterial isolates were identified as lactic acid bacteria on the basis of morphological, biochemical and physiological characterization and later identified as *Lactobacillus* species (78.94%), *Enterococcus* species (15.78%) and *Lactococcus* species (5.26%). Thirteen out of the nineteen lactic acid bacteria showed phytase activity. Low phytase activity was observed in eight of the lactic acid bacteria isolates while five of the isolates produced significant extracellular phytase activity (>6mm). The most predominant *Lactobacillus* species were also found to be the most potent phytase producers. This can be exploited for industrial production of phytase in upgrading the nutritional status of feed and combating phosphorus pollution from poultry waste.
Keywords: Phytase, Gastrointestinal tract, Lactic acid bacteria, Phosphorus pollution, Poultry industry.

[Full text-PDF]

Archive

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EFFECT OF PELLETED BROWSE-BASED FEED WITH A BASAL DIET OF *Andropogon gayanus* FOR SHEEP ON INTAKE, NUTRIENT DIGESTIBILITY AND SOME HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PARAMETERS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The study was designed to evaluate the nutritional quality of pelleted diets based on four of the major feed resources fed to small ruminants by farmers in the Accra Plains. Leaves of *Samanea saman, Acacia auriculiformis* and *Ficus exasperata* and cassava peels were dried, mixed with other ingredients and pelleted. A preference trial showed sheep accepted all the four supplements with a marked preference for cassava peels-based (CP-B) and *Samanea saman*-based (SL-B) supplements compared with *Acacia auriculiformis*-based (AL-B) and *Ficus exasperata*-based (FL-B) supplements (P<0.05). The supplements were subsequently fed to Eight West African Dwarf sheep on a basal diet of *Andropogon gayanus* (Gamba grass) hay in Latin square design. Dry matter intakes (DMI) did not differ by the type of supplement (P>0.05). However, crude protein intake (CPI) was higher (P<0.05) in sheep fed AL-B and FL-B than those fed SL-B and CP-B. Dry matter and neutral detergent fibre (NDF) digestibility were lowest (P<0.05) for sheep fed CP-B. Dietary treatments did not affect haematological parameters, except for neutrophil percentage which was higher (P<0.05) in sheep fed CP-B than those fed SL-B. Animals fed CP-B had the lowest monocyte concentrations (P<0.05). Furthermore, all the serum biochemical parameters were not affected by dietary treatment except total protein concentration which was highest (P<0.05) in sheep fed on AL-B. It is concluded that the feed resources available to the small ruminant keepers can be used to prepare pelleted supplements that are acceptable to sheep and help sustain appreciable performance on low quality forages during the dry season without any deleterious effects on intake, digestibility, physiology and health.

Keywords: Acceptability, Browse plants, Dry season, Accra Plains, Blood parameters, Feed intake

Abbreviations: AL-B: *Acacia auriculiformis*-based supplement; ADF: Acid detergent fibre; ADFD: Acid detergent fibre digestibility; ADFI: Acid detergent fibre intake; ANOVA: Analysis of variance; AOAC: Association of Official Analytical chemists; CP: crude protein; CPD: crude protein digestibility: CPI: crude protein intake; CP-B: cassava peel-based supplement; DM: dry matter; DMD: dry matter digestibility; DMI: dry matter intake; FCE: feed conversion efficiency; FL-B: *Ficus exasperata*-based supplement; LIPREC: Livestock and Poultry Research Centre; MCV: mean corpuscular volume; MCH: mean corpuscular hydrogen; MCHC: mean corpuscular hydrogen concentration; NDF: Neutral detergent fibre; NDFD: neutral detergent fibre digestibility; NDFI: neutral detergent fibre intake OM: organic matter: OMD: organic matter digestibility; OMI: organic matter intake; PCV: packed cell volume; RBC: red blood cell; SEM: standard error of mean; SL-B: *Samanea saman*-based supplement; T. Cholesterol: total cholesterol; WAD: West African Dwarf; WBC: white blood cell

INTRODUCTION

In the savannah areas of West Africa, where most livestock in the sub region are kept, the dry seasons are much longer than in the humid areas and are characterised by declines in forage availability and quality. For both cultivated pastures (Olanite et al., 2004) and natural pastures (Adjorlolo, 2014) forage biomass have been shown to decline drastically in the dry season. Other studies have indicated significant decreases in forage quality during the dry season. Even for forage legumes, decreases in crude protein content to as low as 5-7% (Peters et al., 1997) and increases in neutral detergent fibre (Fujihara et al., 2004) during the dry season have been reported. Supplementation, either to increase the dry matter intake or to increase crude protein intake is often necessary for maintenance and possibly production. The use of fodder tree and shrub leaves as supplement is widely practiced by farmers in Ghana. However, during the late dry season, many trees shed their leaves and availability of tree leaves decline. Many small ruminant keepers resort to buying agro-industrial by-products such as wheat bran, rice bran and cassava peels from processing facilities for supplementary feeding.

An earlier study (Nsoh, 2019) identified feed resources commonly used by small ruminant keepers in the Accra Plains. This study sought to use four of the most important feed resources identified to develop pelleted multi-nutrient feed supplements with long shelf life, which can be stored and fed anytime during the year. It therefore assessed the effects of supplementary feed packages based on three browses and cassava peels on intake, metabolism and physiology of the West African Dwarf sheep.

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MATERIALS AND METHODS

Study area

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

Experimental animals and their management

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

Preparation of experimental diets

Three browse plants, *Ficus exasperata, Samanea saman* and *Acacia auriculiformis*, and cassava peels were identified in an earlier study (Nsoh, 2019) as the most important feed resources used in small ruminant feeding in the Accra Plains. These were selected for evaluation. Leaves of the browses were harvested from trees around LIPREC. The leaves as well as cassava peels, which was bought from cassava processors were shade dried for four to six days and ground in a hammer mill (1 mm screen) into meals. The meals were each mixed with conventional feed ingredients and micro-nutrients and pelleted (Table 1).

Table 1 - Ingredient composition of supplements used in the acceptability study

Ingredients: (g/kg) \ Supplements	SL-B	AL-B	FL-B	CP-B
Maize	159	124	165	0
Wheat bran	120	135	108	650
Mineral salt	5	5	5	5
Dicalcium phosphate	5	5	5	5
Sulphate of ammonia	5	5	5	5
Urea	6	26	12	15
Cassava peels	0	0	0	320
Samanea saman	700	0	0	0
Acacia auriculiformis	0	700	0	0
Ficus exasperata	0	0	700	0
Total (Kg)	1000	1000	1000	1000
Crude protein (calculated)	160.6	160.1	160.7	160.7

CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements

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

Treatment 1 (SL-B) = Gamba grass hay + *Samanea saman* leaf meal-based supplement
Treatment 2 (AL-B) = Gamba grass hay + *Acacia auriculiformis* leaf meal-based supplement
Treatment 3 (FL-B) = Gamba grass hay + *Ficus exasperata* leaf meal-based supplement
Treatment 4 (CP-B) = Gamba grass hay + Cassava peel meal-based supplement

Preference study

Four female sheep with an average live weight of 13.7±1.5 kg were used for this trial. Each animal was penned individually and given free access to fresh water. Each sheep was offered the four supplements in a cafeteria style at 08:00 hours each day and were allowed one hour to select. After the one hour, the refusal was deducted from the feed offered to determine the amount of each supplement consumed. The *Andropogon gayanus* hay which acted as the basal diet, was then offered *ad libitum*. An adjustment period of 14 days was followed by a data collection period of seven days.

Voluntary feed intake and digestibility study

Eight female sheep with an average initial body weight of 14.9±1.5 kg were randomly allotted to four experimental diets in a replicated Latin square design. Animals on each treatment were offered *Andropogon gayanus* hay as the basal feed and supplemented with either SL-B, AL-B, FL-B or CP-B. A daily supplement allowance of approximately 25% of voluntary intake was offered as single meals at 08:00 hours followed by the grass hay offered *ad libitum*. An adjustment

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period of 14 days was followed by 74 days of data collection. Feed intake was determined daily as the difference between weight of feed offered and refusals. Rectal faecal samples were taken from each animal and bulked for each sheep for six days during the feed intake trial. The faecal samples were stored in a refrigerator. The faecal samples were then oven dried at 55℃ to a constant weight for dry matter (DM) determination. The dried faeces were ground using a laboratory mill through 1mm sieve and bagged for subsequent analysis.

Apparent digestibility (AD) of dry matter and other fractions of the feed were calculated as:

AD (%) = 100 – $(100 * (\frac{Lignin\ in\ feed}{Faecal\ lignin}) \times (\frac{Faecal\ lignin}{Total\ dry\ matter\ intake}))$ (de Oliveira et al., 2012)

Lignin was used as internal marker.

Chemical analysis of feed and faeces

Dry matter, organic matter, crude protein, and ash for the feed and faeces were determined using the method of AOAC (2004). Neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, cellulose, hemicellulose and silica were determined according to Van Soest et al. (1991).

Blood sampling

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

Haematological analysis

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

Total RBC count was determined using the formula given by Samour (2006): RBC (10^{12}/L)= $\frac{N}{100}$, Where: L= Litre; N= Number of cells counted in 160 small squares.

The total WBC counts was estimated using the formula given by Campbell (1994): WBC (10^9/L) = $\frac{N \times 10 \times 200}{9}$, Where: L= litre; N= number of cells counted in nine small squares

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

MCV (fL)= $\left(\frac{PVC}{RBC}\right)$ x 10; MCH (pg)= $(\frac{Hb}{RBC})$ x 10; MCHC (%)= $(\frac{Hb}{PCV})$ x 100

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

Blood biochemical analysis

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

Statistical analyses

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

RESULTS

Chemical composition of feed ingredients and supplements

The chemical composition of the basal diet (Gamba grass hay), the three browses *(Samanea saman, Acacia auriculiformis* and *Ficus exasperata*) and cassava peels are presented in Table 2. The basal diet, the leaf meals of the three browses and cassava peels had comparable dry matter contents (range 89.9 to 94.6%) and organic matter (range 80.8 to 87.2%) contents. The chemical composition of the supplements are shown in Table 3. The dry matter, organic

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matter, NDF and lignin contents were similar. FL-B had the highest crude protein content (21.5%) while CP-B had the least (16.3%) crude protein but highest ADF.

Preference of sheep for the pelleted supplements

The sheep accepted all the supplements but preferred (P<0.05) SL-B and CP-B to the rest. The supplement least preferred (P<0.05) was FL-B. The preference of sheep for the three browses and cassava peel meal supplements is shown in Table 4.

Influence of supplements on voluntary intakes in West African Dwarf sheep

The influence of the supplements on voluntary intake in sheep is shown in Table 5. The total dry matter intake was similar (P>0.05) across the treatments. Crude protein intake ranged from 59.63 to 67.01%. Sheep fed AL-B and FL-B had similar crude protein intakes but significantly higher (P<0.05) crude protein intake than those fed SL-B and CP-B. Organic matter intake was least (P<0.05) in FL-B Sheep. However, intake of NDF was significantly higher (P<0.05) in sheep fed FL-B than those fed the other treatments. The ADF intake on the other hand was in the range of 25.44 to 54.65g/day and was found to be significantly higher (P<0.05) in sheep fed SL-B and CP-B than those fed AL-B and FL-B. Sheep CP-B had lower (P<0.05) lignin intake than those fed SL-B, AL-B and FL-B.

Digestibility of nutrients by West African Dwarf sheep

Dry matter digestibility was lowest (P<0.05) in sheep fed CP-B (Table 6). SL-B had the highest dry matter digestibility value of 62.35% and this was significantly (P<0.05) higher than the digestibility of 60.33% for AL-B. The crude protein digestibility followed a similar pattern as dry matter digestibility. The organic matter digestibility in this study ranged from 46.31 to 52.25%. Sheep fed SL-B had the highest (P<0.01) organic matter digestibility. Also, sheep fed FL-B had higher (P<0.05) organic matter digestibility than those fed AL-B and CP-B. The NDF digestibility in this study ranged from 34.9 for CP-B to 41.57% for FL-B. NDF digestibility was similar (P>0.05) for sheep fed SL-B and FL-B, but both were higher (P<0.05). The ADF digestibility in this study ranged from 22.30 to 33.47%. The ADF digestibility was similar (P>0.05) in sheep fed SL-B and CP-B, but both were higher (P<0.05) than for AL-B and FL-B.

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

Fraction (%)	Andropogon hay	Samanea	Acacia	Ficus	Cassava Peels
Dry matter	89.9	92.7	93.4	91.9	94.6
Crude protein	6.7	21.9	16.4	15.9	2.1
Organic matter	80.8	83.8	87.2	87.2	80.9
Neutral detergent fibre	73.8	59.8	60.7	42.9	36.3
Acid detergent fibre	44.9	39.7	49.5	36.4	27.4
Lignin	6.1	6.8	6.2	3.7	9.7
Total ash	12.6	8.9	6.2	3.7	7.3

Table 3 - Chemical composition of the experimental supplements

Fraction (%)	Supplement (%)			
	SL-B	AL-B	FL-B	CP-B
Dry matter	92.5	91.7	90.2	91.2
Crude protein	18.3	20.5	21.5	16.3
Organic matter	85.9	84.7	83.7	84.8
Neutral detergent fibre	41.6	44.5	43.4	41.3
Acid detergent fibre	30.2	29.6	14.6	30.6
Lignin	3.8	4.7	4.5	3.4

CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B*: Acacia auriculiformis*-based; FL-B*: Ficus exasperata*-based supplements

Table 4 - Preference of West African Dwarf sheep for the supplements

Supplements	Means of intake (g)
CP-B	223.3[a]
SL-B	195.8[a]
AL-B	111.3[b]
FL-B	57.6[c]
SEM	*24.16*
P-Value	*<0.001*

[a,b,c.] Means within a column with different superscripts differ significantly at P<0.05.

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Table 5 - Influence of supplements on voluntary intakes in West African Dwarf sheep

Parameter (g/day) \ Supplements	SL-B	AL-B	FL-B	CP-B	*SEM*	*P-value*
Dry matter intake	649.5	636.8	629.6	653.3	11.52	0.143
Crude protein intake	63.69^{b}	67.01^{a}	66.32^{a}	59.63^{c}	1.277	< 0.001
Organic matter intake	533.6^{a}	520.9^{a}	497.3^{b}	534.4^{a}	9.40	< 0.001
Neutral detergent fibre intake	74.36^{b}	76.75^{b}	85.98^{a}	72.98^{b}	2.042	< 0.001
Acid detergent fibre intake	54.65^{a}	50.81^{b}	25.44^{c}	53.79^{a}	1.158	< 0.001
Lignin intake	35.49^{a}	36.56^{a}	36.17^{a}	34.14^{b}	0.629	< 0.001

^{a,b,c,}Means within a row with different superscripts differ significantly at P<0.05.; CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements.

Table 6 - Digestibility of components of feed as influenced by supplementation (%)

Fraction (%) \ Supplements	SL-B	AL-B	FL-B	CP-B	*SEM*	*P-value*
Dry matter	62.35^{a}	60.33^{b}	61.22^{ab}	57.10^{c}	0.655	<0.001
Crude protein	57.25^{a}	56.30^{b}	56.25^{ab}	51.10^{c}	0.553	<0.001
Organic matter	52.25^{a}	47.22^{c}	49.43^{b}	46.31^{c}	0.892	<0.001
Neutral detergent fibre	40.62^{a}	36.29^{b}	41.57^{a}	34.90^{b}	0.993	<0.001
Acid detergent fibre	32.87^{a}	22.30^{c}	25.64^{b}	33.47^{a}	1.034	<0.001

^{a,b,c,} Means within a row with different superscripts differ significantly at P<0.05.; SEM = Standard error of mean. CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements

Haematological and serum biochemical parameters in West African Dwarf sheep

Details of the effects of the supplements on haematological and serum biochemical parameters of sheep are shown in Table 7. There was no significant treatment effect (P>0.05) on most of the haematological parameters measured except neutrophils and monocyte levels. Sheep that were fed the CP-B had significantly (P<0.05) higher neutrophil value than those fed on SL-B. Values for sheep on AL-B and FL-B, however, were not significantly different (P>0.05) from those on SL-B and CP-B. Sheep on SL-B and AL-B had significantly (P=0.05) higher monocyte concentrations than those on CP-B. Dietary treatment did not significantly (P>0.05) affect all the serum biochemical parameters determined except total protein concentration which was significantly (P<0.05) higher in sheep fed on AL-B than those fed on SL-B, FL-B and CP-B. Generally, the concentrations of most of the haematological and serum biochemical parameters remained relatively stable and showed similar trends across dietary treatments during the period of study (Figures 1 and 2).

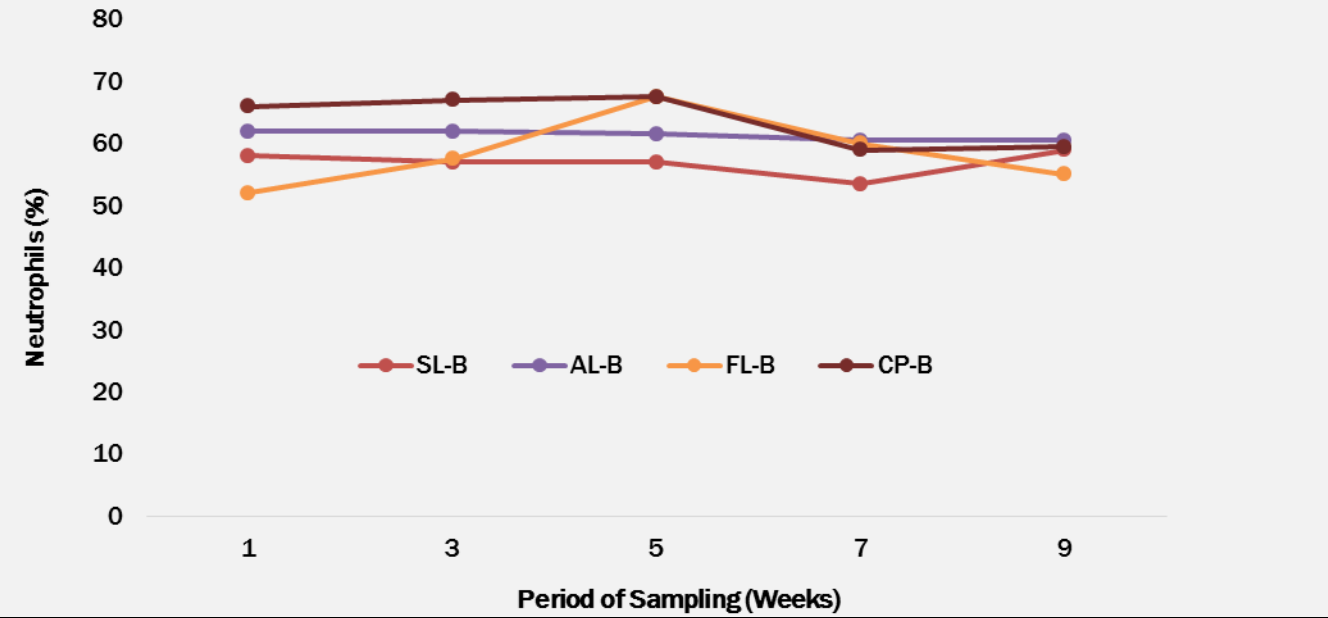

Figure 1 - Changes in neutrophil concentration in West African Dwarf Sheep. CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements

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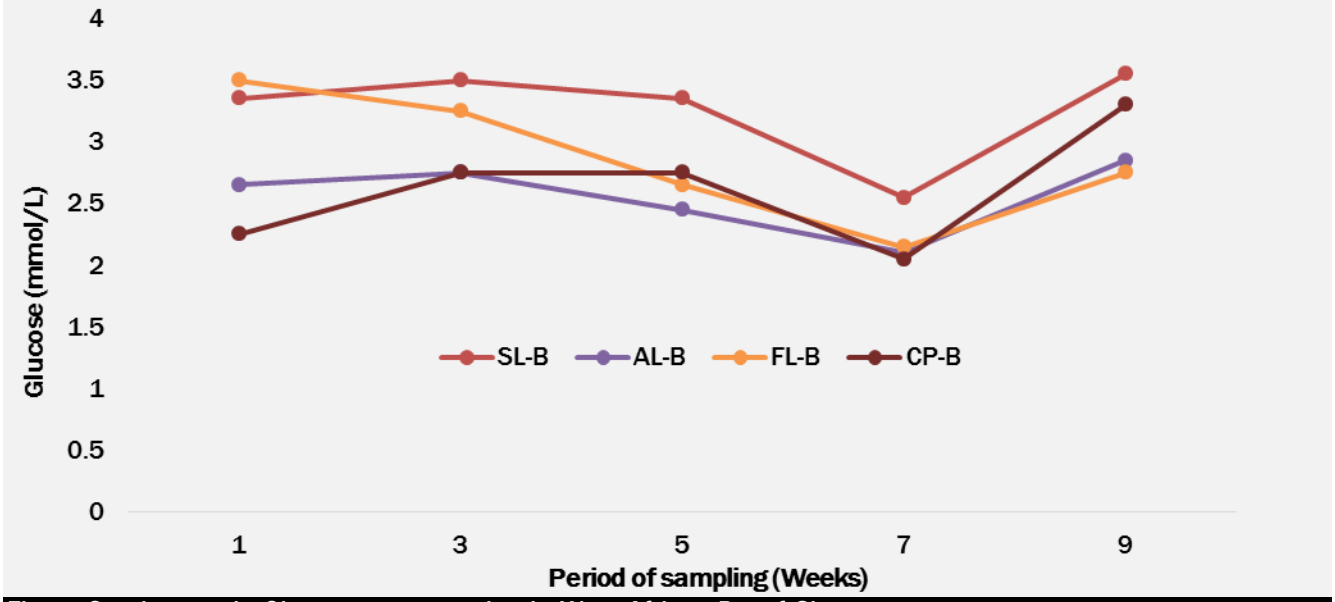

Figure 2 - changes in Glucose concentration in West African Dwarf Sheep. CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements

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

Parameters	Treatments				SEM	P-value
	SL-B	AL-B	FL-B	CP-B		
Haematological Indices						
Haemoglobin (g/dL)	13.27	15.27	15.33	15.46	1.01	0.162
PCV (%)	29.20	33.60	32.80	35.60	2.05	0.291
RBC (x10^{12}g/L)	10.63	11.94	11.80	11.24	0.66	0.160
MCV (fL)	27.80	27.87	28.73	29.45	0.14	0.577
MCH (pg)	12.40	12.87	13.26	13.92	0.07	0.157
MCHC (g/dL)	45.44	45.24	46.74	43.43	0.15	0.053
WBC(x10^{9}/L)	4.37	4.96	4.34	4.87	0.39	0.641
Neutrophils (%)	56.90^{b}	61.30^{ab}	58.40^{ab}	63.80^{a}	4.37	0.037
Lymphocyte (%)	39.20	34.60	39.30	34.70	4.10	0.487
Eosinophils (%)	1.60	1.50	0.80	0.80	0.80	0.625
Monocytes (%)	2.20^{a}	2.30^{a}	0.90^{ab}	0.50^{b}	0.73	0.50
Basophils (%)	0.09	0.11	0.22	0.18	0.10	0.596
Serum Biochemical Indices						
Glucose (mmol/L)	1.96	1.54	1.72	1.57	0.20	0.108
Total protein (g/L)	60.46^{b}	64.38^{a}	61.48^{b}	61.57^{b}	0.65	0.030
Albumen (g/L)	36.96	38.43	37.07	37.37	0.78	0.374
Globulin (g/L)	23.50	25.95	24.41	24.14	1.23	0.388
T. cholesterol (mmol/L)	1.40	1.50	1.48	1.79	2.87	0.497
Urea (mmol/L)	9.51	9.67	8.71	9.21	0.36	0.492

^{a,b} Means within a row with different superscripts differ significantly at P<0.05.; T.cholesterol = total cholesterol. CP-B: cassava peels-based; and SL-B: *Samanea saman*-based; AL-B: *Acacia auriculiformis*-based; FL-B: *Ficus exasperata*-based supplements

DISCUSSION

All the supplements were acceptable but SL-B and CP-B had the highest preference by sheep. A number of factors may influence acceptability of feed by small ruminants. Provenza and Cincotta (1994) reported that plant physical structure and chemical composition are the most vital factors that influence preference for feed. Oldham and Alderman (1982) reported that *ad libitum* intake by animals is increased by an increase in crude protein content of diets. However, in this study, no association between crude protein concentration and preference could be established.

The similarity in dry matter intakes may suggest that the supplements stimulated intake of the basal diets to similar extents despite the differences in the crude protein concentrations. The higher crude protein intake in sheep fed AL-B and FL-B compared to the SL-B and CP-B could be attributed to higher crude protein concentration of the supplements of AL-B and FL-B. High crude protein intake makes available nitrogen needed to improve the rumen eco-system and increase the animal's ability to digest fibrous portions of feed. Odedire and Oloidi (2014) reported a decrease in crude protein intake

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due to reduced palatability of the diet when West African Dwarf goats were fed supplements containing increasing levels of wild sunflower. In the current study however, dry matter intakes were similar. The lower organic matter intake in sheep fed FL-B could be attributed to the high levels of anti-nutritional factors, such as alkaloids, saponins, cyanogenic glycosides and tannins contained in that supplement that could potentially have adverse effects on nutrient utilization as reported by Ljeh and Ukwemi (2007). The higher NDF intake of sheep fed FL-B could be attributed to the higher crude protein level in FL-B (21.5%) which could have improved rumen environment aiding rumen microbial fermentation thereby increasing dry matter intake and consequently, NDF intake. The high intake of ADF in sheep fed SL-B and CP-B may be due to moderate quantities of antinutritional factors in SL-B (Obasi et al., 2010) and tolerable levels of cyanogenic glycosides in CP-B which might not have adversely influenced the rumen environment but rather aided in ADF digestion thereby increasing its intake. The lower lignin intake in sheep fed CP-B compared to those fed SL-B, AL-B and FL-B could be attributed to an imbalance or inadequacy of nutrients especially crude protein intake in sheep fed CP-B which might have resulted in reduced rumen ammonia production and microbial growth and activity. This could indirectly slow down the rates of digestion and passage and subsequently reduce intake as reported by Preston and Leng (1987).

The lower dry matter digestibility in sheep fed CP-B compared to the other treatments could be attributed to lower crude protein intake of this supplement. Also, anti-nutritional factors such as cyanogenic glycosides in the cassava peels might have slowed down microbial action and thereby decreased dry matter digestibility. Anti-nutritional factors are known to interfere with normal digestion, metabolism and absorption of nutrients (Gilani et al., 2005). The leaves of forages are high in readily degradable nitrogen as reported by NRC (2000) and some by-pass protein. Inclusion of such browses in ruminant diets cause faster fermentation rate and substrate degradation hence increasing dry matter intake. The higher crude protein intake of sheep fed SL-B, AL-B and FL-B over CP-B could have enhanced the digestibility of crude protein in these supplements than the CP-B. The presence of cyanogenic glycosides in the cassava peel meal supplement could have inhibited the effective digestion of protein by the rumen microbes. The leaves of forages are high in readily degradable nitrogen as reported by NRC (2000) and some by-pass protein. Inclusion of such browses in ruminant diets will cause faster fermentation rate and substrate degradation hence increasing dry matter intake.

The high digestibility of organic matter of sheep fed SL-B and FL-B could be due to the provision of adequate nutrients to the rumen microbes with consequent improvement in organic matter intake whilst higher levels of flavonoids and triterpenoids in AL-B and cyanogenic glycosides in CP-B adversely affected rumen microbial activity resulting in lower organic matter digestibility. Also, the lower crude protein digestibility in sheep fed CP-B may account for their lowest organic matter digestibility.

The high NDF digestibility in sheep fed SL-B and FL-B is likely due to moderate concentrations of secondary metabolites in the Samanea and Ficus leaf meals that might have had positive influence on rumen microbes as several researchers have reported secondary metabolites having positive impacts on rumen fermentation due to their low or moderate concentrations (Jiménez-Peralta et al., 2011; Salem et al., 2014). The low crude protein level in CP-B could have inhibited rumen activity thus decreasing digestibility of NDF of sheep fed that diet. However, ADF digestibility in sheep fed CP-B was higher probably as a result of low lignin contents in CP-B compared with the others.

Haematological and blood biochemical indices provide useful information on the physiological status of animals and hence serve as a tool in determining normal healthy state of animals (Onasanya et al., 2015). The similar concentrations of the haematological parameters in most of the test diets suggest that the inclusion of the supplements did not have adverse or detrimental effects on the health of the sheep. This suggests the quality of the supplementary diets were good to help sustain growth of sheep during periods when animals have to rely of poor quality fodder. The haemoglobin and PCV levels of 13.27 to 15.46 g/dL and 29.20 to 35.60% respectively obtained in the present study were within the normal physiological range of 9 to 15 g/dL and 27 to 45% respectively reported for sheep (The Merck Veterinary Manual, 2010). This suggests similar ability of the dietary treatments in augmenting the production of haemoglobin and RBCs for efficient transportation of gases during respiration. Konlan et al. (2012) and Dougba (2017) in earlier studies reported haemoglobin and PVC ranges of 12.41 to 13.60 g/dL and 27.45 to 29.43% respectively for the same breed of sheep fed diets containing various agro-industrial by-products. Total RBC counts range of values (10.63 to 11.94 x 10^{12} g/L) was within the normal physiological range of 9 to 15 x 10^{12} g/L reported for sheep (The Merck Veterinary Manual, 2010) indicating the efficient synthesis of RBCs across the dietary treatments. The MCV, MCH and MCHC values obtained in the present study were also comparable to the normal physiological range for sheep. The total WBC counts (4.34 x 10^9 to 4.96 x 10^9 g/L obtained in the present study were within the normal range of 4 x 10^9 to 12 x 109/L reported for sheep (The Merck Veterinary Manual, 2010). This suggests the test diets supplied enough nutrients for the production of WBCs to adequately defend the body against infections. Konlan et al. (2012) reported a range of 8.37 x 10^9 to 9.30 x 10^9 for the West African Dwarf sheep fed a basal diet of rice straw and groundnut haulms with graded levels of shea-nut cake supplement. Also, the WBC differential counts across dietary treatments were within the normal ranges reported for sheep (The Merck Veterinary Manual, 2010). This suggests similar ability of the sheep to fight infection when fed the supplements. The distribution of WBC observed in the present study were comparable with the range of values reported for the same breed of sheep by Baiden and Obese (2010) and Konlan et al. (2012).

The nonsignificant difference in the concentrations of most of the blood biochemical parameters across the dietary treatments suggest that the inclusion of leaf meal supplements based on Samanea, Acacia, Ficus, and Cassava peel meal-based supplements did not have adverse effects on the physiology of the West African Dwarf sheep. The similar concentration of serum glucose across dietary treatments suggest the inclusion of the browse species leaf meal and

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cassava peel-based supplements did not adversely deprived the sheep of energy for metabolic activities. The range of values (1.54 to 1.96 mmol/L) obtained in the present study was however, lower than the 2.85 to 3.10 mmol/L reported for West Africa Dwarf sheep fed basal diet of rice straw and supplemented with varying levels of neem leaf meal concentrate diets (Dougba, 2017). Serum concentrations of total protein, albumin and globulin serve as indicators of protein status (Ndlovu et al., 2007). Also, circulating concentrations of globulin usually give indication of an animal's immune state and its response to fighting diseases and infections (Kapele et al., 2008). The higher crude protein intake for sheep fed AL-B than those fed the other three supplements may account for its high total protein value. The values obtained for total protein concentrations, 60.46 to 64.38 g/L were within the normal physiological range of 59 to 78 g/L reported for sheep (The Merck Veterinary Manual, 2010). The total protein concentrations were comparable to the 56.00 to 61.34 g/L reported for the same breed of sheep fed basal diet of rice straw and groundnut haulms with graded levels of shea nut cake concentrate supplement (Konlan et al., 2012), but lower than the 72.3 to 83.3 g/L reported for the same breed of sheep (Dougba, 2017). The age, type of diet fed and physiological state of the sheep used may account for the differences. The concentrations of serum albumin (36.96 to 38.43 g/L) were similar to the reported normal physiological values of 27 to 37 g/L reported for sheep (The Merck Veterinary Manual, 2010). However, globulin concentrations (23.50 to 25.95 g/L) in the present study were lower than the reported normal physiological values of 39 to 60 g/L in sheep (The Merck Veterinary Manual, 2010). The low globulin concentrations in the sheep may indicate low ability of the sheep to resist infections or diseases. All the sheep used in the study were however healthy and did not show any signs of disease throughout the study. The normal and similar total protein and albumin concentrations in sheep fed the various supplements indicates that the inclusion of leaf meal and cassava peel - based supplements did not adversely influence the availability of protein to the sheep, their immune status and ability to fight diseases. The range of values for total cholesterol (1.40 to 1.50 mmol/L) was within the reported normal physiological range of 1.1 to 2.3 mmol/L in sheep (the Merck Veterinary Manual, 2010). However, the concentrations of serum urea (range 8.71 to 9.67mmol/L) in the present study was close to the normal physiological upper range value of 9.3 mmol/L reported for sheep (The Merck Veterinary Manual, 2010), but lower than the values 13.26 to 16.32 mmol/L reported for West African Dwarf sheep fed basal diet of rice straw and supplemented with varying levels of neem leaf meal concentrate diets (Dougba, 2017). The difference may be attributed to the type of diet fed to sheep in these studies.

CONCLUSION

From the above studies, feed resources available to the small ruminant keepers can be used to prepare pelleted supplements that are acceptable to sheep and help improve performance on low quality forages. Feeding these supplements did not adversely affect the health and physiology of sheep as indicated by the blood parameters. These supplements, which have high bulk density and long shelf life, can help prevent the major losses in ruminant production during the dry season.

DECLARATIONS

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

Conflict of interests
The authors have not declared any conflict of interests.

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ASSESSMENT ON DEFECTS OF WET-BLUE HIDE AND PICKLED SKIN AT MODJO TANNERY

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Supporting Information

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ABSTRACT: Across-sectional study was conducted from February to June 2015 with the objectives of identifying the major types of hide and skin defects and determining their prevalence in pickled skins of sheep and wet blue goat skins and hides originated from two districts namely Hitosa and Dodota of East Arsi Zone at the Colba and Gelan tanneries in Modjo town. A total of 389 wet blue cattle hides, 385 wet blue goat skin and 399 pickled sheep skin were examined. The study finding showed that there exist various defects responsible for the decline in quality of skin and hide. The major defects at the wet blue hide were flay cut (59.1%), gouge mark (42.2%), and putrefaction (35.2%). In sheep pickled skin higher percentage of cockle (36.9%), gouge mark (28.3%) and scratch (27.0%) were observed. In wet blue goat skin, cockle (48.1%), veininess (44.6%) and crack (41.9%) were the major defects observed. The prevalence of cockle, veininess, scar, corduroying, crack, gouge mark, poor pattern and brand mark were significantly higher ($P<0.05$) in goat skin at wet blue stage than pickled sheep skin while putrefaction and shoat pox were significantly higher ($P<0.05$) in sheep skin compared to goat wet blue skin. The major defects that leads to rejection of wet blue hide were flay cut while cockle in sheep and goat skin. In pickled sheep skin, grade of 1-3 accounts 14% and grade 4-7 accounts 86% of the total observation. This study showed large proportion of skin and hides were subjected to rejection because of poor quality and this implies that integrated efforts towards improved livestock husbandry and better health care are vital issues for production of better-quality hide and skin. Furthermore, effective extension system and programs that could raise public awareness on ectoparasites and skin diseases control as well as on methods of flaying, preservation and handling of skins should be implemented.

Keywords: Cockle, Fly cut, Grade, Hide, Quality, Rejection, Skin

Abbreviations: CSA: Central Statistical Authority; UNIDO: United Nations Industrial Development Organization; QSAE: Quality Standard Authority of Ethiopia; SPSS: Statistical Package for Social Science; FAO: Food and Agricultural Organization

INTRODUCTION

Ethiopia has 53.4 million cattle, 25.5 million sheep and 22.7 million goats. These numbers illustrate a considerable potential for the leather industry in the country (Central Statistical Authority, CSA, 2011/2012). This places the country as one of the richest countries in livestock resources. It has a huge potential for production of hide and skins. For instance, its potential was estimated at 3.78 million cattle hides, 8.41 million sheep skins and 8.42 million goatskins in 2012/13 (CSA, 2013). This raw material of the leather industry is mainly derived from local areas of the country where basic amenities for slaughtering and subsequent marketing are either not in existence or lacking. Additional sources of hides and skins include slaughter slabs, municipal slaughterhouses and the limited number of export abattoirs. With regard to skin production, except the export abattoirs engaged in the production of chilled mutton and goat meat for export, the contribution of other slaughtering premises in terms of skin supply is very negligible (Ahmed, 2001).

The leather industry is one of the fastest-growing economic sectors in Ethiopia (Abadi, 2000; Bayou, 2007). The 26 operational tanneries in the country have a soaking capacity for 153,650 sheep and goat skins and 9,725 cattle hides per day (United Nations Industrial Development Organization (UNIDO, 2008). Nevertheless, they are not working to full capacity, as the hides and skins become available only when meat is needed and are not supplied for sustained leather processing (Bisrat, 2013).

The leather industry processes raw hides and skins and produces semi-processed and finished leather both for export and for local markets (Abadi, 2000). The semi-processed products are pickled sheep skin, wet blue goat skin and wet blue hides. Pickling denotes to treating unhaired, limed, delimed and bated hides or skins with a solution of salt and acid (e.g. sulphuric acid or formic acid) to preserve them or prepare them for the tanning process. Wet blue skins or hides refer to products that have been chrome tanned but not dried (Quality Standard Authority of Ethiopia, QSAE, 2008).

The leather industry sector is one of the growing Agricultural export commodities in Ethiopia. However, the sector is constrained by different factors like external parasites, inappropriate management of animals, faults during slaughtering

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and improper handling of skin before reaching to the tanneries. Hence the sector is losing large amount of money due to decline in quality and fall in export price (CSA, 2007). Lower quality hides and skins negatively impacts not only tanneries, but also Ethiopian footwear and other leather goods producers who sell their product domestically and abroad. There exists a paucity of research output in identifying pre and post-slaughter hide and skin causes of defects, and the measures to be taken under different agro-ecologies. To date there are no reports on type of defects on processed hides and skins in tanneries that sourced from East Arsi Zone especially Hitosa and Dodota districts which have high potential of livestock production. Therefore, this study was carried with the objective of identifying the major types of hide and skin defects and to determine their prevalence in pickled skins of sheep and wet blue goat skins and hides originated from the two districts of East Arsi Zone at the Colba and Gelan tanneries in Modjo town.

MATERIALS AND METHODS

Study area

The study was carried out at Colba and Gelan tanneries which are found in Modjo town. Modjo town is found in East Shoa Zone of Oromia Region, located 75 km south East of Addis Ababa situated between 8°35′N latitude and 39°10′E longitude at an altitude of 1,777 meters above sea level (CSA, 2008). Gelan tannery obtains the raw materials for processing from skin collection centers in and around the East Arsi Zone, Addis Ababa and Sheno and it has a soaking capacity of 2,000 sheep and 1,000 goat skins per day. Whereas, Colba tannery get cattle hide, sheep and goat skins from its main collection centers in East Arsi, Adama, Bishoftu, and Addis Ababa as well as from sheep and goats slaughtered in Modjo modern export abattoir which is a sister company of the tannery. It has a soaking capacity of 400 to 500 hides and 9,000 pieces of sheep and goat skins per day (Kebed and Yonas, 2015, Personal communication).

Study design and sample size determination

A cross-sectional study was conducted from February to June 2015 on skins and hides collected from the two districts of the East Arsi Zone namely Dodota and Hitosa after reaching and processed in their destination, cattle hide at wet blue stage in Colba tannery and sheep skin at pickle and goat skin at wet blue stage in Gelan tannery were randomly sampled and type of defect and their grading value were registered on pre-prepared data collection sheet. Systematic stratified sampling was used, whereby only 20% of each delivered batch was randomly selected and considered in this study. Each selected skin or hide was examined for defects in natural light by trained skin selectors of the company and the research groups (Figure 1). The defects were identified and graded according to the quality standards as indicated by the QSAE (2008). Various forms of skin defects appearing beyond 2.5 cm in sheep and goat skin and 5.0 cm in cattle hide from the edges towards the center of the skin were registered from grain and flesh surfaces.

Figure 1 - Observation of defects and grading wet blue goat skins in properly prepared and lighted point at Gelan tannery in Modjo

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The total number of hide and skins included in the study was determined using the formula described by Thrusfield (2005). Based on the formula, with the assumption of 50% expected prevalence as there were no reports from study area, 95% level of confidence (CL), and 5% desired level of precision the sample size was calculated as 384 for skins of each animal species. Accordingly, 1,173 (389 cattle hide, 399 sheep and 385 processed goat skins) were selected for the study.

$$N = \frac{(1.96)^2 P_{exp} (1-P_{exp})}{d^2}$$

Where: P_{exp} = expected prevalence (50%)
n = required sample size
d = desired absolute precision

Statistical analysis

Data collected were coded, entered, managed and stored into Microsoft Excel and imported to Statistical Package for Social Sciences (SPSS, version 20) software for analysis. Descriptive statistics were used to summarize the data with regard to frequencies and percentage. The Chi-square (X^2) test were used to observe the association of different skin defects and species. Significance was considered at $P<0.05$.

RESULTS

Defects on cattle hide at wet blue stage

A total of 389 cattle hide were examined for the presence of defects after being processed in Colba tannery and all examined hides revealed one or more defects (Figure 2). The study showed higher prevalence of flay cut followed by gouge mark, putrefaction, corduroying, scratch, scar and cockle/ekeke (Table 1). The grade distribution of this study on wet blue cattle hide revealed that the higher grades, grade 1 accounts 8 (2.1%), grade 2, 8 (2.1%), grade 3, 18 (4.6%) and the lower grades, grade 4 accounts 56 (14.4%), grade 5, 73 (18.8%) grade 6, 115(29.6%) and grade7/reject accounts 111 (28.5%). Out of the total observed 389 hide 76.9% were distributed in the lower grades 5-7, accordingly only few cattle hide went into first grades 1-3.

Table 1 - Types of defects observed on hide at wet blue stage (n=389)

Type of defect	Frequency	Percent
Flay cut	230	59.1
Gouge mark	164	42.2
Corduroying	137	35.2
Purification	137	35.2
Scratch	110	28.3
Scar	78	20.1
Cockle/ekeke	49	12.6
Brand mark	25	6.4
Machine defect	22	5.7
Wound	21	5.4

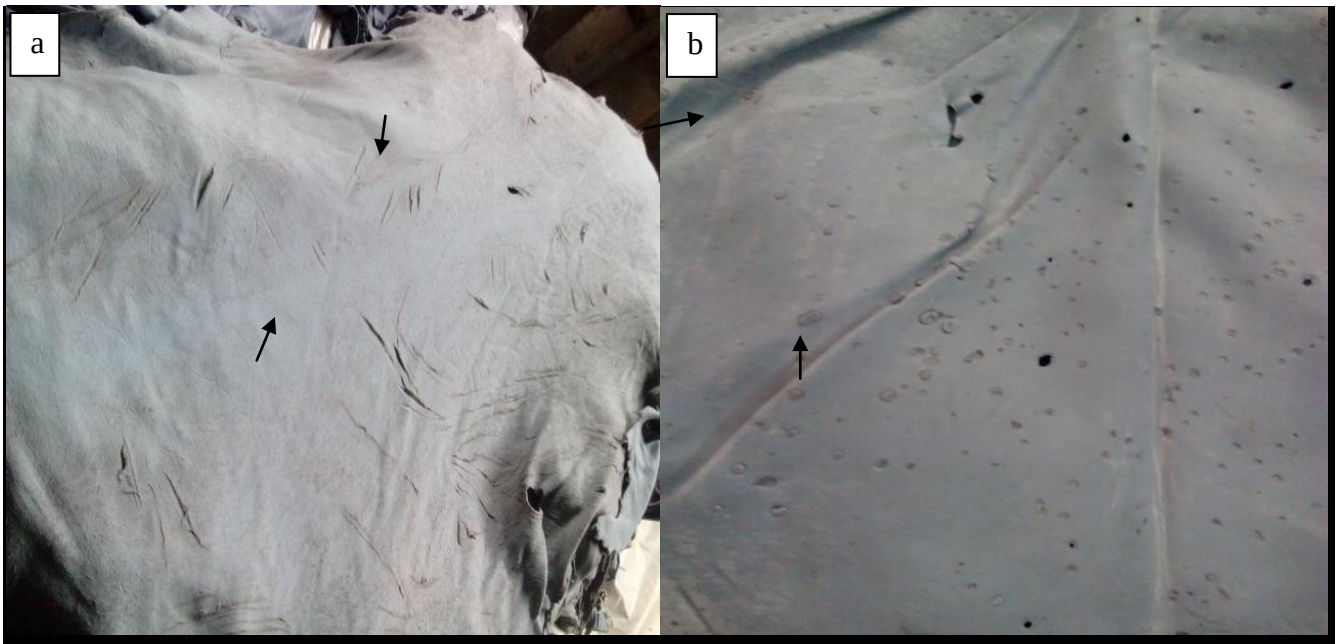

Figure 2 - Wet blue stage Hide defects. a. Flay cut and corduroying b. Pox like defects (holes).

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Distribution of defects to hide quality grades

The distribution of skin defects in different quality grades on cattle hide processed at wet blue stage is presented in Table 2. Flay cut was most important in quality grades 2 to 7 especially in quality grads 5 to7 in a higher proportion comparing with the higher quality grades 1 to 3. The other defects scratch, scar, pox, crack, brand mark and poor pattern were distributed in higher proportion in lower grades 4 to 7.

Table 2- Distributions of defects on cattle wet blue stage in different grades

Type of defects \ Grade	1	2	3	4	5	6	7/Reject	Total
Cockle/'ekeke'	0*	0*	0(0.0)	1(0.9)	2(1.2)	14(4.6)	32(8.0)	49
Scratch	0	0	4(16.7)	12(11.9)	16(9.8)	30(9.8)	48(12.0)	110
Flay cut	0	4(28.6)	5(20.8)	25(24.8)	50(30.9)	68(22.1)	78(19.6)	230
Scar	0	0	4(16.7)	3(3.0)	7(4.3)	29(9.4)	35(8.8)	78
Crack	0	0	0	4(3.9)	1(0.6)	2(0.7)	3(0.8)	10
Corduroying	3(27.3)	3(21.4)	4(16.7)	13(12.9)	30(18.5)	44(14.4)	40(10.0)	137
Gouge mark	5(45.5)	2(14.3)	3(12.5)	27(26.7)	27(16.6)	53(17.3)	47(11.8)	164
Veniness	0	0	0	1(0.9)	0	2(0.7)	0	3
Putrefaction	2(18.2)	3(21.4)	3(12.5)	5(5.0)	13(8.0)	45(14.7)	66(16.6)	137
Poor pattern	0	0	0	2(1.9)	3(1.9)	2(0.6)	4(1.0)	11
Pox	0	0	0	0	0	0	2(0.5)	2
Machine defect	11(9.0)	1(7.1)	1(4.2)	3(2.9)	2(1.2)	5(1.6)	9(2.3)	22
Brand mark	0	0	0	1(0.9)	3(1.8)	5(1.6)	16(4.0)	25
Poor pattern	0	0	0	2(1.9)	3(1.9)	2(0.7)	4(1.0)	11
Wound	0	1(7.1)	0	2(1.9)	4(2.5)	4(1.3)	10(2.5)	21
Tick hole	0	0	0	0	1(0.6)	2(0.7)	3(0.8)	6
Total defects	11	14	24	101	162	307	397	1016

*Figures in parentheses are percentages

Defects on pickled sheep skin and wet blue goat skin

The prevalence of different defects on pickled sheep skin and wet blue goat skin examined in Gelan Tannery is presented in Table 3. On the assessment of sheep pickled skin, the study showed higher prevalence of cockle/ekeke, followed by gouge mark, scratch, flay cut, putrefaction, scare and crack. Whereas the prevalence of defects on goat wet blue skin according to their importance were cockle/ekeke, veininess, crack, scratch, gouge mark, flay cut, scar and corduroying. The prevalence of cockle/ekeke, veininess, scar, corduroying, crack, gouge mark, poor pattern and brand mark were significantly higher ($P<0.05$) in goat skins while putrefaction and shoat pox were significantly higher ($P<0.05$) in sheep skin at pickled stage than goat wet blue skin. On defects like scratch, flay cut, poor pattern and machine defect in sheep and goat skin there was no statistically significance ($P>0.05$) difference.

The result of current study on proportion of skins in different quality grades of sheep and goat processed skin revealed that higher proportions of skins distribution in lower grades 4-7. Out of the total 385 pickled sheep skin observation the result showed that 0(0%) were in grade 1, 7 (1.8%) in grade 2, 47(12.2%) in grade 3, 85(22.1%) in grade 4, 77(20%) in grade 5, 80 (20.8%) in grade 6, and 89 (23.1%) in grade 7/reject. Moreover, the proportion of wet blue goat skin in different quality grade were 0 in grade 1, 0(0%) in grade 2, 1(0.3%) in grade 3, 67(17.3%) in grade 4, 135 (33.8%) in grade 5, 107 (26.8%) in grade 6 and 87 (21.8%) were distributed in grade 7. Significant number of skins in both species was classified as reject (Grade 7).

Quality of sheep skin at pickled stage

The distribution of skin defects in different quality grades on sheep pickled stage is presented in table 4. Ekek/cockle, scratch, scar, flay cut and venines were distributed from grade 2-7. Whereas corduroying, gouge mark, putrefaction, crack and poor pattern were distributed from grade 3-7. Moreover, cockle, flay cut and scratch were highly distributed in grade 7/reject.

Quality of goat skin at wet blue stage

The distribution of skin defects in different quality grades on goat wet blue stage is presented in table 6. Veinines was distributed from grade 3-7. Whereas cockle, scratch, flay cut, scar, crack, corduroying and putrefaction were distributed in the lower grades 4-7. Moreover cockle (ekek), scar, crack, scratch corduroying and gouge mark were highly distributed in grade 7/reject.

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Table 3 - Proportion of defects between sheep and goat skins

Types of defects	Pickled Sheep (N= 385)	Wet blue Goat skin (N= 399)	*P*-value
	Number of defects (%)	Number of defects (%)	
Cockle/ekeke	142 (36.9) *[a]	192 (48.1)	0.001*
Scratch	104 (27.0)	127 (31.8)	0.139
Flay cut	58 (15.1)	75 (18.8)	0.164
Scar	46 (11.9)	167 (41.9)	0.000*
Crack	22 (5.7)	150 (37.6)	0.000*
Corduroying	44 (11.4)	166 (41.6)	0.000*
Gouge mark	37 (9.6)	79 (19.8)	0.000*
Veniness	38 (9.9)	178 (44.6)	0.000*
Putrefaction	58 (15.1)	14 (3.5)	0.000*
Poor pattern	29 (7.5)	19 (4.8)	0.106
Pox	12 (3.1)	4 (1.0)	0.036*
Brand mark	0	14 (3.5)	0.000*
Machine defect	7 (1.8)	3 (0.8)	0.183
Poor substance	6 (1.6)	0	0.012*
Ring worm	2 (0.5)	0	0.149
Tick hole	0	1 (0.3)	0.326
Wound	0	1 (0.3)	0.326

*p<0.05; *[a] Figures in parentheses are percentages

Table 4 - Distribution of defects on sheep pickled stage in different quality grades

Type of defects \ Grade	1	2	3	4	5	6	7/reject	Total
Cockle/ekeke	0	1(14.3)	14(26.9)	28(24.3)	30(21.7)	27(19.4)	42(27.3)	142
Scratch	0	1(14.3)	12(23.0)	18(15.7)	31(24.5)	26(18.7)	16(10.4)	104
Flay cut	0	2(28.6)	3(5.8)	10(8.7)	13(9.4)	11(7.9)	19(12.3)	58
Scar	0	1(14.3)	3(5.8)	3(2.6)	12(8.7)	12(8.6)	15(9.7)	46
Crack	0	0	5(9.6)	4(3.5)	4(2.9)	7(5.0)	2(1.3)	22
Corduroying	0	0	3(5.8)	11(9.7)	12(8.7)	13(9.4)	5(3.3)	44
Gouge mark	0	0	2(3.8)	2(1.74)	13(9.4)	13(9.4)	7(4.6)	37
Veniness	0	2(28.5)	4(7.7)	19(16.5)	8(6.0)	2(1.4)	3(2.0)	38
Purification	0	0	5(9.6)	11(9.7)	11(8.0)	19(13.7)	12(7.8)	58
Poor pattern	0	0	1(1.9)	8(7.0)	3(2.2)	4(2.9)	13(8.4)	29
Pox	0	0	0	0	0	3(2.2)	9(5.8)	12
Machine defect	0	0	0	0	0	0	7(4.6)	7
Ring worm	0	0	0	1(0.9)	0	0	0	2
Poor substance	0	0	0	0	1(0.7)	1(0.6)	0	6
Total	0	7	52	0	138	139	154	605

*Figures in parentheses are percentages

DISCUSSION

In the present study out of 389 cattle wet blue hide examined in Colba tannery, all hides had one or more defects. The various defects observed includes flay cut, gouge mark, putrefaction, corduroying, scratch, scar and cockle in their order of prevalence. This finding is in argument with the report by Bisrat (2013) who studied the case of tanneries in Addis Ababa and Modjo who reported lower prevalence of flay cut (21.3%), putrefaction (15.8%), scratch (13.5%), branding (2.5%) and scar (0.3%). Likewise, present finding of 12.6% prevalence of cockle is also in contrary with report of Bisrat (2013) who reported higher percentage of cockle (42.5%).

In the present study high proportion of wet blue hide grade lied in lower grades 4-7 which accounts 91.2% and the higher grades 1-3 accounts only 8.8% of the total observations. This finding is nearly similar with the report of Bisrat (2013) reported 99.6% of wet blue hide in grade 4-7 in Addis Ababa and Modjo tanneries. In contrary the present finding disagreed with his report which had very lower proportion in higher grade 1-3 (0.5%). The current finding of low proportion of higher grade 1-3 is in agreement with the report of Mekonen and Gezahegn (2008) who indicated, the tanneries receiving raw hide and skin are often complaining the decline in the quality and quantity from time to time.

The present study on pickled sheep skin and goat wet blue skin defect assessment result showed a higher prevalence of cockle in goat (48.1%) than sheep (36.9%) skin which is in line with the findings of Worku et al. (2011) who reported 54.6% prevalence in goat and 45.4% in sheep from Modjo export tannery. On the contrary the current finding disagreed with the result of Zenaw and Mekonnen (2012) who reported that high prevalence of cockle (76%) in pickled sheep than (22.4%) in goat wet blue skin from Bahir Dar tannery. Furthermore, this study was not comparable with the findings of

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Hagos et al. (2013) who reported higher prevalence of cockle in sheep pickled skin (35%) than goat wet blue (21.5%) in Sheba tannery. In current study the higher prevalence of cockle/ekek in goat wet blue skin might be probably the direct reflection of high infestation of external parasites like mange, lice, flea in goat than sheep which were responsible for skin irritations and the mid altitude of the study area might not favorable for sheep ked which is common ectoparasite in sheep skin at higher altitude.

The higher prevalence of cockle in the present as well as other previous studies indicates the impact of cockle on the tanning industry is a serious concern. This is mainly due to the fact that cockle lesion cannot be detected at the raw skin and selection cannot be made prior to processing. The defect appears only after processing the skin into pickled stage. Therefore, the losses to the tanning industry is three times with regard to each cockle affected skins: first through the purchase of raw skins of undetectable inferior quality, secondly by the cost of processing of these skins and thirdly by the fact that such skins are downgraded after processing and therefore they are not suitable for sale in export markets (FAO, 1998; Kassa, 2006).

The current study revealed high prevalence of scratch (31.8%) in goat wet blue stage than in sheep pickled (27.0%) skin which were most important for downgrading of sheep and goat skin in Gelan tannery which is supported by Assefa et al. (2012), who reported a higher prevalence of scratch (73.3%) in goat wet blue than sheep pickled skin (26.7%) at Bahir Dar tannery and with that of Hagos et al. (2013) who reported higher prevalence of scratch (53%) in goat wet blue than in sheep (43.4%) pickled stage from Sheba tannery. However, the present finding was not in line with the findings of Worku et al. (2011) who indicated higher prevalence of scratch (57.3%) in sheep pickled than goat wet blue (42.7%) from Modjo export tannery. The higher prevalence of cockle and scratch on both pickled sheep and wet blue goat skin in the current study showed the association between cockle and scratch that could be attributed to the effect of ectoparasites on animals causing intense itching and rubbing against bushes, thorns, posts and barbed wires leading to the formation of scratches on their skin (Urquhart et al., 1996; Wall and Shearer, 1997).

A statistically significant higher prevalence of scar was observed in goat wet blue skin than sheep pickled skin. This finding is in line with report of Zenaw and Mekonnen (2012) who observed higher prevalence of scar in goat wet blue skin (15.2%) than in sheep pickled skin (9.9%).

Veinness /poor bleeding/ corduroying, gouge mark and brand mark which have statistically higher prevalence in goat wet blue skin than sheep pickled skins were the other important defects encountered in this study. Whereas, the prevalence of skin putrefaction in sheep pickled skin has higher than in goat. The higher prevalence of veniness or poor bleeding in goat was the most important defect in downgrading the wet blue skin of goats which was also observed in this study. This is in line with Alemu (2009) who observed the area with the congealed blood has a degrading effect to the leather quality. Veiny leather is the result of blood vessels in the skin where the blood is not completely drained (poorly drained). This is an unwanted effect which shows very clearly in suede leather. Veininess is a prominent defect in goat skins and very prominent in glazed kid leather.

The mentioned defects lead to downgrading or rejection of skins and hides. In this study, large proportion of sheep pickled skin are rejected. Grade 1-3 accounts only 14% and grade 4-7 accounts 86% of the total observation. This finding is not in agreement with the study conducted of Bisrat (2013) who reported only (5.3%) a proportion of 1-3 the higher grade and a proportion of lower grade 4-7(94.7%) in Addis Ababa and Modjo tanneries. However, the present finding is in line with the report of Assefa et al. (2012) who reported 20% proportion of higher grade/1-3 and 80% a proportion of lower grade/ 4-7. According to Mekonnen and Gezahegn (2008) the percentage of the highest-grade skins from grade 1-3 is very low in a randomly packed hides and skin on its arrival to the tannery. The present study confirmed that all defects have a high number of distributions in lower quality grades. Most of the sheep pickled skin defects were distributed under grade 7/reject sheep pickled skins in order of importance were cockle/ekek, flay cut, scratch, scar, poor pattern, putrefaction and pox. The current finding is also supported by Kassa et al. (1998) stated that, as one quarter to one-third of all the skins processed at tanneries are unsuitable for export due to various defects.

The high proportions of goat wet blue skin were graded in the lower grades (4-7) of grade 5, grade 6, grade 7, grade 4, grade 3 in and none of the goat skins were in grade one and two confirming once more a very few proportions lied in grade 1-3. The present result is supported by the report of Bisrat (2013) who recorded a proportion of 1-3 the higher grade only (0.6%) and a proportion of lower grade 4-7(99.4%) in Addis Ababa and Modjo tanneries. Nevertheless, the present finding is not concords with the report of Assefa et al. (2012) which came up a proportion of higher grade/1-3 (5.6%) and a proportion of lower grade/ 4-7(84.4%) from Bahir Dar tannery. This difference between the two studies might be due to the variation in agro climate, management and the efficiency of ectoparasite control program conducted in the two study regions.

The most prevalent defects which leads to rejection (grade 7) of wet blue goat skin were cockle/ekek, scar, crack, and scratch. The present finding indicated that cockle were the dominant defects that leads to rejection of both wet blue goat and pickled sheep skin rejection. Similarly, scratch, scar and crack are also the cause for rejection or lower grade wet blue goat and pickled sheep skin. The present finding is accordance with the result of Berhanu et al. (2011) out of the rejected skins from goats and sheep, 98.8% of them had ekek or cockle and scratch, whereas 85.6% of them contained sheep and goat pox and 52.2% of them were having knife cuts. Likewise, it is also comparable with the report of Assefa et al. (2012) which stated the most important defects in rejected skins were ekek/cockle (54.2%), scratch (25%) and pox (18.8%).

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CONCLUSION

The study showed that all examined processed hide and skin originated from the two districts of East Arsi Zone had encountered one or more defect. Higher prevalence of various defects was observed, causing rejection of skin and hide. Such defects also cause depreciation in the value of the hides and skins and the consequence is that farmers, traders and the tanning industry suffer considerable financial losses. The low proportion of higher grades of 1-3 observed in this study is the direct reflections of poor live animal management (feeding, livestock disease managements), faulty animal slaughtering practices, post slaughter preservation, transportation of the hide and skins to the tanneries and as well as lack of agricultural extension services addressing hide and skin quality management. The higher prevalence of cockle on processed sheep and goats' skins demands attention to be given to external parasites control programs by the responsible stakeholders. Furthermore, effective extension system and programs that could raise public awareness on ectoparasites and skin diseases control as well as on methods of flaying, preservation and handling of skins should be implemented.

DECLARATIONS

Consent to publish

Not applicable

Authors' contributions

BA: Conception and design of the study, data collection, data analysis, draft writing and correcting the manuscript; YGH: Design of study, Data analysis and interpretation, revising the manuscript and final approval of the version for publication.

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Competing interests

The authors declare that they have no competing interest.

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TRANSMISSION OF *Salmonella* Spp FROM WATER SOURCES TO FISH IN THE MUDDY SEASONAL WATER OF THE RIVER NILE STATE, SUDAN

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study was conducted in river Nile state, north Sudan aimed to give base line information on the potentialities of transmission of *Salmonella* spp from water source to fish in muddy season, in AL-fadlab and Al-akad stations. Twenty samples of water and *Schilbidae* spp fish were taken from the two stations and transferred to the laboratory for physiochemical and microbial analysis of water and studding fish species. Samples were performed using standard bacteriological procedures. Swaps from each fish gill were microbiologically analyzed for *Salmonella* spp and total plate count. Results indicated that studied fish infected by *Salmonella* spp in AL-fadlab station was 44.83±8.6 while in Al–akad station was 9.33±1.4, *Salmonella* spp in water was 5.00±1.0 in AL-fadlab station while it has no growth in Al–akad station. On the other hand, total plate count in fish gills was uncountable in AL-fadlab station and 30.40±7.1 in Al–akad station. Total plate count in water, was 8.13±1.87 for AL-fadlab station and 11.67±2.04 for Al-akad station. Statistical analysis showed significant difference ($P<0.05$) in all studied parameters except the total plate count in water. There was also no significant difference in weight and length of studied fish species and also in water turbidity and temperature from both stations, but water pH showed significant difference ($P<0.05$, 7.62±0.04 and 9.53±0.08 for Al-fadlab and Al-akad, respectively). *Schilbidae* spp fish infected by *Salmonella* spp in studied stations is an indicator of the contamination by untreated municipal sewage, runoff, and storm-water. Therefore, *Schilbidae* spp fish from studied areas have to be carefully handling and heating before consumption to avoid the pathogenic bacteria risks.

Keywords: Chemical, Foods, Genetically, Health, Organisms, Risk

INTRODUCTION

Fishes are vertebrates, poikilotherms and live predominantly in water. Their body shapes may be elongate, dorsoventrally, laterally compressed or rounded in cross section but recognizable into head, trunk and post anal tail. They have been one of the main foods for humans anciently (Ibemenuga et al., 2014). Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins constituting the major part of human diet (Hastein et al., 2006). Fresh water fish are subjected to the risk of contamination with various pathogens from different sources, primary during their presence in aquatic environment and secondary after being harvested through handling and marketing as well as storage. Such contamination may render these food articles unfit for human feed or even harmful to them (Elsherief et al., 2014). Fish and shellfish appear to be passive carriers of *Salmonella*, demonstrate no clinical disease and can excrete *Salmonella spp*. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella* spp (Novotny et al., 2004). The presence of *Salmonella* as enter pathogens in farm fish may reflect the bad hygienic conditions during harvesting, transporting and marketing of the fish. The presence of considerable numbers of Salmonellosis indicates bad hygienic measures during catching and distribution of the fish (Valdivia et al., 1997).

It is clear that fish are continuously exposed to the microorganisms present in water and in sediment. These organisms will undoubtedly influence the microflora on external surfaces including the skin, gills of fish. And the digestive tract will receive water and food that is populated with microorganisms. On the other hand, colonization may well start at the egg and or larval stage, and continue with the fish live (Olafsen, 2001). If the fish are exposed to environmental stress, or injury, it causes sever outbreaks of disease and mortalities. Environmental stresses such as high temperature, poor water quality and high organic content primarily contribute to the onset and severity of Enterobacteriaceae infections in fish (Zheng, et al. 2004; Thillai Sekar et al., 2008). *Salmonella* spp have been found to survive and multiply in the gut,

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mucus and tissues of fish and that render fish acting as potential vector of human disease over long periods (David et al., 2009). The particular isolation of *Salmonella* spp, which when isolated from fish and fish products gives an indication about environmental fecal pollution of fish (Wogu and Maduakol, 2010).

This study aimed to isolate the total viable bacteria and *Salmonella* spp from the water and shilbidae spp fish in the belt of river Nile from two stations (AL- fadlab and AL- akad) and to determine water physiochemical characteristics in studied stations (pH, temperature and turbidity).

MATERIALS AND METHODS

Study area

Study was carried out in river Nile state north Sudan at (Al-fadlab) station in Atbara city and (Al-aked) station in Al-damar city during muddy season (2018), on the upstream of the River Nile.

Water and Fish Sample Collection

Twenty samples of water and fish shilbidae spp were collected from the studied stations using gills net between (6:00-8:00 am). Ten swabs samples were obtained by rubbing the sterilized cotton swab over the gills placed on ice in polythene bag and conveyed to the laboratory for microbiological examinations, water carried in test tube and transferred to Atbara water laboratory for the physiochemical and microbiological analysis turbidity was measure in the site.

Materials

Swab, test tube, picker, flask, sensitive balance, gloves, tips, micro pipette, loops, petri dish, autoclave, incubator, distil water, broth agar, nutrient agar, SSA agar, glass containers, cotton, pH meter, thermometer and alcohol.

Microbiological analyses

Five ml broth agar was added for each swab and inoculated for at 37 °C for 18 hour, after that the sample were serially diluted and 1ml of each diluted sample were plated for microbiological analysis.

Enumeration and Isolation of Bacterial

Preparation of the media and Isolation of the bacteria were done according to Cheesbourgh (1984). Sterilization of the media was done by autoclaving at 121°C for 15 min. Pour plate method was employed for the determination of microbial load of samples. Tenfold serial dilution of the samples was made and 10 dilutions of the samples were plated out on: Nutrient agar medium for total viable count (TVC), *Salmonella*/Shigella agar (SSA) for *Salmonella* isolation. All samples were incubated at 37°C for 24 - 48 h. After incubation the colonies were counted and isolated.

Statistical analysis

The obtained data were analyzed using independent samples T. test at 0.05 levels of significant, data were presented as mean ± standard error of mean. IBM SPSS statistics for Windows program, Version 20.0. Armonk, NY: IBM Corp was used in data analysis.

RESULTS AND DISCUSSION

Fish diseases due to bacterial infections are the major problems in the water sources as it found naturally in the fish environment and under certain stress condition causes severe economic losses to fish (Olsson et al., 1998). Fish and shellfish appear to be passive carriers of *Salmonella,* demonstrate no clinical disease and can excrete *Salmonella spp*. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella* spp (Metz, 1980; Minette, 1986; Chattopadhyay, 2000).

From the results of the microbial and physiochemical analysis of water it reveals that *Salmonella* spp bacteria were obtained in water and studied fish spp, that may be due to the occurrence of some contaminant sources in surrounded area., and from folded water in rainy season. This implies that studied fish are passive carrier of *Salmonella* spp bacteria pathogens this finding agree with Salihu et al. (2012). Fish harvested from contaminated waters can carry *Salmonella* spp. (Pelczar et al., 1993) which is pathogenic to man and other animals. Total plate bacteria detected in both location fish was higher than reported by Mandal et al. (2009). Who found that total plate count in fish was (2.55±0.15). The study revealed that the bacterial load was high in muddy season in the studied locations, one of the reasons possibly being that the high ambient temperature in the water body was close to optimum for many Mesophilic bacteria in natural systems and the bacterial load in fish might be increased with the increase of water temperature (Fernandes et al., 1997; Hossain et al., 1999). Also these results agree with the finding of Rekhari et al. (2014) and Abd-Elall et al. (2014), they found that the bacterial load is higher in summer season in cultured fish. *Salmonella* spp was transmitted to the studied fish gills throw water reflect the risk of the contamination of water by pathogenic bacteria, the physiochemical characters of the water were in suitable numbers of water for aquaculture.

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Table 1 - *Salmonella* spp, total plate count in fish *Schilbidae* spp gills and water from Al-fadlab and Al-akad stations

Parameters	Al-fadlab Station	Al-akad Station	Sig
Salmonella Spp (fish gills)	44.83±8.67	9.33±1.45	**
Plate count (fish gills)	Uncountable	30.40±7.18	**
Salmonella Spp (water)	5.00±1.00	No growth	**
Plate count (water)	8.13±1.87	11.67±2.04	NS

Table 2 - Weight and length of *Schilbeidae* spp fish from Al-fadlab and Al-akad stations

Parameters	Al-fadlab station	Al-akad station	Sig.
Weight (g)	39.92±2.48	43.47±6.97	NS
Length (cm)	11.75±0.51	11.78±0.61	NS

Table 3 - Water turbidity, pH and temperature °C in Al-fadlab and Al-akad stations.

Parameters	Al-fadlab station	Al-akad station	Sig.
Turbidity	42.32±6.85	51.68±12.72	NS
pH	7.62±0.04	9.53±0.08	**
Temperature °C	26.00±0.00	25.00±0.00	NS

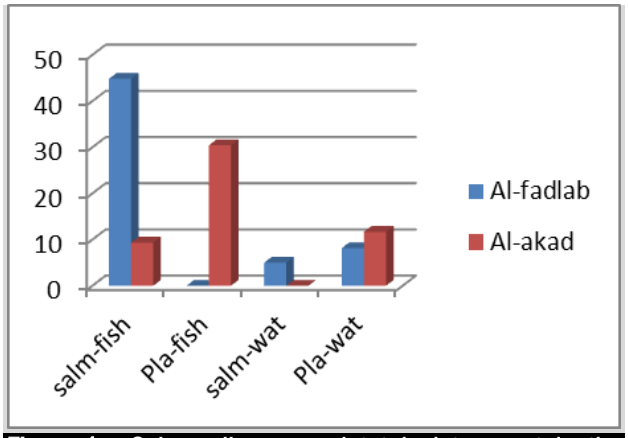

Figure 1 - *Salmonella* spp and total plate count in the studied station fish and water

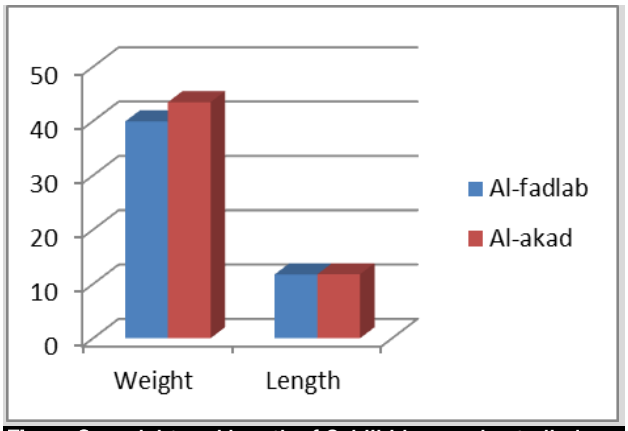

Figure 2 - weight and length of *Schilbidae* spp in studied stations

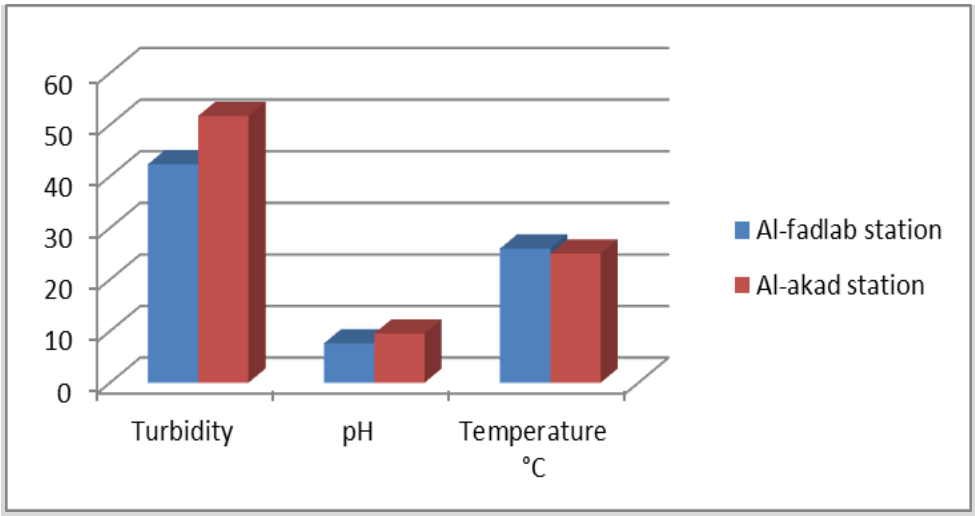

Figure 3 - Water parameters in studied stations

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CONCLUSION

Salmonella spp in fish was higher in Al-fadlab station than Al-akad station while the plate count was in the opposite situation. The *Salmonella* spp in water was high in Al-fadlab station compared with no growth in Al-akad station. There was no significant difference in weight and length of fish from the two locations. No significant difference in water temperature and turbidity from the two locations while there was significant difference in pH which is high in Al-akad location.

Recommendations

- *Shilbidae* spp fish from Al-akad Location and Al-fadlab Location areas have to carefully handling and heating before consumption to avoid the pathogenic bacteria.
- Continuous studies have to be conduct to assess the effect of contamination on these areas in fish health using different fish species and different season.
- Water quality has to be monitoring to evaluate any water risk that may affect the aquatic life.

DECLARATIONS

Authors' Contributions

All authors contributed equally to this work.

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Competing interests

The authors declare that they have no competing interests.

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MAJOR DISEASES OF NILE CROCODILE (*Crocodylus niloticus*) WITH FOCUS ON CURRENT STATUS IN ARBA MINCH CROCODILE RANCH, ETHIOPIA

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Supporting Information

REVIEW ARTICLE

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ABSTRACT: *Crocodylus niloticus* is found in 26 African countries including Ethiopia, the largest recorded specimen measuring 17.0 feet Nile crocodile from the Gambela Upeno River in 1969. Its presence and absence also depend on the climatic conditions and the environment (i.e. the landscape for basking and feeding). In Ethiopia, Nile crocodiles have a mating period during September to October, Nesting occurs in the dry season December to January, and hatchling takes place at the onset of the rainy season, i.e. March/April months. Over the period of 2007-2016 an average of 201,000 *Crocodylus niloticus* skins were exported globally per year, with an increasing trend over the period 2009-2016. Besides the management problems, at Arba Minch Crocodile Ranch, Nile crocodiles are suffering from nutritional abnormalities and health problems. The diseases of the Nile crocodile are classified as infectious (transmissible) and non-infectious (non-transmissible). A transmissible crocodile disease includes bacterial, viral, fungal, protozoan, and parasitic diseases; non-transmissible crocodile diseases are nutritional, toxic poisonings and metabolic disorders; other diseases like nutritional bone diseases and skin lesions are the major health problems at Arba Minch Crocodile Ranch. The main aim of this review is to highlight the major diseases and management status of *Crocodylus niloticus* in Arba Minch ranches, Ethiopia. In conclusion, the Arba Minch Crocodile Ranch (AMCR) should exercise care and caution when introducing hatchlings from the wild into the Ranch and enhance its husbandry methods to reduce the occurrence of infection and disease. It must also work with professionals and research groups.

Keywords: Arba-Minch, *Crocodylus niloticus*, Diseases, Nile crocodile.

INTRODUCTION

Reptiles are considered ectothermic (Seebacher, 1999, Modesto and Anderson, 2004) and due to their environmental thermal limit, most reptile species distribution tends to be near the tropics (Summers, 2015). Crocodiles are classified as the largest reptiles grouped under family Crocodylidae (Huchzermeyer, 2003). They belong to the great group called archosaurs (ruling reptiles), which also included extinct thecodonts. Crocodilians of today are the most social reptiles (Shine, 1988) all belong to the clade Eusuchia (Summers 2015); For the last few decades, and until quite recently, 23 species of modern crocodilians in eight genera were recognized currently there are 27 species in nine genera (Grigg, 2015). Which comprises of 27 species and sub-species, all belonging to a single-family called the Crocodylidae (Summers 2015). When the taxonomy is resolved, there are likely to be ~30 species recognized (Grigg, 2015).

Crocodiles are widespread throughout sub-Saharan Africa (Leslie and Spotila, 2001). There are five species of crocodiles in Africa, the African dwarf crocodile *(Osteolaemus tetraspis)*, the Central African slender-snouted crocodile *(Mecistops leptorhynchus)*, the West African slender-snouted crocodile *(Mecistops cataphractus)*, the West African crocodile *(Crocodylus suchus)* and the Nile crocodile *(Crocodylus niloticus)* (Shirley et al., 2018). The Nile crocodile is among the largest and best known biologically of all the crocodilians. Nile crocodiles are widely distributed throughout sub-Saharan Africa, and historical records indicate its range formerly extended into southern Israel and Jordan. The species was also established on the Comoros Islands and still exists in Madagascar (Ross, 1998).

The Nile crocodile was once abundant in Ethiopia's rivers and lakes. By 1971, the head of the Wildlife Conservation Department had already considered the Nile crocodile to be seriously depleted but they were protected only in reserves like Omo Game (Nechsar National) Park. Subsequently, in 1972 commercial hunting of crocodiles was prohibited in Ethiopia and the Nile crocodile was listed as a game animal that could be hunted under permit only. Ethiopia ratified the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in 1989. The Nile crocodile was transferred from Appendix I, the highest order of protection, under the convention, to Appendix II to allow an export quota

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for ranched skins (referring to crocodiles raised from wild-collected eggs and/or hatchlings). The initial export quotas approved by CITES were 9370 for 1990 and 8870 for 1991-92 (Whitaker, 2007), but this has reduced to 3000 for ranched skins for 2018 as well as a quota of 5 trophy hunting (Isberg et al., 2019).

A ranching program for *Crocodylus niloticus* was implemented in Ethiopia in 1985 when the government created Arba Minch Crocodile Ranch (AMCR) (Shirley et al., 2014). The mortality rate of *Crocodylus niloticus* at AMCR of hatchlings collected from wild have a very high mortality rate up to 67.9% basically from hatchlings, 1-year-old, and juveniles (Whitaker, 2007; Shirley et al., 2014). Main factors affecting the production management of *Crocodylus niloticus* and health-related problems that decrease the number (number of mature individuals are 50,000-70,000). Some of the lives threatening factors of Nile crocodiles are urban sprawl, environmental pollution, and habitat destruction, subsistence agriculture and deforestation, and diseases (Isberg et al., 2019).

The Crocodiles in AMCR were provided mainly with feed items like fish and meat. The pathological finding was most frequently arise from nutritional deficiency as a result of crocodiles being fed meat meal, such as frozen fish and frozen meat without a bone meal. There is known to be deficient in important minerals and vitamins (Gilber, 2000). Skin lesions were the second most common problems observed at AMCR after paralysis of hind legs (Shirley et al., 2014). Therefore, this was prepared to review the major diseases and management status of *Crocodylus niloticus* in Arba Minch ranches, Ethiopia.

Husbandry and breeding of Nile crocodile

Understanding the habitat requirements and habitat use of a specific Nile crocodile population requires a combination of understanding the specific landscape mosaic dynamics that the population is found in and the resource requirements of the selected Nile crocodile (Champion and Downs, 2015). Its presence and absence also depend on the climatic conditions and the environment i.e. the landscape to basking and feed (Botha et al., 2011). In farm situations, the basic requirements for the well-being of farm crocodiles include ensuring that: 1. appropriate and sufficient food and water are provided to sustain health and vitality; 2. sufficient area is provided to maintain well-being and to allow crocodiles to exhibit normal behavior; 3. they are protected from predation; 4. they are protected from disease, including disease that can be exacerbated by management practices; 5. they are protected from extremes of climate, particularly during certain phases of their lives; and 6. pain, distress, suffering, and injury are minimized or avoided (Anon, 1992; Tosun, 2013). The mean body temperature of a crocodile is 25.6°C and a range of 6 degrees, with fluctuations from the mean of -2.6 to +4° C. Under optimum raising conditions, with adequate temperatures, hatchlings have high metabolic rates, high food requirements, and they grow rapidly; in countries with "cold" winters (e.g. USA), crocodilians are grown very successfully in heated sheds, but not all species appear to require or can tolerate high and constant temperatures. Some require a mosaic of temperatures, where they can spend part of, rather than the whole day with body temperatures in the 30-33° C range (Manolis and Webb, 2016).

Ranching Nile crocodiles

Ranching is a commercially viable strategy for crocodile farming which is widely used and demonstrates accepted conservation advantages. Ranching entails harvesting crocodile eggs from wild and incubating them to produce hatchlings (Khosa et al., 2012) it can also include the harvesting of hatchlings immediately post-hatching. The collections of eggs, hatchlings, and juveniles from the wild give natural populations a conspicuous economic value (Luxmoore, 1992; Thorbjarnarson et al., 1992; Whitaker, 2007). In Ethiopia, there are two Crocodile ranches, the State-owned Arba Minich Crocodile Ranch (AMCR) and the privately owned, Blen Development PLC, AMCR was created at the mid of 1984 and Blen was created in the mid of 2006 (Whitaker, 2007; Mahammed, 2008).

Food and feeding behavior of Nile crocodile

Crocodylus niloticus has a similar ontogenetic shift in diet to that of other crocodilians (Wallace and Leslie, 2008). The powerful enzymatic digestive juices of crocodiles completely digest bones, hooves, and feathers but being poikilothermic animals, digestion is promoted by higher temperatures. Juveniles feed on insects, spiders, snails, gastropods, and mussels in the shallows and onshore. Young crocodiles feed mostly on toads, frogs, and small fish such as Clarias, Labeo, and Tilapia species (Whitaker, 2007; Furstenburg, 2008). With age the diet changes to catfish (barbel) and larger mammals up to the size of young giraffe, buffalo, and elephant. On average catfish comprises 70% of the diet of adult Nile crocodile. During summer a 4 m adult will consume a large meal once in 2-3 weeks. Sub-adults of 1.5m eat once a week while juveniles feed daily. Carrion is taken only when fresh food is not available. It is estimated that up to 60% of the food intake is converted to fat for storage in the tail and trunk (Bolton, 1997; Johan and Frits, 2000; Davis, 2001; Furstenburg, 2008).

Reproduction in Nile crocodiles

Copulation takes place in water and all species lay eggs (Bolton, 1997). Reproductive females had a plasma testosterone surge corresponding to the time of courtship and mating. Both reproductive and non-reproductive females showed increased plasma progesterone at several times of the year (Kofron, 1990). Courtship and mating occurred in water during the day, usually directly in front of the shared basking ground (Kofron, 1991). Gender assessment in crocodilians is typically achieved by digital examination of the cranio-ventral cloaca; males have a penis while females have a clitoris. The penis may be exteriorized for visual examination if there is doubt on palpation. Adult male crocodilians typically grow to a larger size than females (Pooley, 1982; Kofron, 1991; Timothy, 2018). In Ethiopia the mating period for

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Nile crocodiles is September/October, nesting occurs in the dry season of December/January, and hatchling occurs at the onset of the rainy season of March/April months. Range and average clutch size are 25-70 eggs; average: with an average size of 45 for Lake Chamo, in the south of Ethiopia. Clutch size increases and is directly proportional to female size and age, with average fertility rates for first nesters 20 to 50%, increasing to 80 to 90% for older and mature females (Whitaker, 2007).

Normal health conditions of Nile crocodiles

Husbandry techniques are continually evolving to ensure animals are maintained in good health. There are major approaches to assessing the normal health status of crocodiles in ranches; anatomical and physiological assumptions, health correlations such as body condition, growth rates, and size, survival rates (Isberg et al., 2009), Frequency of injuries, disease incidence, parasite incidence, reproductive performance; biochemical Indicators (such as comparative corticosterone levels, which are an indicator of stress) (Elsey et al., 1990a,b; Turton et al., 1997; Franklin et al., 2003; Isberg et al., 2009, 2013; Finger et al., 2015); behavioral observation and stimulus-response. Over and above genuine concerns about animal welfare, the media is often used to promote information that is deliberate manipulation of factual evidence (Manolis and Webb, 2016). Husbandry strategies for different ages and sizes of crocodilians have evolved separately in different farms and for different species; however, some fundamental principles can be applied to most, if not all, species. General considerations like suitable incubation and hatchling characterize, initiation of hatchling feeding, treatment of hatchlings, nutritional deficiencies and imbalances, metabolic rate and temperature, water quality, effects of hatchling size on growth, effects of sex on growth, density and social behavior (Huchzermeyer, 2003).

Economic importance of rearing *Crocodylus niloticus*

Over the past five decades, the captive rearing and managed harvests of crocodilians have been held up as a success story in the search for balanced, sustainable use of wildlife and the generation of wildlife products for international trade (Thorbjarnarson, 1999). Wild animals and their derivatives are traded worldwide to meet demands for food, clothing, decorative items, traditional medicines, and pets (Challander et al., 2015). The overall volume of world trade in classic crocodilian and caiman skins has been variable over the 10 years 2007 to 2016, with an average of 1.44 million skins exported annually. Over the period 2007-2016, an average of 201,000 *Crocodylus niloticus* skins were exported globally per year, with an increasing trend over the period 2009-2016 (Caldwell, 2018). In 2016, Zambia was the leading (112,434 Crocodile skins) exporter of Nile crocodile skins to the global market. Data provided by Ethiopian Wildlife Conservation Authority (EWCA), show that Ethiopia, exported 594, 492, 77, and 400 Crocodile skins in 2007, 2008, 2011, and 2012, respectively. The country's crocodile skin export capacity is declined due to international market in the years 2010, 2015, and 2016 to 4, 6 and 7 skins, respectively and as the report shows there was Zero export of skin in the years 2009, 2013 and 2014 (Caldwell, 2018). In 2017, Crocodile hatchlings can be sold at USD 2 each to foreign investors, 15 birrs (USD 0.75) to Ethiopian investors, and 5 birrs (USD 0.25) to farmers for quantities over 100. Crocodile eggs are sold at half those prices. The Nile crocodile is also hunted by foreign tourist hunters for a USD 2000 trophy fee (Whitaker, 2007; Shirley et al., 2014; Nisagurwe, 2017). Crocodiles are not only involved in the skin trade but also traded as live animals as crocodile meat and for their teeth (Caldwell, 2018).

Constraints to Nile crocodile management

Crocodile ranch management in Ethiopia requires a formal management plan in terms of national legislation. The species is currently subject to both consumptive (e.g., ranching and trophy hunting) and non-consumptive (e.g., tourism) uses, as well as implicated in human-wildlife conflict. Crocodiles are theoretically managed by federal, state, zonal, and woreda (district) administrations under national laws for biodiversity protection and utilization, ranching, and trophy hunting. A formal management plan should also provide a framework for overcoming some current management deficiencies but this is not yet the case, in Ethiopia (Whitaker, 2007; Shirley et al., 2014). Local threats for crocodile conservation management includes; (a) lack of regulation enforcement, (b) illegal fishing gear and unsustainable growth of the fishing industry, which has resulted in overfishing, a decline in a population of the main target species to almost non-viable levels and the apparent extinction of one of them; (c) increase in cattle grazing such that many areas previously used by crocodiles for basking and nesting have been destroyed. (d) Cultivation up to the lakeshore which is impacting negatively on crocodile behavior, recruitment, and survival. Droughts due to climate change and other effects will likely increase this threat (Whitaker, 2007). In addition to management problems in the wild, Nile crocodiles are suffering from nutritional abnormalities and health problems at AMCR. The main health problems are a combination of mal-nutritional diseases and skin diseases. The mortality rate of almost 70% of hatchlings and juveniles has been ascribed to nutritional bone diseases caused by continuous feeding of meat and fresh frozen fish meat. AMCR harvested more than 8890 crocodile skins from 1982-1998 E.C, they would earn more than USD 3,744,000.00 if they harvest first-grade skin, but the reality is 769,000 ETB (USD 1 was 8 ETB when the author reports the data) (Shirley et al., 2014).

Common diseases of Nile crocodiles

According to Radostits et al. (2006) definition of animal diseases, crocodile diseases are 'inability to perform physiological functions at normal levels even though nutrition and other environmental requirements are provided at adequate levels'. The diseases of Nile crocodiles are classified as infectious (transmissible) and non-infectious (non-

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transmissible). A transmissible crocodile disease includes bacterial, viral, fungal, protozoan, and parasitic diseases; the rest are nutritional, toxic poisonings and metabolic disorders are non-transmissible crocodile diseases (Huchzermeyer, 2003).

Infectious diseases of Nile crocodile

Ippen and Zwart (1996) postulated as the most reptiles in captivity were taken from the wild. Their infectious and parasitic diseases will have been imported with them, and that husbandry practices would have an influence on disease outbreaks there are several crocodile-specific viral and bacterial infections, some of which may even be species or genus specific. However, their present distribution may also be due purely to geographical limits. The specificity of parasites also varies. Besides, there are many non-specific infections, particularly bacterial and fungal (Huchzermeyer, 2003).

Bacterial infections

Only a few bacteria cause specific diseases in crocodiles, and even fewer of these are crocodile-specific. However, many different species of bacteria can cause nonspecific septicaemias. These bacteria are recruited either from the aquatic environment, the intestinal flora or from food contaminants, particularly where raw meat is used as feed. All septicaemias, specific and non-specific, are triggered, if not caused, by stress. Bacteria are allowed to escape under severe stress from the intestine into the blood circulation, and if the stress continues, the resultant immune suppression prevents the crocodile from overcoming the initial escape and allows the bacteria to gain a foothold (Huchzermeyer and Cooper, 2000). Some bacterial genera that cause infection in Crocodiles are included in this review.

Mycoplasmosis

Mycoplasmas were isolated from lungs and synovial fluid of the Nile crocodiles and the isolates were identified as *Mycoplasma crocodyli* (Kirchhoff et al., 1997). The joints of infected crocodiles were had swollen joints and filled with excessive quantities of turbid fluid, in chronic cases with dry fibrinous exudate, and some of the animals were found to have lesions of pneumonia. As *M. crocodyli* is a relatively recently described (in Zimbabwe in 1995) pathogen, aspects of other *Mycoplasma spp.* (Mohan et al., 1995). *M. crocodyli*, as with other Mycoplasmas, lacks true cell walls and has a typical fried-egg appearance on solid medium, but grows relatively well in an artificial medium. Glucose and mannose are both fermented, and cholesterol or serum is required for growth. It is one of the few *Mycoplasma spp.* that fulfills Koch's postulates for disease causation (Kirchhoff et al., 1997). Serological assays are often used to test animals for exposure to infectious agents and include many of the common laboratory procedures such as the ELISA, agglutination, precipitation, neutralization, etc. Indirect ELISA (iELISA) for the detection of antibodies (Ab) to *M. crocodyli* infection in crocodile sera was developed using Ag and anti-crocodile conjugate (Dawo and Mohan, 2007). An immune-blotting protocol for the detection of antibodies to *Mycoplasma crocodyli* was developed using the sonicated antigen of the reference strain 266/93. Immunoblotting detected nine reacting antigens, of which the 33 and 40 kDa antigens were immune-dominant (Dawo and Mohan, 2008). The complete genome sequence of *M. crocodyli* has recently been reported but, although at least five potential virulence factors have been identified, their role and significance are still unclear (Brown et al., 2011), polyarthritis is the best described clinical and pathological sign including progressive weakness, ranging from stiffness to complete immobility. Different stages of exudative polyarthritis are encountered at necropsy, ranging from turbid mucous containing *Mycoplasma spp.* in acute and sub-acute cases, to yellow, inspissated exudates in chronic cases. Histopathological changes include inflammatory edema of the surrounding tissue, necrosis of the superficial layers of the synovial membrane, and fibrin deposition, lymphocytic infiltration, and fibrosis of the joint capsule. Apart from polyarthritis, the organism also triggers pneumonia, histo-pathologically characterized by consolidation and edema of affected areas, with a white blood cell (particularly poly-morpho-nuclear cells and mononuclear cells) and erythrocyte infiltration (Mohan et al., 1995; Kirchhoff et al., 1997; Huchzermeyer and Cooper, 2000; Huchzermeyer, 2003).

In general, mycoplasmosis control can be divided into three important sectors, namely vaccination, medication, and keeping disease-free animals (Desrosiers, 2001; Ley, 2006; Caswell and Archambault, 2008; Kleven, 2008). These are generally not mutually exclusive and are used in combination as required. Medication, including parenteral treatment of diseased crocodiles and/or in-feed treatment, have been performed during crocodile mycoplasmosis outbreaks, but treatment failures (Mohan et al., 2002), reports on antimicrobial resistance (Ayling et al., 2000, Reinhardt et al., 2002, Rosenbusch et al., 2005, Antunes et al., 2007) and high costs eliminates this as a long term control strategy (Grobler, 2013). Vaccination against mycoplasmosis is widely used in commercial pig, poultry, and cattle production systems, particularly in multi-age set-ups because it often is the only viable long-term option. Both inactivated and live-attenuated vaccines have been tested and are currently in use (Grobler, 2013).

Chlamydiosis

Chlamydiosis is a disease in farmed Nile crocodiles caused by chlamydiae closely related to *Chlamydia psittaci*, but probably a different species. There are two forms: acute hepatitis and chronic conjunctivitis (Huchzermeyer et al., 1994). On post-mortem examination, the liver is found to be pale, mottled, and enlarged and the spleen slightly enlarged. There are mild ascites and a severe hydro-pericardium: the most severe histopathological changes are found in the liver: a severe portal to diffuse lymphoplasmacytic hepatitis with congestion, mild bile duct proliferation, vacuolar degeneration of the hepatocytes and multifocal to coalescing necrosis. Numerous colonies of intra-cytoplasmic organisms are present in the hepatocytes (Huchzermeyer, 2003). The mode of transmission is not identified, yet. But the contamination of surface water by wild carrier crocodiles is suspected. The diagnosis is based on the demonstration of the agents either microscopically or by culture (Huchzermeyer, 2002). An investigation into the cause of acute mortality in the farmed

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hatchling, *Crocodylus niloticus* led to the isolation of chlamydia from the livers of affected animals (Huchzermeyer et al., 1994). Both forms of chlamydiosis respond to tetracycline (Huchzermeyer, 2002); Terramycin soluble powder (10g/kg of feed), or pure oxytetracycline (1g/kg of feed) (Huchzermeyer, 2003). The prevention of chlamydiosis must be based on stress prevention as well as on strict hygienic measures, such as the use of borehole or well water in the rearing section, as well as the disinfection of footwear when moving from section to section (Huchzermeyer, 2002; 2003).

Salmonellosis

Salmonellosis is caused by bacteria of the genus *Salmonella* and manifests itself either as enteritis, particularly in hatchlings (Huchzermeyer, 2003). Reptiles may be considered a natural reservoir for *Salmonella* bacteria, but except for pet turtles, the role of poikilothermic vertebrates in the transmission of *Salmonella* to other animals and men is common (Madsen, 1996). From 1985-1994 scientists, Huchzermeyer and Agnagna were isolated Salmonella from 148 out of 173 from farmed Nile crocodiles (Huchzermeyer, and Agnagna, 1994; Walt et al., 1997). A study from Zimbabwe published that the prevalence of Salmonella was 30% in fresh and 20% in the frozen meat samples (Madsen, 1996), but the prevalence of the bacteria has not been adequately studied. Bacterial septicemia is often precipitated by severe stress with frequent change in temperature, the ongoing infection may cause depression and anorexia, the enteritic form of the disease may either cause fibrinous exudation and occlusion of the intestine, or diarrhea, hemorrhagic enteritis due to *S. choleraesuis* (Ocholi and Enurah, 1989; Huchzermeyer, 2003). Diagnostic procedures are performed by bacterial culture of blood, feces, or synovial aspirate. The treatment of clinical cases comprises oral or parenteral administration of an antibiotic selected by an antibiogram and the elimination of the precipitating stressor(s). The prevention methods require strict sanitary feed, hygiene, washing with a detergent to remove protective layers of fat, and vaccinating via a calf paratyphoid vaccine (Huchzermeyer, 2003).

Non-specific septicaemias

The non-specific septicaemias of crocodiles are caused by a large variety of bacteria of enteric or environmental origin, many of which are opportunistic rather than obligatory pathogens, mostly part of the normal intestinal flora, although the intestinal flora of farmed crocodiles may be modified by antibacterial treatments and the introduction of potential pathogens when feeding meat, particularly from farm mortalities (Huchzermeyer, 2003). Septic wounds rarely lead to septicaemias and this adds support to the hypothesis of the enteric origin of septicemia in crocodiles (Huchzermeyer and Cooper, 2000). Some of the isolated cases of septicaemias in Nile crocodiles (Table 1). The course of the disease depends on the environmental temperature (the course is fast in hatchlings kept at 32–34°C, but slows in juveniles at low temperatures, while it can take several months in adults) and the size of the affected crocodiles. In some chronic cases, the affected crocodiles develop white patches around the nostrils and eyes, as well as on the dorsal surface of the body and limbs. In advanced cases, the likelihood of a treatment being successful is minimal. For the prevention of septicaemias, it is necessary to maintain optimal temperature (Huchzermeyer, 2003).

Table 1 - Isolated cases of septicaemias in Nile crocodiles

Septicaemic agents	Reported author/s	Reported Year/s
Aeromonas hydrophila and *A. shigelloides*	Foggin	1992
Citrobacter spp. and *C. freundii*	Foggin	1992
Corynebacterium spp. and *C. pyogenes*	Foggin	1992
Enterobacter agglomerans	Foggin	1992
Escherichia coli	Foggin	1992
Providencia rettgeri	Foggin	1992
Pseudomonas spp. and P. aeruginosa	Foggin	1992
Pasteurella multocida	Dziva and Mohan	2000

Source: Huchzermeyer and Van Wyk (2003)

Viral Diseases of Nile crocodiles

The etiology of reptilian viral diseases can be attributed to a wide range of viruses occurring across different genera and families. Forty to fifty years ago, studies of viruses in reptiles focused mainly on the zoonotic potential of arboviruses in reptiles and much effort went into surveys and challenge trials of a range of reptiles (Ariel, 2011). The diagnosis of viral infections should be based on the presence of serological tests and the isolation and characterization of the virus. Regarding the crocodile viruses, there is a serious problem. None of them can be isolated in embryonated chicken eggs, the most common tool in veterinary virology laboratories, nor can they be grown in any of the cell culture lines presently in use. Nobody has yet isolated or established crocodile embryonic cell lines that could be used for this work (Huchzermeyer, 2003).

Adenoviral infection

These viral infections most commonly affect the liver of hatchlings under 5 months, less often the intestines and pancreas, and sometimes the lungs as well, but rarely all at the same animal (Jacobson et al., 1984; Foggin, 1987; 1992). Diagnosis of adenovirus is now largely done by molecular tools such as PCR directly on swabs or organs followed by

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sequencing (Wellehan et al., 2004), or in situ hybridization of formalin-fixed tissues (Perkins et al., 2001), or by transmission electron microscopy in negatively stained feces of three Nile crocodile (Huchzermeyer et al., 1994). Apart from its indirect diagnosis, there is no reported successful isolation of the crocodile adenovirus virus (Huchzermeyer, 2003). Lethargy and anorexia are the only clinical symptoms associated with a massive mortality rate (Foggin, 1987). On post-mortem diagnosis, there may be slight icterus, swollen and pale liver, and pale yellow bile; swollen and pale intestines sometimes filled with fibrous exudate (Jacobson et al., 1984; Foggin, 1992). Repeated findings in chronic hepatitis are fibrosis of the portal tracts and bile duct hyperplasia (Foggin, 1992). Since there is no specific treatment for adenoviral infections secondary antibiotic drugs are administered infections may have a beneficial effect in serious outbreaks. Prevention should be based on strict hygienic measures aimed at preventing the horizontal spread of the virus, including not using water from rivers inhabited by wild crocodiles, and preventing stress, particularly thermal stress caused by wide temperature fluctuations in open-air rearing pens in winter (Huchzermeyer, 2003).

Parapoxvirus infection

Crocodile pox (Afonso et al., 2006; Huchzermeyer et al., 2009) is an infection of hatchling and juvenile crocodiles with a Parapoxvirus, characterized by brown crusty lesions (Pandey et al., 1990) in the oral cavity, on the head and the ventral (Marschang, 2011) and lateral surfaces of the body and tail (Foggin, 1987; Horner, 1988; Huchzermeyer et al., 1991; Buoro, 1992). Lesions on the eyelids may cause blindness, and lesions on the head may cause a shrinking of the skin, leading to deformities (Foggin, 1987; Horner, 1988). The skin lesions appeared as dark brown, crusty pox-like lesions up to 3mm in diameter, with a sharply outlined central depression. The lesions are situated between the scales and can occur over the entire body. They intended to be concentrated mainly on the ventral and lateral surfaces of the body and tail, the upper and lower surfaces of the limbs, and around the jaws and eyes (Huchzermeyer et al., 1991). It is presumed that the virus can be carried and shed by clinically healthy carriers. Adult breeding stock on the farm also is a possible source of the virus. While the virus could possibly be transmitted by mosquito bite, it is much more likely to be transmitted by contaminated water, or the acquisition of hatchlings from a farm where the disease had occurred (Horner, 1988; Huchzermeyer et al., 1991; Huchzermeyer, 2003). There is no specific treatment against crocodile pox infection (Huchzermeyer, 2003), A crude autogenous vaccine prepared from scabs from affected animals reduced the recovery time (Horner, 1988), but there is the danger of causing generalized infection amongst unvaccinated individuals, when the live vaccine virus is introduced into the rearing environment (Foggin, 1992). The prevention of crocodile pox infection is based on avoiding the use of potentially contaminated water and the avoidance of stress, particularly heat stress (Huchzermeyer, 2003)

Other viral infections

Other viruses found in *Crocodylus niloticus*, with less economic importance, include Coronavirus-like particles (found by transmission electron microscopy in negatively stained feces of four 2-3-year-old crocodiles at a farm with severe mortality in that age group; Filamentous forms of influenza C virus (found by transmission electron microscopy in negatively stained feces of eight Nile crocodiles (length 31-81cm) from one farm associated with high mortality over 1 month (Huchzermeyer et al., 1994); Newcastle disease virus, although it does not cause clinical disease in crocodiles. But, when Nile crocodiles are fed fowl that had died from Newcastle disease, they seroconvert; Paramyxovirus was found in the feces of a single crocodile from a farm where no poultry had been fed (Thomson, 1972; Huchzermeyer et al., 1994; Pfitzer et al., 2000).

Parasitic diseases of Nile crocodile

Nile crocodiles are infected by many ecto and endo-parasites, with trypanosome being the most common. The trypanosomes of crocodiles are harmless flagellate blood parasites transmitted by biting flies and possibly also mosquitoes (Hoare, 1928; 1929; 1931). Other blood parasites that have been isolated from Nile crocodiles are *Hepatozoon species* such as *Hepatozoon petite* and *Hepatozoon sheppardi* (Travassos Santos Dias, 1952). Coccidiosis in Nile crocodiles is caused by a complicated parasitic protozoan and several coccidian parasites of crocodiles have been described from fecal suspensions according to their oocyst morphology such as *Eimeria spp.* (Hoare, 1932; Huchzermeyer, 2003), *Goussia spp.* (Gardiner et al., 1986) and *Cryptosporidia* (Siam et al., 1994; Lane and Mader, 1996) (Table 2). The pentastomid parasites of chelonians and crocodilians are currently divided into the family Sebekidae and Subtriquetridae (Riley et al., 1990; Riley 1994; Riley and Huchzermeyer 1996; Riley et al., 1997; Junker and Bookmker, 2006; Junker et al., 2016). Pentastome assemblages comprised seven species in three Sebekid genera, *Alofia Leiperia,* and *Sebekia*, for example, *Alofia nilotici* (Riley and Huchzermeyer, 1995), *A. simpsoni* (Riley, 1994), *Leiperia cincinnalis* (Sambon, 1922), *Sebekia cesarisi* (Giglioli, 1922), *S. minor* (Junker et al., 1998; 2016) and *S. okavangoensis* (Riley and Huchzermeyer, 1995), *Subtriquetra rileyi* (Junker et al., 1998).

In the lungs, the parasites suck blood and thereby can cause infection and inflammation. In cases of stress septicemia, the bacteria present in the blood can invade the lung tissue in the lesions caused by the pentastomes, and thus create the abscesses found associated with pentastome infestations. In severe infestations, pentastome eggs may be found in the host's feces. On post-mortem examination, the parasites are found in the larger air passages of the lungs. The treatment requires antiparasitic Dectomax® (Doramectin 1%) dose of 1 ml per 50 kg of body mass, while Ivermectin at effective doses is toxic. For prevention, it is vital to control the fresh fish food as a fish are intermediate hosts, and distress prevents Nile crocodiles from forming lung abscess (Huchzermeyer, 2003).

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Table 2 - Major parasitic agents of the Nile crocodile

Endoparasites	*Genus and species*	Authors	Reported years
Ascaridoids	*Dujardinascaris dujardini*	Bayliss	1947
	Dujardinascaris gedoelsti	Sprent	1977
	Dujardinascaris madagascariensis	Sprent	1977
	Dujardinascaris puylaerti	Sprent	1977
	Dujardinascaris tasmani	Ortlepp	1932
	Gedoelstascaris vandenbrandeni	Sprent	1978
	Hartwichia rousseloti	Sprent	1983
	Multicaecum agile	Sprent	1983
	Ortleppascaris nigra	Graber	1981
	Terranova crocodile	Machida et al.	1992
	Trispiculascaris assymmetrica	Sprent	1983
	Trispiculascaris trispiculascaris	Sprent	1983
Capillarioids	*Paratrichosoma spp.*	Foggin	1987
Trichinellae	*Trichinella spiralis*	Mukaratirwa and Foggin	1999
Filariae	*Micropleura vivipara*	Foggin	1987
	Oswaldofilaria versterae	Bain et al.	1982
Trematodes	*Acanthostomum productum*	Hughes et al.	1941
	Acanthostomum vicinum	Hughes et al.	1941
	Allechinostomum crocodile	Hughes et al.	1941
	Cyatocotyle fraternae (fraterna?)	Bisseru	1957
	Neoparadiplostomum kafuensis	Bisseru	1956
	Neoparadiplostomum magnitesticulatum	Bisseru	1956
	Neoparadiplostomum africana	Bisseru	1956
	Neoparadiplostomum leiperi	Bisseru	1956
	Nephrocephalus sessilis	Hughes et al.	1941
	Prostrigea arcuata	Bisseru	1956
	Pseudoneodiplostomum bifurcatum	Huchzermeyer and Agnagna	1994
	Stephanoprora ornate	Hughes et al.	1941
	Exotidendrium spp.	Foggin	1992

Source: Huchzermeyer and Van Wyk (2003).

Fungal Infections in Nile crocodiles

Crocodiles are farmed mainly for their skin, and most fungal infections are affecting the skin of Nile crocodiles (in both farms and wild). Many of the fungi involved in these infections are part of the normal intestinal flora and are excreted daily with the feces into the water (Huchzermeyer, 2003). Normally, the fungi are inhibited in the intestine by the bacterial flora. If the latter is suppressed by prolonged antibacterial treatment, the fungi can multiply more freely. Reported cases of most fungal diseases were diagnosed by histopathological examinations of the host tissue (Gilber, 2000). The tissue reaction to fungal infections is granulomatous and not exudative as it is in most localized bacterial infections. The granulomata are characterized by the presence of multinucleated giant cells (Huchzermeyer, 2003). *Beauveria bassiana* (Keymer, 1974) has been isolated from the lungs of captive Nile crocodiles and *Trichosporon species* has been isolated from the tongue and gingivae of a captive Nile crocodile (Kuttin et al., 1978). Systemic and respiratory infections are often diagnosed too late for treatment to be considered, Oral mycosis and gastro-intestinal mycosis are treated with antifungal injections, whilst skin dermatophytes are treated by injections and topical routine of administration. Avoiding excessive fungal build up prevents the diseases and an aggressive antibiotic also delays the development of fungus (Huchzermeyer, 2003).

Non-infectious diseases of Nile crocodiles

Captive crocodiles frequently are given a monotonous diet, which may be deficient in one or more essential constituents. This may lead to deficiencies of certain minerals and vitamins that used for fast growth rate, and this can further accentuate potential imbalances in their artificial nutrition. These also enhance some conditions like osteomalacia, rickets, secondary hyperparathyroidism, metabolic bone disease, fibrous osteodystrophy and osteoporosis, diaphanous teeth, Vitamins deficiencies (Vitamins A, B_1, C, D, E, and K), mineral deficiencies (Ca^{++}, K^{+}, P and Zn), and hypoproteinaemia. Poisoning occurs not only through the deliberate or accidental ingestion of substances but also human activities such as organophosphates poisoning from pesticides, algicides, algal toxicity, rodenticides, radionucloides, and fire ants delays the production of Nile crocodiles (Huchzermeyer, 2003).

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Figure 1 - Persisting Kyphoskoliosis in a juvenile Nile crocodile after recovery from osteomalacia. Source: Huchzermeyer, and Van Wyk (2003).

CROCODILE DISEASES AT ARBA MINCH CROCODILES RANCH, ETHIOPIA.

Arba Minch Crocodile Ranch (AMCR) is found in Arba Minch, where it is located 500 km south of Addis Ababa with an altitude range from 1100-2800 m above sea level. Arba Minch district covers 173,108 hectares and has three climatic zones; lowland (37.5%), midland (40.5%) and highland (22%) areas with an average mean temperature range of 15°- 31° C. The area has grasses, bushlands and deciduous forests with sandy and clay soil type (Kebede, 2006). In order to conserve and optimally utilize the Nile crocodile ranch was established in 1984 (Yeshdenber, 1994; Graham and Gebre, 1997; Kebede, 2006; Whitaker, 2007; Shirley et al., 2014).

Although crocodile ranching practiced in Ethiopia, for almost 35 years, it is difficult to find any work on health problems of crocodiles and management activities. The government of Ethiopia collaborated with CSG to study the crocodile ranching in the country and highlighted the documents housed at EWCA that focused mainly on surveys and conservation issues. Mahammed (2008) researched crocodile health at AMCR but his report remains unavailable. In his cross-sectional study, no parasites or their ova were found (n= 80; 60 Juvenile and 20 Adults). Clinical investigation of the same animals showed 20/80 abnormalities, predominantly hind legs, and skin lesions.

The clinical assessment of the previous study 25% (20 out of 80) had clinical abnormalities; 5 of the crocodiles were unable to move on dry land during basking, showing paralysis of two hind legs and slight swelling of the tail muscle, and yet were able to swim 'normally'. A further 5 animals showed multiple skin lesions, 5 showed circling movement while swimming in the water, and the remaining animals were dead before the examination. During post mortem examination, there was excessive yellow and hard fatty accumulation was identified in tail muscles. 6.25% (5 out of 80) had multiple skin lesions which were multiple and dominated by small erosions, on the ventral aspect of the abdomen and One crocodile showed ulcerative types of skin lesion over its head, neck, back, and tails. The sick crocodiles were found dead after 2-4 weeks. And showed accumulation of fluid in the pericardial sac slightly enlarged pale heart and liver. Most of these problems were observed on juveniles (young) and yearlings and occurred after they were provided with frozen fish meat (Mahammed, 2008).

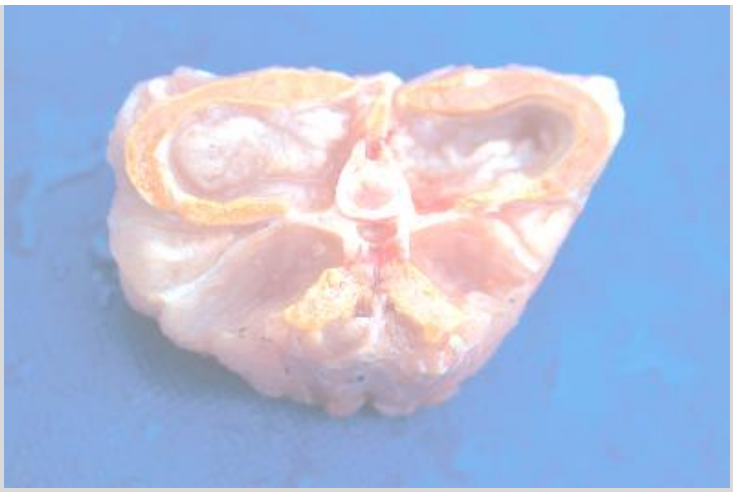

Figure 2 - Fat necrosis: hardened yellow fat between the tail muscles of a Nile crocodile. Source; Huchzermeyer and Van Wyk (2003).

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Nutritional Diseases of Nile crocodiles in AMCR

Nutritional bone disease is an umbrella term that covers a range of related conditions and names, such as osteomalacia, rickets, metabolic bone disease, fibrous osteodystrophy, and osteoporosis. Metabolic bone disease shifts the emphasis on calcium (Ca) and phosphorus (P) metabolism. Osteo-malacia and fibrous osteodystrophy are the terms for the condition in young hatchlings where their bones fail to harden due to the lack of calcium. Rickets applies to malformations of the growing bone when due to the lack of vitamin D_3, the bones also fail to harden and become bent. Osteoporosis occurs in older juveniles and adults, where the already hardened bone structure becomes weakened by the withdrawal of calcium for metabolic needs (Huchzermeyer, 2003). The Crocodiles in AMCR were provided mainly with feed items like fish and meat. The meat that was used as a feed for crocodiles includes meat from dead old horses, donkeys, dogs, cattle, sheep, goats, and crocodiles themselves. The crocodiles are fed three times per week or once every two days while they were young. The pathological finding was most frequently arising from nutritional deficiency as a result of crocodiles being fed meat meals, such as frozen fish and frozen meat without a bone meal (Mahammed, 2008). There is known to be deficient in important minerals and vitamins (Gilber, 2000).

Feeding frozen fish has two limitations; the first is that fresh and frozen fish often contain large amounts of the enzymes thiaminases. Freezing appears to increase the concentration of the thiaminases in the tissue of fish, which destroys the vitamin B_1 (thiamine); the second problem is an accumulation of fats in the subcutaneous and intramuscular tissue leading to paralysis of the legs, which is caused by particularly oily fish meals (Huchzermeyer, 2002; Huchzermeyer, 2003). For the treatment of nutritional bone disease, it is necessary to rectify the diagnosed deficiency, usually that of calcium. If the affected hatchlings are too weak to feed by themselves, they can initially be dosed or injected intra-peritoneal (IP) with calcium borogluconate (250 mg/ml), at a dosage of 1.5ml/kg body mass. The corrected ration should contain additional calcium carbonate, dicalcium phosphate, or sterilized bone meal, to give a final composition containing 1.5–2% calcium and a Ca:P ratio of 1.5:1 (Huchzermeyer, 2003).

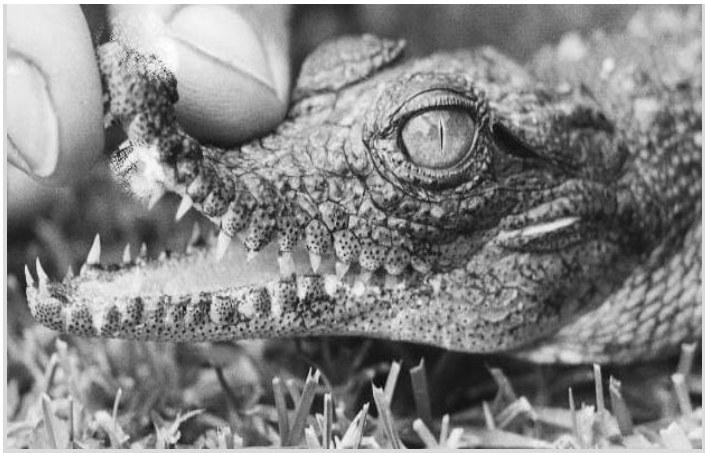

Figure 3 - 'Rubber jaws' and 'glassy teeth' in a Nile crocodile hatchling with osteomalacia. Source; Huchzermeyer and Van Wyk (2003).

Skin Diseases of Nile crocodiles in AMCR

Skin lesions were the second most common problems observed at AMCR after paralysis of hind legs (Mahammed, 2008). Dermatophilosis is one of the two-specific bacterial skin infections after Erysipelothrix. The other two known forms, 'winter sores', with yellow-brownish crusty lesions, and chronic stress dermatitis, with patches of white discoloration, particularly on the head around eyes and nostrils, are non-specific and many bacterial species can be involved. No occurrence of dermatophilosis has been reported, yet (Gilber, 2000; Huchzermeyer, 2003). Crocodile pox is one of the viral skin diseases of Nile crocodile, caused by Parapoxvirus. Based on the histopathological diagnosis of the skin lesions, showed the finding of the typical intra-cytoplasmic inclusion bodies. Moreover, fungal infections of the skin occur either locally or generalized under unhygienic conditions in animals with reduced immune capacity due to stress or cold. Superficial infections in the epidermis do not provoke much of an inflammatory response. Deeper infections cause a granulomatous reaction and not an exudative one (fibriscess), as in the case of bacterial infections. The treatment of deep granulomatous lesions may need the application of systemic fungicides, such as ketoconazole. There is no specific treatment; hygienic sanitation is the best prevention provision (Gilber, 2000; Huchzermeyer, 2003).

CONCLUSION AND RECOMMENDATIONS

Crocodylus niloticus is found in 26 African countries, including Ethiopia. In 1972 commercial hunting of crocodiles was prohibited in Ethiopia and *C. niloticus* was listed the Nile crocodile in Appendix II of CITES. The decreasing number of species in AMCR affected by low management skills, environmental factors, anthropogenic effects, health problems, and misguided hunting. The isolated causative agents of the species abnormalities require more attention and commitment to resolve the problems. Infectious diseases of bacterial, viral, parasitic, and fungal infections have to be managed wisely and crocodile farmers and other stakeholders in the crocodile industry must focus on causes of the degenerative and

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metabolic disorders. EWCA is losing its foreign currencies income from consumptive and non-consumptive advantages. In conclusion, the office of AMCR must work with professionals and research groups. Besides, the Arba Minch Crocodile Ranch (AMCR) should exercise care and caution when introducing hatchlings from the wild into the Ranch and enhance its husbandry methods to reduce the occurrence of infection and disease. The recommendations by Crocodile Specialist Group (CSG) on Management of Crocodile in Ethiopia should be addressed and implemented.

DECLARATIONS

Authors' contributions

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interest.

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EVALUATION OF THE CHEMICAL COMPOSITION OF ARGAN (*Argania spinosa* L.) OIL ACCORDING TO ITS EXTRACTION METHOD, ORIGIN OF PRODUCTION AND ALTITUDE

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ABSTRACT: In this study the chemical composition of Argan (*Argania spinosa* L.) oil was evaluated according to its mode of extraction, origin of production and altitude of the Argan tree. To carry out this work, the physico-chemical characteristics and chemical composition of 5 samples differing by their mode of extraction or coming from different regions was compared. The study of the physicochemical characteristics of the 5 samples showed that the roasting of the almonds of the Argan fruit as a parameter can increase the value of the peroxide index, decrease the percentage of α-tocopherol and the unsaponifiable rates in percentage. Also it found that geographic origin can influence fatty acid values (behenic acid, C22:0). The results of the specific extinction and the refractive index did not give any precise information on the origin, the altitude and the method of extraction of Argan oil. The study of the triglyceride fraction showed that the geographical origin of northeastern Morocco can increase the value of triglyceride. Present study has indicated that the high quality of Argan oil can be extracted by mechanical pressing and hence, the present results may support the commercialization of Argan oil.

Keywords: Argan, Chemical composition, Extraction method, Nutritional value, Sapotaceae.

INTRODUCTION

Argan (*Argania spinosa* L. Skeels) is a specifically Moroccan endemic plant (El Youbi et al., 2010), it is a rustic, xero-thermophilic species, which belongs to the tropical family of Sapotaceae, of which it is the only northern representative in the Mediterranean region (Algeria and Morocco) hence its marked endemism in Morrocoregion (Véla et al., 2007). Morocco is one of the countries in North Africa to have a set of endemic ecosystems of remarkable biodiversity (Faouzi et al., 2015). It has great medicinal and therapeutic benefits (Moukal et al., 2004; Lizard et al., 2017; Idm'hand et al., 2020). In addition, it is highly sought after in cosmetics as a skin and hair-conditioning agent (El Abbassi et al., 2014). This ecosystem is based on a balance between resources and human exploitation and plays an important role in the fight against desertification and erosion (Bellefontaine et al., 2010). Argan oil is the main product of the Argan tree. It is extracted in an ancestral way and sometimes under very precarious conditions (Khallouki et al., 2017). The artisanal extraction of a liter of oil requires 20 hours of strenuous and intense work (Charrouf et al., 2007).

Argan oil is rich in oleic acid, which makes this oil particularly interesting in the regulation of cholesterol. In addition, Argan oil is also rich in phytosterols which have an important activity and whose incorporation in a diet is supposed to offer cancer prevention (Cherki, 2016). Studies showed that polyphenols and phytosterols as well as a certain number of their derivatives have anti-tumor properties (Benani et al., 2007).

Great efforts have been made to develop Argan oil by improving its extraction technology and allowing forest users to benefit from this benefit by creating cooperatives in the region that produces and sells Argan oil (Faouzi et al., 2012). This work had repercussions in the production region, both socio-economic and environmental. Argan oil has a fatty acid composition close to the fraction of peanut or sesame oil, and their unsaponifiable fraction is of the same order of magnitude as that commonly observed in vegetable oils (Hanan et al., 2018).

Present work tried to make a study of the exhaustive physico-chemical/biochemical composition of Argan oil according to its mode of extraction and its origin of production, proving to be essential. The aim of this work is to study the influence of the region or the altitude near or far from the sea and the extraction method on the physicochemical characteristics and the chemical composition of Argan oil, in order to know the parameters that can degrade the quality of Argan oil.

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MATERIALS AND METHODS

Preparation of different samples of Argan oil

Biological material

This present work, we have selected 5 samples of the Argan fruit having different regions and its different mode of extraction (southwest, northeast and northwest of Maorc). Table 1 gives information on the origin, the extraction method and the altitude of the Argan tree of each sample.

Argan oil extraction

After the selection of five different samples by the mode of extraction or from different regions of Morocco. Argan oil is prepared by two different methods (Charrouf et al., 2007): 1) APR: Argan oil is extracted by mechanical pressing from roasted almonds; 2) APNR: Argan oil is extracted by mechanical pressing from unroasted almonds. These oils are then analyzed in the Official Laboratory of Analysis and Chemical Research (LOARC) of Casablanca in Morocco, the physico-chemical characteristics and the chemical composition of all the samples are determined (fatty acid, sterols, triglycerides, tocopherols). The oils are analyzed according to the analysis methods already described in the literature (European Standard, 1999). Table 1 provides information on the origin and method of extraction of each sample of Argan oil.

Table 1- Origin and method of extraction of the 5 samples

No.	Extraction mode	The region	Province	Altitude	The distance between the region and the sea
1	Roasted almond extracted by mechanical press (APR)	Tidzi	Essaouira North-West of Morocco	150 meters	25 Km
2	Unroasted almond extracted by mechanical press (APNR)	Tidzi	Essaouira North-owest of Morocco	150 meters	25 Km
3	Unroasted almond extracted by mechanical pressing (APNR)	Beniznassen	Oujda North-eastern of Morocco	1532 meters	100 km
4	Unroasted almond extracted by mechanical pressing (APNR)	Ait mzal	Chtouka ait baha Southwest of Morocco	933 meters	75 Km
5	Unroasted almond extracted by mechanical pressing (APNR)	Ighrem	Taroudant Southwest of Morocco	1277 meters	170 Km

Physicochemical analyzes of oils

All analyzes were done in the Official Laboratory of Chemical Analysis and Research (LOARC) in Casablanca, Morocco. Determination of acidity (Européenne Norme, 1999), the peroxide value (Lagardere, 2004), the refractive index (ISO, NFEN-2000) of the absorbance in the ultraviolet (Denormalisation, 2002), the saponification number (Denormalsation, 2002), the un-saponifiable content (Sylvester et al., 1945) were measured according to the standardized methods of reference.

Determination of composition and nature in total sterols

All of process was in according to reference ISO 6799 (Aïssi et al., 2009).

Operating mode

Weigh 2.5 g of Argan oil and put into a 20 ml flask. 25 ml of a solution of potassium hydroxide (1N of ethanol) is added. The flask is heated under reflux for 30 minutes until the solution becomes clear. Then, 25 ml of distilled water is added to stop the reaction. The extraction of the unsaponifiable is carried out using 75 ml of hexane or petroleum ether. The organic phase is subjected to a series of washing with 15 ml of mixture (water/ethanol 95°) (90/10) in a separatory funnel. The hexane phase is transferred from the top of the ampoule into a 100ml flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material is recovered. The unsaponifiable agent, diluted with 300 µl of hexane or petroleum ether, is filtered on a silica column (25cm × 4mm). The HPLC device is equipped with a 205 nm-254 nm UV detector. The eluent is an isooctane/isopropanol (99/1) mixture whose flow rate is 1.2 ml/min. The duration of the analysis is 15 min, the sterol fraction recovered according to standard NF 12228 May 1999 is evaporated to dryness. The sterols are converted to silylated derivatives (TMS) using a mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), (9/1/1), (v/v/v). The pyridine isevaporated to dryness and the silylated derivative is diluted with 60 µl of heptane or hexane. The TMS sterols are analyzed by gas chromatography (GC) on an apolar column (Chroma pack) (30m × 0.32mm, DI: 0.25µm, phase: CPSIL8CB). The HP Hewlett Packard 6890 GC Series Chromatograph is equipped with FID detector (T°: 300°C). The carrier gas is nitrogen and its flow rateis 1 ml/min (P.E: 8.6 bar). The analysis is performed in temperature programming (200 °C up to 270 °C with a speed of 10 °C/min and an isotherm at 270 °C for 35 min).

Analysis of cis-fatty acids

Reference: NF ISO 5509 COFRAC code: CC30 (Normalization 2015).

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Operating mode

The test sample of Argan oil 1g is supplemented with 0.5 ml of methanolic KOH for HPLC (minimum 98%) and 10 ml of methanol in a 100 ml flask. The mixture is refluxed for 15 minutes until the solution is clear. Then 1 ml of heptane is added to the reaction mixture after cooling. The heptanic phase containing the methyl esters is transferred to a test tube and then a solution of sodium carbonate Na_2CO_3 is added. This neutralizes all free acids by giving sodium salts with a release of carbon dioxide. The methyl esters, which are in the organic phase, are removed using a 2 ml cone pipette and placed in a test tube. The methyl esters undergo a series of washing20ml are taken from the esters, which are placed in a tube of nominal capacity of 2 ml and then filled with heptane. The fatty acid methyl esters are analyzed by GC gas chromatography. The HP Hewlett Packard 6890 GC Series GC chromatograph is equipped with a divider (T: 240 °C) and a FID (T: 260 °C) injector. The carrier gas is nitrogen (PE: 12.4 bar). The analysis is carried out in temperature programming (140 °C to 200 °C with a speed of 10 °C/min and an isotherm at 200 °C for 40 min) on a capillary column (polyethylene glycol) (30 m × 0,32 mm, DI: 0.25 µm).

Tocophérols analysis (Lara-Ortega et al., 2017)

Operating mode

In a 25 ml volumetric flask, 2 g of Argan oil was diluted with 2,2,4-trimethyl pentane. The test sample is added to 2, 2, 4-trimethyl pentane up to the mark, then mixed thoroughly. The tocopherols are analyzed by HPLC, on a silica column (25 cm × 4 mm), according to the AOCS method, official method CE8-89 revised 1990 updated 1992. The SHIMADZU brand device is equipped with a fluorimetric detector (excitation wavelength 290 nm - emission wavelength 330 nm). The elution is carried out with a mixture (isooctane/isopropanol) (99/1) with a flow rate of 1.2 ml/min during the analysis time (20 min).

Triglyceride analysis

Reference: IUPAC No. 2.0 324 (Brand et al., 2014).

Operating mode

To 0.15 g of the Argan oil are added 0.5 ml of hexane and 15 ml of a mixture of hexane/diethyl ether (87/13). This solution is poured into a supelco brand cartridge with 0.5 g of silica gel previously activated with hexane. The triglyceride fraction is thus separated from the diglycerides and monoglycerides. It is recovered in a 100 ml flask. It is subjected to analysis after evaporation of the solvent and dilution with 1.5 ml of acetone. The triglycerides are analyzed by HPLC on a reverse phase C18 column (250 mm × 4.6 mm, Φ silica 5 µm), according to IUPAC Method No. 2.0324. The HPLC apparatus is equipped with an HP refractometric detector 10 47A. Elution is carried out with a mixture (acetonitrile/acetone) (v/v) with a flow rate of 0.5 ml/min during the analysis time (90 min).

RESULTS

Analysis of physico-chemical characteristics

Table 2 shows the results of the acidity value, the unsaponifiable rate, the saponification index and the specific extinction values at 270 nm (k270). All the acidity values observed are less than 1%. This result shows that Argan oil is characterized by low acidity compared to other vegetable oils (acidity of olive oil ≤ 2%) (Hilali et al., 2005). The acidity of samples 1 and 2 (0.33%, 0.50% respectively) (belonging to the same batch of Tidzi) is higher compared to other samples such as 4.5, (0.28%, 0.14 respectively) (belonging to different lot). These results suggest that the origin may influence the acidity values of Argan oil. Roasting also appears as a parameter influencing the acidity value of Argan oil (sample 1 and 2). The acidity value of sample 3 is higher compared to that of other samples. This result can be linked to the geographic origin of the sample because this sample comes from the higher elevation lot (1532m). The unsaponifiable rate of Argan oil is less than 0.8% (for virgin olive oil, it is less than or equal to 1.50%) (Brajol, 2014). Argan oil extraction technology can influence the level of unsaponifiable matter in Argan oil. Indeed, the unsaponifiable rate of sample 1 obtained by extraction by mechanical pressing from roasted almonds is lower (0.55%) than that which is prepared by mechanical press from non-roasted almonds (0.71 %). The Argan oil saponification index (Table 2) was found between 180.0 and 199.0. For virgin olive oil, it is between 180 and 198) (Hilali et al., 2005).

Table 2 - The main physico-chemical constants of the 19 samples of Argan oil studied.

No.	1	2	3	4	5
Acidity in%	0.33	0.50	0.67	0.28	0.14
Unsaponifiable rate in%	0.55	0.71	0.63	0.56	0.54
Saponification index	197.9	180.0	189.6	183.3	183.5
Peroxide index in meq of O_2 / kg	1.23	0.24	2.40	1.46	1.68
Specific extinction at 270 nm (k270).	0.228	0.282	0.291	0.392	0.277
The refractive index 20°C	1,4705	1,4705	1,4691	1,4667	1,4682

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The study of this work shows a great variation between the values of the saponification index of Argan oil extracted from roasted and non-roasted almonds of the same batch (Tidzi). Indeed, samples 2 have a low saponification value (180). The specific extinction of Argan oil was determined at 270 nm. In general, the values found are higher than that of olive oil, they vary between 0.228 and 0.426 for Argan oil. The peroxide index results for the 5 samples of Argan oil. For all samples, a peroxide index lower than that required for virgin olive oil was observed. The peroxide index in sample 1 is higher. Indeed, this sample is taken from a lot nearest the Atlantic Ocean and at the same time is extracted from roasted almonds. This result clearly indicates that some components of Argan oil are extremely sensitive to oxidation. The high peroxide content is observed for sample 1. This is probably related to the extraction method, the hygienic and extraction conditions and the use of water in the preparation of the oil and also related to the geographic location. The determination of the peroxide index seems to be a critical measure for the evaluation of the quality of Argan oil. The determination of the refractive index, in general, is used for a quick and reliable verification of the purity of a substance. Both the refractive index and the density depend on the chemical composition of the oil and its temperature. It grows with the establishment and presence on fatty chains of secondary functions. The refractive index was determined at 20 °C. The results show that this index varies between 1.4667 and 1.4705.

Analysis of fatty acids

The fatty acid composition of the different oils was determined after methylation of the oil and analysis of the methyl esters by gas chromatography on a capillary column. Table 3 groups together the results obtained for the 5 samples. The fatty acid composition corroborates with data from the literature (Rahmani, 2005). Argan oil contains 80% unsaturated fatty acids. It is of the oleic – linoleic type and contains between 29 to 35% of essential fatty acids: linoleic acid (29 to 34%) (Vitamin F). This acid is said to be essential because it cannot be synthesized by the body and must be provided by food. Unsaturated fatty acids play an essential role in the prevention of cardiovascular disease and the omega 6 family (such as linoleic acid) is essential for the growth of the child (Lapillonne 2007). Its oleic acid content makes Argan oil particularly interesting in regulating cholesterol.

The other fatty acids present are: myristic acid C14: 0 (0.10 to 0.15%), palmitic C16: 0 (11 to 13%) and stearic C 18: 0 (5 to 7%). The percentage of linolenic acid (C18: 3) in Argan oil does not exceed 0.1%. Note the presence in Argan oil of long chain fatty acids such as C20: 0 (0.4%), C20: 1 (0.5%), and C22: 0 (0.1%). No significant variation was observed between the different samples. Sample 3 contains a higher percentage of behinic acid (C22: 0) (0.38%). On the other hand, this percentage does not exceed 0.1% for all the other samples; this sample is prepared from Argan almonds gathered in the Benaiznassen plantation. These variations can be considered useful markers to ascertain the geographical origin of Argan oils. No major significant variation was observed between samples. This demonstrated that the origin and the geographical process cannot influence the dietary qualities of Argan oil. These results agree with those reported by Louni (2009) and Kechairi (2009) which showed that climatic conditions have no marked influence on the fatty acid composition of the oils of Argan fruit from different localities.

Table 3 - Fatty acid composition of samples 1 to 5 (%).

Samples	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$
1	0.12	0.04	12.45	0.04	0.08	5.44	47.11	33.53	0.09	0.36	0.44	0.11
2	0.11	0.04	12.06	0.01	0.08	5.77	47.76	32.69	0.08	0.40	0.47	0.14
3	0.15	0.04	12.06	0.09	0.07	6.35	48.32	31.73	-	0.35	0.41	0.38
4	0.11	0.04	12.56	0.07	0.08	6.94	45.05	33.74	0.10	0.47	0.43	0.17
5	0.11	0.05	12.75	0.08	0.08	6.12	47.64	31.73	0.08	0.44	0.49	0.17

Trans-fatty acid analysis

The trans-fatty acid composition of the different oil samples was determined after methylation of the oil and analysis of the methyl esters by gas chromatography. Table 4 groups together the results obtained for the 5 samples. It appears from this result that the percentage of trans-oleic and linoleic acid (C18: 1 and C18: 2), (elaidic acid) in Argan oil is low and varies between 0.01% and 0.02%. The results are similar to those found for olive oil (Hilali et al., 2005). The presence of trans-fatty acids in "virgin" Argan oils, suitable for consumption, is an indication of the fraudulent presence of refined oil. For this reason, the trans-fatty acid content has been limited by the standard to 0.05% for both elaidic acid and the sum of the trans-isomers of linoleic and linolenic acids.

Table 4 - Composition of trans fatty acids in samples 1 to 5

Samples	1	2	3	4	5
%C18 :1trans	0.02	0.01	0.01	0.01	Trace
%C 18:2trans	0.02	0.02	0.02	0.02	0.01

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Triglyceride analysis

The triglycerides of the different Argan oil samples analyzed by high performance liquid chromatography are grouped in Table 5. Analysis of the triglyceride fraction of Argan oil by HPLC allowed the separation of the individual triglycerides. We note the predominance of triglycerides LLO (12 to 14%), LOO (13 to 16%), LOP (14%), OOO (11 to 14%) and POO (15 to 16%). It is also noted that the oleic and linoleic acids occupy most of the Sn-2 position. Our results are in agreement with the data in the literature (El Youbi et al., 2010; De Normalisation, 2010, 2015; Gharby et al., 2013) which indicate that the triglycerides LLL, LLO, LOO, LOP, OOO and POO are predominant in Argan oil. Samples 3 has a high percentage of triglycerides SOP (4%) this result clearly shows that the geographical location or the origin of the Argan fruit can influence the chemical compositions.

Table 5 - Triglyceride composition of samples 1 to 5 (%)

Samples	LLL	LLO	LLP	LOO	LOP	PPL	OOO	POO	OPP	LPS	SOO	SOP
1	6.89	13.22	5.85	15.96	14.18	1.94	13.77	16.05	4.06	0.31	4.58	2.25
2	7.43	13.80	6.17	16.27	13.96	2.05	13.74	15.74	3.76	0.32	4.69	1.91
3	6.84	12.20	6.00	14.31	13.22	2.08	13.98	15.48	4.39	0.66	6.04	4.00
4	7.69	13.41	6.55	13.93	14.57	2.14	11.32	15.17	4.37	0.11	4.61	2.11
5	7.54	12.91	5.77	14.80	13.64	1.86	14.03	16.32	4.16	0.21	4.10	1.77

LLL: trilinoleoylglycerol, LLO: linoleoyl-linoleoyl-oleoylglycerol, LLP: linoleoyl-linoleoyl-palmitoylglycerol, LOO: linoleoyl-oleoyl-oleoylglycerol, LOP: linoleoyl-oleoyl-palmitoylglycerol, PPL: palmitoyl-plamitoyl-linoleoylglycerol, OOO: trioleoylglycerol, POO: palmitoyl-oleoyl-oleoylglycerol, OPP: oleoyl-palmitoyl-palmitoylglycerol, LPS: linoleoyl-palmitoyl-stearoylglycerol, SOO: stearoyl-oleoyl-oleoylglycerol, and SOP: stearoyl-oleoyl-palmitoylglycerol.

Sterol analysis

The sterol composition of the various Argan oil samples was determined by gas chromatography after silylation of the sterol fraction. The latter is obtained by fractionation of the unsaponifiable matter of Argan oil by HPLC on a normal phase. This analysis was carried out in the presence of an internal witness: 0.2% α-cholestanol in chloroform. The various sterols encountered were identified by gas chromatography coupled to mass spectrometry and by comparison with data from the literature (Gharby, 2013). Their individual and total assay was possible by GPC using an internal standard: α-cholestanol 0.2% in chloroform. Table 6 summarizes the results obtained for the 5 samples selected.

The total sterol content of all samples of Argan oil ranges from 130 to 206mg / 100g of fat. This is not negligible compared to other seed and olive oils. The sterolic composition is in accordance with data from the literature (Hilali et al., 2007). They are essentially Δ-7-stigmasterols. The main products are schottenol (or Δ-7-stigmasterol) and spinasterol. Their proportion varies respectively between 42 and 48%, and 34 and 42%. Schottenol and spinasterol are rarely found in vegetable oils and are characteristic of this oil. Two minority sterols were identified on the basis of their mass spectrum obtained by GC / MS and by comparison with data from the literature (Hamia and Yousfi, 2007). These are stigmast-8,22-diene and stigmasta-7,24-28-diene (or Δ-7-avenasterol). Their proportion varies between 2.6% and 6.9% of the mixture of total sterols. It's found that the content of campesterol in Argan oil is very low (0.3%) compared to other seed oils and olive oil. This parameter can be taken as a marker to detect adulteration of Argan oil. Also the percentage of total sterols is higher for the sample extracted from unroasted almonds (2 to 5). The variation in the sterol composition of the different samples is not significant.

Table 6 - Composition in sterols of samples 1 to 5 (mg/100g)

samples	Campest.	Stigma 8,22	Spinast.	Schott.	Stigma 7,24	Total
1	0.20	4.31	37.07	46.66	4.81	142.0
2	0.17	4.57	38.50	43.39	5.94	158.2
3	0.11	4.85	35.44	48.47	2.57	206.3
4	0.24	4.77	39.17	44.99	4.71	147.4
5	0.31	5.40	39.29	46.12	3.55	130.0

*Campest.: Δ5-campesterol; 8.22 Stigma: Stigmasta-8,22-diene-3β-ol; Spinast .: spinasterol; Schott: schottenol; Stigma 7.24: stigmasta-7.24-diene-3β-ol.

Tocopherol analysis

The tocopherols were analyzed by HPLC on a column in the normal phase, directly from vegetable oil without saponification. They were identified by comparison of their chromatogram with controls injected under the same conditions. Their dosage was possible by the use of α-tocopherol. The results obtained are grouped in Table 7. Argan oil is richer in tocopherol (633 to 775 mg / kg) than olive oil (50 to 150 mg / kg) and, than hazelnut oil (300 to 550 mg / kg) (Hilali et al., 2007). Tocopherols have vitamin E activity. This vitamin is a powerful antioxidant that captures free radicals and neutralizes destructive oxidation (Nkhili, 2009). Present study shows that our samples are rich in γ-tocopherol (80 to 90%), Tocopherols are natural antioxidants, and gamma tocopherol has the highest antioxidant power. Rich in gamma tocopherol, Argan oil is a valuable nutraceutical. Tocopherols and polyphenols are natural antioxidants. These play an

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essential role in the prevention of several diseases (Jager, 1968), because they are anti-free radicals. We found that samples 5, have a low content of total tocopherols (633 mg/kg). The roasting of almonds has an influence on total tocopherols. Indeed, the oils extracted from unroasted almonds have a higher total α-tocopherol content compared to the samples extracted from roasted almonds (sample 1 versus 2).

Table 7- Composition of tocopherols in samples 1 to 5 (mg/kg)

Samples	γ-tocopherol	δ-tocopherol	α-tocopherol	β-tocopherol	total
1	631.3	59.5	26.6	-	717.4
2	621.1	50.9	32.7	-	704.7
3	701.1	37.2	37.2	-	775.5
4	615.6	38.0	33.2	-	686.8
5	545.9	38.7	49.3	-	633.9

DISCUSSION

As part of the development of Argan oil, we conducted a comparative study of the different physico-chemical parameters of Argan oil according to its mode of extraction and its origin of production. To carry out this work, we selected 5 samples of Argan fruit located in different geographical localities of Morocco and extracted in different ways (by mechanical pressing from roasted and non-roasted almonds). The study of the physico-chemical characteristics shows that all the acidity values of Argan oils are less than 1.40%. This result shows that Argan oil is characterized by low acidity compared to other vegetable oils (acidity of olive oil ≤2%).

Present study indicates that roasting appears as a parameter influencing the acidity value of Argan oil. This is because the acidity value is higher in Argan oil samples prepared from unroasted almonds. We also found that the Argan oil sample from Tamanar batch had a higher acidity value compared to the samples. This finding suggests that geographic origin may influence acidity values. The results concerning the unsaponifiable rate shows that Argan oil is characterized by a low unsaponifiable rate (unsaponifiable rate ≤ 0.81%) (Olive ≤ 1.50%) (Charrouf et al., 2008). Argan oil extraction technology can influence the unsaponifiable level of Argan oil. In fact, the level of unsaponifiable matter in the sample prepared from roasted almonds is lower (0.56%) than that prepared from non-roasted almonds. Our study also shows that the roasting and the origin of the Argan fruit have an influence on the reduction of this parameter (samples 1 and 2). Analysis of the peroxide index shows that the sample of Argan oil extracted from roasted almonds has a higher peroxide content compared to sample 2 (same batch). The determination of the peroxide index seems to be a critical measure for the evaluation of the quality of Argan oil. The specific extinction and the refractive index give no precise information on the origin and the method of extraction of Argan oil.

Analysis of fatty acids shows that Argan oil contains 80% unsaturated fatty acids. It is of the oleic – linoleic type and contains between 29 to 35% of essential fatty acids: linoleic acid (29 to 34%). Its oleic acid content makes this oil particularly interesting in regulating cholesterol. Our results showed that the percentage of behinic acid (C22: 0) is higher in the sample which was prepared from the Argan almonds gathered in the Benaiznassen plantation. These variations can be considered useful markers to ascertain the geographical origin of Argan oils. Sterol analysis shows that the total sterol levels of Argan oil vary between 130 to 206 mg/100g of fat. The sterolic composition consists essentially of Δ-7-stigmasterols. The main products are schottenol (or Δ-7-stigmasterol) and spinasterol. It is noted that schottenol and spinasterol, which are very rare in vegetable oils, can be a parameter for the detection of adulteration of this oil. Two minority sterols were identified on the basis of their mass spectrum obtained by GC / MS. These are stigmast-8,22-diene and stigmasta-7,24-28-diene (or Δ-7-avenasterol).

The sterol composition does not show any significant variation. These results agree with those reported in the literature (Monfalouti et al., 2010). Argan oil is richer in tocopherols (633 to 775 mg / kg) than olive oil (50 to 150 mg / kg) and hazelnut oil (300 to 550 mg/kg). The results for tocopherols show that the extraction method and roasting can influence the composition of tocopherols. In contrast, the sample obtained from roasted almonds has a lower content of total α-tocopherols. Roasting decreases the total α-tocopherol content (Hilali et al., 2005). Analysis of the triglyceride fraction of Argan oil allowed the separation of individual triglycerides. We note the predominance of triglycerides LLO (12% -14%), LOO (13% -15%), LOP (14%), OOO (12% -14%), and POO (14% -17%) in l 'Argan Oil. These triglycerides represent approximately 73% of each fraction of triglycerides in Argan oil. The triglyceride results do not give any specific information on the geographical origin and the extraction process of the Argan fruit.

CONCLUSION

The results of this study indicated that the extraction method and the origin of the fruit of the Argan tree can influence the peroxide index, the rate of unsaponifiable matter, fatty acids (including behinic; C22: 0), the content of α-tocopherol and triglycerides (SOP). Present study has demonstrated the high quality of Argan oil extracted by mechanical pressing and the results of this work have helped support the commercialization of Argan oil worldwide.

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DECLARATIONS

Availability of data

The data can be availed to the journal upon request.

Conflict of Interest:

The author declare that there is no conflict of interests regarding the publication of this paper

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MATERNAL IMMUNOGLOBULIN IN THE SERUM OF NEWBORN LAMBS AND ITS RELATION WITH NEONATAL MORTALITY

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ABSTRACT: The study was conducted on 153 neonatal lambs of one of the highland breeds of sheep, locally called "Menz sheep" in North-Eastern part of Ethiopia, with the aim of assessing the relationship of total serum immunoglobulin level and neonatal lamb mortality in the first one month of life. The overall mortality in neonates was 8.5%. Surviving lambs (2.43±0.35 kg) were significantly heavier than those that died during the neonatal period (2.21±0.55 kg). Males (2.45±0.31 kg) were significantly heavier than females (2.37±0.43 kg). The lambs that survived the neonatal period had a significantly higher level of immunoglobulin (31.71±12.88 Zinc Sulphate Turbidity units) than those that died (12.77±5.25 Zinc Sulphate Turbidity units). Neonatal lambs with total serum immunoglobulin levels below 12 Zinc Sulphate Turbidity units may be considered as an indication of failure of passive transfer of colostrum immunoglobulins and consequently increased the susceptibility of lambs to diseases and subsequent deaths. The neonatal lambs with lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 Zinc Sulphate Turbidity units) had found dead before the first 30 days of their age. Most deaths of lambs occur in the first few days of birth that are typically associated with lower birth weight which also led to weakness, taking longer time to stand up and reduced chance of survival than lambs of heavier weight. Hence, several works have to be done to further improving the birth weight of newborn lambs as well as the nutritional status of the dam so that lambs may receive sufficient and good quality amount of colostrum from the first few hours of birth.

Keywords: Colostrum, Immunoglobulins, Neonatal lamb, Mortality.

INTRODUCTION

Neonatal lambs are extremely vulnerable to infectious diseases as they are born immunologically nave. A major factor affecting neonatal sensibility to pathogens is the permeable immature gut. This permeability of the gut allows the initial immunoglobulin passage, but increases also the risk for pathogens to enter (Fischer et al., 2019). However, colostrum ingestion itself accelerates the process of intestinal closure; thereby it is also preventing the route of neonatal infection (Dwyer, 2008).

The maternal immunoglobulins acquired through the colostrum play a pivotal role in the defense mechanism of lambs against neonatal diseases until their own immune system is primed and produces a protective amount of antibodies. Immunoglobulins are not detected in the serum of lambs before the first intake of colostrum (Klobasa and Werhahn, 1989). The absorption of immunoglobulins from the intestine is maximum during first six hours of life and no absorption occurs 24-36 hours postpartum. Hence, the peak Ig levels are obtained in the serum of neonatal ruminants around these times in the immediate postpartum period (Tizard, 1992). Ideally, the maternal immunity should be transferred in utero to their fetuses so that they are brought into the world protected against the microorganisms (Fisher, 1980; Godden et al., 2019). However, placental barriers in ruminants do not allow the passage of immunoglobulins from dams to neonates, and therefore the lamb has to be dependent entirely on antibodies received via colostrum (Tizard, 1992). Colostrum is not only rich in immunoglobulins as compared to milk but is also an excellent source of energy, vitamin A and essential minerals (Khan and Khan, 1996).

Globulin proteins are serum proteins that are classified into three groups in ruminants; α-, β-, and γ- globulins (Tizard, 1987). The γ-globulin fraction contains mainly immunoglobulins, which are proteins with antibody activity (Tizard, 1987). According to Tizard (1987), sheep have four different types of immunoglobulins; IgG, IgA, IgM and IgE and IgG is the immunoglobulin found in highest concentration in serum. Sheep have an epitheliochorial placenta, the immunoglobulins do not cross the placental barrier and the lamb is born without any circulating antibodies. The passive immune transfer from the ewes' colostrum to the lamb is of utmost importance for the survival of the offspring, providing it with some resistance against infectious diseases (Nowak and Poindron, 2006). Immunoglobulin-synthesis is initiated at approximately 3 weeks of age in neonatal lambs (Klobasa et al., 1985).

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Intestinal closure happens approximately 24 hours after birth, meaning that the passive absorption of immunoglobulins in the intestine seize. When suckling begins; the level of immunoglobulins in the blood starts to rise rapidly during the first hour and reaches a peak around 24 hours after parturition (Nowak and Poindron, 2006). Shubber et al, (1979), concluded that larger volumes of colostrum correlate with larger amounts of immunoglobulins. Adequate passive immune transfer (PIT) has been determined in some studies to be reached when the lambs IgG intake was above 30 g during the first 24 h of life (Alves et al., 2015). Consequently some studies suggest that the failure of passive immune transfer (FPIT) for the neonatal lamb has a significant effect on neonatal mortality and losses because of infectious causes correlate positively with low concentrations of serum immunoglobulins (Ahmad et al., 2000). The intestine is unselectively permeable, therefore, all immunoglobulins types can be absorbed (Sawyer et al., 1977). The permeability remains highest immediately after birth to 6 hours of life. Then immature fetal type of cells capable of transfer of intact immunoglobulins is gradually replaced by a digestive type of cells (Tizard, 1992). Smeaton and Simpson-Morgan (1985) also observed that the layer of cells responsible for absorption of colostral antibodies progressively disappears from the villi, resulting in closure which usually completes 24-36 hours after birth (Khan and Khan, 1991 b). So, lambs absorb intact immunoglobulins from ingested colostrum only during the first day of life (Klobasa et al., 1986). Immunoglobulins reach peak level on day I of lamb life, then decline during the next 3 weeks (Smith et al., 1976).

According to Bekele et al. (1992), failure and partial failure of Ig transfer from dam to lambs are observed in 1.8 and 15.3 per cent lambs, respectively. Passive transfer failure was observed in 14 per cent of apparently healthy lambs and in 46 per cent of lambs dying of natural causes between 24 hours and 5 weeks of age (Sawyer et al., 1977). The results of Logan and Irwin (1977) showed that about 20.2 % of lambs born were hypogammaglobulinaemic and were more susceptible to neonatal diseases. Findlay (1973) also observed that all lambs with immunoglobulins less than 20 ZST units die during first week of life, mortality in lambs with 20-40 ZST units was very low and no mortality in lambs with 50 ZST units.

Failure of passive transfer of immunoglobulins to neonatal lambs has a significant effect on neonatal mortality, and losses due to infectious causes are positively correlated with low concentrations of serum immunoglobulins (Sallam, 2019; Ibrahim et al., 2020). According to Hodgson et al. (1992), morbidity and mortality rates are higher in colostrum-deprived lambs (80 and 67%) than colostrum fed lambs (20 and 13%) and 20% of colostrum-deprived lambs die within the first week of life. The concentration of these maternal immunoglobulins in the circulation at 24 hours after birth can be used as an indication of sufficient immunity for the survival of neonatal lambs or susceptibility of lambs to neonatal diseases (Reid, 1972). There are several simple tests that can be used to verify whether or not neonates have received adequate colostrum. The most popular test is the zinc sulphate turbidity test (Roy, 1990). This test has been used by different authors in different species of animals and has been found to be in good agreement with immunoglobulins values determined by other laboratory techniques and it is also simple to use in the laboratory (Ahmad et al., 2000). Hence, the objective of this study is to measure the influence of failure of maternal immunoglobulin transfer on mortality of lambs in the first 30 days of life.

MATERIALS AND METHODS

Study area

The study was conducted in Debre Birhan Agricultural research Center (DBARC). DBARC is found in North Shewa Administrative Zone of the Amhara National Regional State, North eastern part of Ethiopia. It is located in the central part of the Nation, at a road distance of about 120 kilometers from Addis Ababa, the capital city of the country. Geographically, the area lies between 09 0 35'45" to 09 0 36'45" north latitude and 39 0 29'40" to 39 0 31'30" east longitude with an average elevation of about 2828 meters above sea level. It has an average annual rain fall of about 897.8mm and mean annual temperature of about 19.9 °C.

Study population

The lambs which were born from indigenous breeds of sheep (locally named as Menz sheep) were included in the study. The study animals were sourced from the dams which were kept in semi-intensive management system in DBARC. These animals were provided harvested hay and commercial concentrate feed in addition to the morning and afternoon pasture grazing. Both broad and narrow-spectrum anthelmintic drugs were administered against internal parasites based on the laboratory findings and the sheep were also vaccinated against major infectious diseases which include pasteurellosis, sheep and goat pox and peste des petits ruminants (PPR).

Study design and sampling method

An observational longitudinal study design study was conducted from August 2019 to October 2019 to evaluate the impact of level of maternal immunoglobulin transfer on mortality of neonatal lambs in the first 28 days of life. For this study, all the 153 lambs that were born during the activity season were included. Blood samples without anticoagulant were collected from the jugular vein of these new born lambs at the age of 24–48 hours post partem. Serum was separated and stored at -20 °C for further processing and birth weight of lambs was recorded. The health of all lambs under study was monitored daily during the neonatal period. All the lambs that were included in the study were followed up starting from the date of sampling up to the first 28 days of age. By this, the lambs that died were recorded and their

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level of serum immunoglobulin was measured using zinc sulphate turbidity (ZST) test based on the principle of McEwan et al. (1970).

Zinc sulphate turbidity test (ZST)

The principle of the test is that Zinc sulphate at a specific concentration precipitated the gamma globulin. This creates turbidity which is proportional to the quantity of gamma globulin in the sample and can be quantified in a calorimeter at 525 nm/Spectrophotometer 460 nm.

Test procedure

About 250 mg $ZnSO_4 \cdot 7H_2O$ was diluted in 1 L freshly boiled water (to remove CO_2) and 6-mL of the zinc sulphate solution was placed into sealed 7–10 mL plain blood collection tubes. Then, 0.1 mL serum was added to it and each tube was shaken by repeated inversion of the tube. After that, the mixture was kept for 1 hour at room temperature for the turbidity to develop. Finally, the turbidity developed in each tube was read in a spectrophotometer at a wavelength of 460 nm and the absorbance (optical density) of the turbid solution was determined and compared with control and percent turbidity calculated. Before taking the reading, null adjustment was made against the zinc sulphate solution and all the tubes were shaken further to make a uniform turbid solution.

Data analysis

All the data that were collected based on the above procedures were analyzed using multivariate analysis method of the General Linear Model in SPSS version 20.

RESULTS

The study considered 153 neonatal lambs and assessed for the level of serum immunoglobulins with in the first 48 hours of age after birth. 13 out of 153 lambs were died before 30 days of age with overall neonatal mortality of 8.5%. The mortality rates in male and female neonatal lambs were found 10.3 (8/78) and 6.7% (5/75), respectively.

Serum immunoglobulins levels

The mean serum immunoglobulins level recorded was 30.10±13.49 ZST units. The average serum immunoglobulins level of surviving lambs was found 31.71±12.88 ZST units while, the average serum immunoglobulins level of lambs that had died was found 12.77±5.25 ZST units) (Table 1).

Birth weight

The mean birth weight was 2.41±0.37 kg with a range of 1.2 to 3.6 kg. The males (2.45±0.31 kg) were found heavier than the females (2.37±0.43 kg). The surviving lambs (2.43±0.35 kg) were also heavier than those that died during the neonatal period (2.21±0.55 kg) (Table 2).

Table 1 - Mean ± Standard Deviation of serum immunoglobulin level of lambs in relation to survival and mortality during the neonatal period.

Parameter	Sex	Survival Status	Mean± Standard deviation	N
Immunoglobulins (ZST units)	F	Died	11.00±6.63[a]	5
		Survived	31.17±13.85[b]	70
		Total	29.83±14.38[a]	75
	M	Died	13.88±4.29[a]	8
		Survived	32.24±11.91[b]	70
		Total	30.36±12.66[a]	78
	Total	Died	12.77±5.25[a]	13
		Survived	31.71±12.88[b]	140
		Total	30.10±13.49	153

Different letters (a,b,c) in a column show significant differences ($p<0.05$); N: number of animals in each category; ZST: zinc sulphate turbidity test

Table 2 - Mean ± Standard Deviation of birth mass of lambs in relation to survival and mortality during the neonatal period.

Parameter	Sex	Survival Status	Mean± Standard deviation	N
Birth Weight (kg)	F	Died	2.03±0.53[a]	5
		Survived	2.40±0.41[b]	70
		Total	2.37±0.43	75
	M	Died	2.32±0.57[a]	8
		Survived	2.46±0.27[b]	70
		Total	2.45±0.31	78
	Total	Died	2.21±0.55[a]	13
		Survived	2.43±0.35[b]	140
		Total	2.41±0.37	153

Different letters (a,b,c) in a column show significant differences ($p<0.05$); N: number of animals in each category; ZST: zinc sulphate turbidity test

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DISCUSSION

From 153 lambs studied, 13 of them died before 30 days of age with overall neonatal mortality of about 8.5%. According to this study, the mortality rates in male and female neonatal lambs were found 10.3 (8/78) and 6.7% (5/70), respectively. However, there was no significant difference (P>0.05) between immunoglobulins levels of both sexes. Similarly, Cinpercescu (1977) and Esser et al. (1989) also reported no difference in male or female immunoglobulins levels.

Serum immunoglobulins levels

The present study finding, indicates the average serum immunoglobulins level of surviving lambs (31.71±12.88 ZST units) were significantly (P<0.05) higher than those that died during the neonatal period (12.77±5.25 ZST units. The mean serum immunoglobulins level recorded in thisstudy was almost similar to the findings of Reid (1972) and AL salami and Sinclair (1977), who reported serum immunoglobulin levels of about 27.40±1.70 and 30.90 ZST units, respectively. In the present study, the majority of lambs had ZST values between 20 and 40 units which are in accordance with findings recorded by Reid (1972) and Logan and Irwin (1977) in lambs. Based on this study, thirteen lambs out of 153 (8.5%) were found markedly deficient in serum immunoglobulin level (<13 ZST units), and which died later.

The importance of colostrum in reducing the incidence of neonatal lamb mortality is obvious by the fact that the nine out of thirteen lambs that died in the present study had an immunoglobulin level below 10 ZST units. Such lambs would be at high risk of susceptibility to diseases, and subsequent death as the observations reported by Reid (1972), Findlay (1973) and Logan and Irwin (1977). But according to Villar and Vulich (1980), ZST units in the range of 0-20 are indications of high risk of subsequent death. According to the present study, most of the mortalities were recorded during the first week of life. The finding is in agreement with Jordan and Le-Feuvre (1989), Otesile and Oduyo (1991) and Fentie et al. (2020) who reported maximum morbidity/mortality of lambs during the first week of life.

The lambs that survived the neonatal period had a significantly (P<0.05) higher level of immunoglobulins than those that died (Table 1). The result is similar with the findings recorded by Sawyer et al. (1977), Villar and Vulich (1980), Otesile (1994), and Kenyon et al. (2019). However, Bekele et al. (1992) reported no significant differences between mortality during the neonatal period and immunoglobulins concentration. The newborn leaves the sterile uterus to an environment containing many pathogens. The neonates are often overcome by infectious diseases, even by agents that are relatively nonpathogenic to adult animals (Banks, 1982). In the absence of specific immunity at birth due to of placental barriers (Tizard, 1992), ruminant neonates have to rely on antibodies received via colostrum (Khan and Khan, 1991). These antibodies play a significant role in the defense mechanism of newborn lambs until their own immune systems are primed and produce a protective level of antibodies (Tizard, 1992).

In the present study, six lambs out of thirteen (46.15%) showed sign of diarrhea, before death; while four (30.77%) died after signs of a respiratory disorder and the rest three died with no specific clinical signs. According Fisher (1980), IgM was the class of immunoglobulin found to be deficient in neonates that died of septicemic and bacteremic causes, whereas IgG was found to be deficient in neonates that died of diarrhea. IgA seems to be re-excreted and somehow halts the diarrheic process. According to Smith et al. (1976), a small amount of colostrum IgG, after being absorbed, is secreted in the nasal and lachrymal secretions of lambs and this plays a valuable role in preventing respiratory infections before local production of IgA and IgM at the age of 2-3 weeks.

Birth weight

The survival of neonatal lambs was also observed with respect to their birth weight. The mean birth weight was found 2.41±0.37 kg and the males (2.45±0.31 kg) were found heavier than females flock members (2.37±0.43 kg). The surviving lambs (2.43±0.35 kg) were also heavier than those that died during the neonatal period (2.21±0.55 kg) . Being physically weak, the lambs with low birth mass were unable to suckle sufficient amount of colostrum, and as a result, the immunoglobulins level in their serum was low. The physical weakness and low immunoglobulins led to increased mortality in lambs with a low birth mass. The finding of the study is similar with the works which were reported by Purser and Young (1983), Ducrot et al. (1989), Tadich et al. (1990) and Otesile and Oduye (1991). All lambs with higher birth weight survived the neonatal period. However, according to Poonia et al. (1983), as birth mass increases above 3.0 kg, the mortality also increases. Contrary to this finding, Dalton et al. (1980) reported that a lamb with a birth mass of from 3.5 to 5.5 kg had the lowest mortality. According to Hindson and Winter (2002), neonatal lambs with low birth weight often have poor suckling drive or they are unable to compete with stronger lambs for available milk. Hence, they are disadvantaged both from total milk intake and reduced immunoglobulin intake.

CONCLUSION

Based on the finding of the present study, it can be concluded that the total serum immunoglobulin levels in neonatal lambs within the first one to two days of age, had a good indication for the extent of the absorption of colostral antibodies from the dam. The neonatal lambs with lower average birth weight (2.21 kg) and lower average total serum immunoglobulin level (12.77 ZST units) had found dead before the first 30 days of their age. Hence, several works have to be done to further improve the birth weight of newborn lambs as well as the nutritional status of the dam so that lambs can suckle starting from the first few hours of birth and receive sufficient amount good quality colostrum.

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DECLARATIONS

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Authors' contributions

Chekol D contributed to the research design, analysis, interpretation of the data and writing the manuscript. Derib A, Yeshitila W, Firdawok A, Enyiew A and Tadiwos A contributed to the blood sample collection and laboratory work.

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Conflict of interest

None of the authors have conflict of interest.

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THE EFFECTS OF BROILER FEED FORMS ON METABOLIC AND SKELETAL DISORDERS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: A completely randomized study was conducted at the National University of Lesotho farm (altitude 1650 meters) to address the high incidence of metabolic and skeletal disorders in broiler chickens. The incidence of ascites also increases significantly at altitudes greater than 1300 meters above sea level, presumably because of the low oxygen partial pressure. The ascites incidences are very high in Lesotho during the cold winter months, accounting for more than fifty percent of the total mortality. The main objective of the current study was to assess the effect of different feed forms on the occurrence and control of metabolic disorders in broilers. A total of 200 day-old Ross 308 chicks were randomly distributed into two dietary treatments made up of two broiler feed forms namely mash and pelleted diet replicated four times with twenty-five birds per replicate. The two dietary treatments had similar nutritive value across all feeding phases with exception of feed form. Chicks were housed in a well-ventilated house where treatment diets and water were offered on ad libitum basis. Data collection was done on weekly basis for production parameters such as feed intake, feed conversion ratio, live weight and growth rate while mortality, signs of ascites, lameness and Sudden Death Syndrome (SDS) data were collected daily. All dead birds were examined for the signs of ascites by presence or accumulation of fluids in the abdominal cavity. The findings of the current study indicated that dietary treatment had a significant ($P<0.05$) influence on all production parameters namely feed intake, live weight, growth rate, feed conversion ratio and mortality rate. The dietary treatment also had a significant effect on incidences of ascites and lameness in broiler chickens whereby birds offered diet in the form of pellets had better production performance and higher incidences of the ascites, lameness and mortality than birds fed diet in mash form. On the other hand the dietary treatments did not have a significant ($P>0.05$) effect on SDS. However, there were more incidences of SDS in birds offered pelleted diets than mash diet. Birds fed mash diet had fewer incidences because they were experiencing moderate growth rates compared to birds fed pelleted diet with fast growth rates. Birds offered mash spend more time consuming their feed compared to birds fed pellets and therefore, expend more energy in this process resulting in lower feed conversion efficiency. It was evident from the results that diet in mash form can be used to control the incidences of metabolic disorder by reducing growth rates of broilers.

Keywords: Form, Ascites, Mash, Pellets, Growth Mortality

INTRODUCTION

Farmers in Lesotho suffer a huge financial loss during production phase of their broiler chickens as a result of high incidences of metabolic and skeletal disorders that leads to high mortality of chicks and condemnation of carcasses later (Amini et al., 2015). The incidence is common during the cold winter months as is influenced by cold temperatures and poor ventilation methods (Huchzermeyer et al., 1989) and this had led to the seasonality of broiler production in Lesotho as a result (Kuleile and Molapo, 2019). Most of the farmers especially those in the highlands of Lesotho do not produce any broiler during cold months fearing the high mortality rate. Broilers suffer from two forms of heart failure; ascites and sudden death syndrome (SDS) (Maxwell and Robertson, 1998). Ascites and SDS are relatively common and are likely to be due to the fact that the broilers' fast growth requires high levels of oxygen to support metabolic demands (SCAHAW, 2000). Ascites is a common rapid-growth-related problem in broiler chickens grown at high altitude where the partial pressure of oxygen is low and is marginally adequate to support the growth performance and ascites-related variables and it can be recognized by the fluid accumulation in abdominal cavity (Saffar and Khajali, 2010). SDS is an acute heart failure disease that affects mainly male fast-growing chickens which seem to be in good condition. It is characterized by the sudden death of well-nourished broiler chickens after abrupt and brief flapping of their wings (Saki and Hemati, 2011). Death usually occurs within 1-2 minutes with the birds lying on their backs with outstretched wings (Afolayan et al., 2016). Leg problems seen in the absence of infectious agents are often the result of fast early growth and thus can be related to metabolic diseases (Kumari et al., 2016). Poultry metabolic diseases occur primarily in two body systems being cardiovascular disorders, which in broiler chickens are responsible for a major portion of the flock mortality; secondly musculoskeletal disorders, which account for less mortality, but slow down growth (thereby reducing profit), and cause

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lameness, which remains a major welfare concern (Julian, 2005). Lameness is associated with heavy, fast-growing broilers and is of serious welfare concern due to an inability of lame birds to access resources, limited behavioral expression, and pain (Nicol et al. 2017). Lameness can take many forms. It can be infectious, arise due to developmental bone deformities or be degenerative (e.g. due to the consequence of trauma or load-bearing throughout life), and it can involve tendons, joints, ligaments, and bones (Bradshaw et al., 2002).

The broiler growth rate has been found to have a direct relationship with susceptibility to ascites and skeletal disorders (Camacho et al., 2004). Nemati et al. (2017) reported that rapid growth of modern broilers in a relatively short period of time requires a parallel increase in the size or capacity of supply organs, such as those of the cardiovascular and respiratory systems. However, due to the slower development of these organs relative to body growth rate, the capacity to balance body energy is compromised, particularly under extreme environmental conditions, such as cold stress (Shahir et al., 2012; Shinder, 2002). Manipulation of the diet composition and or feed allocation system can have a major effect on the incidence of ascites and skeletal disorders.

Generally, broiler diets could be offered in three forms, Mash, crumble and pellet. Most meat birds are fed crumbled or pelleted diets to achieve maximum growth and feed efficiency (Naderinejad et al., 2017). Feeding mash reduces growth rate (1 to 2 days to market) and reduces mortality and condemnations due to metabolic disease (Baghbanzadeh and Decuypere, 2008). However, this type of feeding programme may not be economically acceptable in all areas and has been demonstrated to increase the incidence of pendulous crops and higher production of inedible parts such as viscera (Kuleile and Molapo, 2019). Broilers that consume pellet feed have frequently been shown to have higher incidences of ascites and skeletal disorders than broilers that consume the same diet in mash form (Bölükbasi et al., 2005). On the same note Farm Animal Welfare Council (FAWC) stated that their Working Group found leg problems of varying degrees of severity on nearly every farm visited (FAWC, 1992). The report stressed that in the worst cases birds were only able to move with great difficulty and such birds were obviously distressed and had problems in reaching food and water. A Danish study in 1999 assessed the prevalence of lameness in a large and representative sample of commercial flocks. This study found that 30.1% of the birds had gait scores of 3, 4 or 5, which indicate that they are suffering from chronic pain (Sanotra, 1999).

Some of the feasible nutritional strategies such as early age feed or nutrient restriction (qualitative or quantitative) and use of appropriate feed form and light restriction lower the growth rate without compromising the final live weight (Baghbanzadeh and Decuypere, 2008). Optimization of the house temperature and ventilation in cold weather are also beneficial management practices to decrease incidences of ascites, SDS and lameness (Singh et al., 2013, 2018).

The objective of the current study, therefore, was to use nutritional strategy in the form of different broiler feed forms to control the incidences of ascites, SDS and lameness.

MATERIALS AND METHODS

Ethical approval

The scientific and ethics committee of the Faculty of Agriculture, National University of Lesotho approved the study protocol.

Study site

The study was conducted at the National University of Lesotho poultry farm located at 29°28'S latitude; 27°44'E Longitude (AfriGIS, 2020); at the altitude of 1650 m a. s. l.

Experimental design

The experimental design was Completely Randomized Design with two dietary treatments replicated four times. Dietary treatments were made up of two broiler feed forms namely mash and pellets. The two diets had similar nutritive value but differ in structural composition.

Birds housing and management

One day old mixed-sex Ross 308 chicks (n=200) were obtained from Letsatsi (local agro dealer) on the hatching day. The birds were reared in deep litter floor pens. The chicks were allocated into 8 pens and they were 25 birds per replicate. The room was lit 24 hours for the first 42 days. The experimental feeds and water were provided on ad libitum basis during the whole experimental period and all necessary prophylaxis and vaccination requirements for broilers were administered equally.

Data collection

Production parameters

Data on production parameters, such as live weight, growth rate, feed intake and feed conversion ratio were collected on weekly basis, while mortality rate, signs of ascites and lameness were recorded on daily basis. Live weight was measured using a platform weighing scale, Feed conversion ratio (FCR) was calculated as feed intake (g) over live weight (g). Feed intake was determined by the difference between feed supplied and leftovers. Growth rate was measured as the final weight minus initial weight divided by number of days. Mortality rate was recorded from 0 weeks

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until the 6 weeks by the following formula: Mortality % = No. of death birds in a replication/No. of initial birds in a replication × 100.

Disorders parameters

Data collection started at the beginning of the growing phase up to the end of finishing phase (17 to 42 days) because pellets were too big for consumption by the day-old chicks. Birds were observed on daily basis for the signs of lameness, abnormal gait and those sitting down all the time not able to reach waterers and feeders. United State gait-scoring system was used to measure the prevalence of leg weakness by assessing the walking ability of broilers. Walking ability was scored according to three category as follows; 0(no obvious signs of problems), 1(obvious signs) and 2(severe signs). Sudden death syndrome was recorded as birds that die without any symptoms of illness and they usually lie on their back with the feet raised. Dead birds were collected daily, weighed, and necropsied for the presence of water accumulation in the abdomen, which was considered as ascites. Any skeletal abnormalities were noted as they were discovered.

Statistical Analyses

Data collected was analyzed using IBM SPSS (version 20.0) and when the existence of difference between treatment means was declared, Least Significant Difference (LSD) test was employed to detect differences between treatments. The model used was; Yijk= μ+ Ti + eij, where: Yij = observation or over all response; μ = the overall mean;

Ti = the effect of treatments (i.e. forms of feed); eij = random error.

RESULTS AND DISCUSSION

Production Parameters

The broiler feed forms had significant (P<0.05) effect on all production parameters (Table 1). Birds that had access to diet in pellet form had significantly (P<0.05) higher feed intake, growth rate, feed conversion ratio , live weight and mortality rate than birds offered diet in a mash form. Feed intake and feed conversion ratio and growth rates results are in agreement with the findings of Kuleile and Molapo (2019), Hasani et al. (2018), Hosseini et al. (2017), Naderinejad et al. (2017), Chehraghi et al. (2013), Dozier et al. (2010), Amerah et al. (2007) who reported that feeding pelleted diets during growing and finishing phases increased broiler feed efficiency. On the contrary, Fasuyi and Odunayo (2015) reported that mash diet resulted in higher feed intake and feed conversion ratio than birds fed pelleted diet. The discrepancy may be due to the size of the pellets which was not suitable for chicks during this growth stage. Broiler mortality rate results are in accordance with the findings of Bricket et al. (2007) and Van Biljon (2006) who reported higher (P<0.05) mortality in chickens fed the crumble-pellet regimen (6.57% at 42 days), compared to chickens on the ground crumbles and pellets (4.03% at 42 days) and all-mash regimen (2.85% at 42 days). They also noted that feeding mash reduced the overall mortality as well as the mortality in every time period, starting at 14 d of age, in comparison with feeding pellet diets. On the other hand Al-Nasrawi (2016), Moayyedian et al. (2011), Dozier et al. (2010), Norollahi (2008), Scott (2002), Engberg et al. (2002), Nir et al. (1995) stated that different broiler feed forms did not have a significant influence on mortality rate. Ommati et al. (2013) also reported no differences in mortality rate. However, they observed that mortality was highest in pellets fed broilers with 12.7% while mash fed birds group had 9%. The inconsistency of reports on the effect of feed forms on mortality may be due to the difference in the duration of feeding.

The observed results on production parameters clearly revealed the superiority of pelleted diets to optimize broiler production during growing and finishing phase items of feed intake, growth and high feed efficiency. Pelleted diet offers a complete nutrient package for broilers because it reduces nutrient segregation and feed wastage as compared to mash diet (Ghazi et al., 2012). Broilers fed pelleted diet had high feed intake than birds fed mash diet because pelleted diet has a bigger particle size than mash and therefore it is consumed relatively faster than diet in mash form. Birds consuming diet in mash form spent a lot of time and energy in the act of eating and hence why low feed conversion efficiency. Moran, (1987), Flemming et al. (2002) and Skinner-Noble et al. (2005) indicated that pellet rations increased available dietary energy for live weight gain, which improved feed efficiency by reducing the time spent eating and increasing the time spent resting. The benefits of pellet feeding on broiler performance have been extensively reported and the current work confirms the benefits in terms of higher feed intake, weight gain and feed efficiency but prone to high incidences of metabolic disorders.

Table 1 - The effects of feed form on broiler production

Parameters	Treatments		Significance	
	Mash	Pellets	P[1]	CV[2]
Feed intake (grams/week)	769	951	0.033	11.26
Growth rate (grams/day)	59	84	0.019	9.34
Feed conversion ratio (g/g)	2.2	2.6	0.024	28.28

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Live weight	1689	2470	0.001	8.91
Mortality rate	0.7	7.5	0.002	0.21

P<0.05 = Means differed significantly, P[1]= Probability at 5% , CV[2]= Coefficient of variation

Metabolic Disorders Parameters

The broiler feed forms had significant (P<0.05) difference on ascites and skeletal disorders however, there was no differences (P>0.05) between feed forms on sudden death incidences. The incidence of ascites and skeletal disorders were significantly higher in broilers fed pelleted diet than birds fed mash diet. Similar trend in results were observed for sudden death syndrome where more incidence were observed in birds offered diet in form of pellets. Van Biljon (2006) results concurred with the findings of the current study on incidences of ascites and skeletal disorders who reported significantly higher mortality mainly caused by ascites (2.11%) and SDS (1.39%) in crumble-pellet treatment than in all mash diets. Skeletal disorders incidences were higher in ground crumble-pellet treatment than in group fed all mash diet. A number of researchers also confirmed the findings of the present study that feeding pellets to broilers lead to fast growth rates that in turn resulted in high incidences of ascites and SDS (Hasani et al., 2018; Meshram and Bijoy 2017; Ghazi et al., 2012; Arce-Menocal et al., 2009; Sarvestani et al., 2006; Bölükbasi et al. 2005; Arce et al., 1985). Arce et al. (1985) observed 15% incidence of ascites in pellets compared to 4% in mash diets. In the current study broilers fed pellets diet grew significantly faster than birds fed mash and hence the high incidence of ascites and skeletal disorders in these group of birds. Variation in observed results amongst researchers could be as a result of combination of feed form treatment with cold induced treatment, different altitudes, lighting programme, stocking density in rearing house as well as the use of bioenzymes. Researchers also reiterated that skeletal disorders, ascites and SDS are the common cause of economic losses due to mortality and downgrades in fast-growing broiler strains.

Table 2 - The effects of feed form on incidences of ascites, sudden death and skeletal disorders

Parameters	Treatments		Significance	
	Mash	Pellets	P[1]	CV[2]
Ascites (%)	0.75	6.25	0.033	11.26
Sudden death syndrome (SDS)	0.00	1.30	0.356	28.28
Skeletal disorders	0.75	5.75	0.001	8.91

P<0.05 = Means differed significantly, P[1]= Probability at 5% , CV[2]= Coefficient of variation

CONCLUSION

The findings of the current study revealed that broiler feed forms had a profound influence on the occurrences of ascites, sudden death syndrome and skeletal disorders whereby feed in the form of mash significantly reduced metabolic disorders in broilers compared to feed in the form of pellets which resulted in significantly high incidences. However, broiler mash diet gave significantly poor growth rates, feed conversion ratio and final live weight. It was concluded that pelleted feeds improved growth rate and feed conversion ratio, albeit by inducing metabolic disorders in broilers. It is recommended that farmers in Lesotho especially those in the highlands should consider feeding their broilers diet in the form of mash during the period of high susceptibility such as winter time. Farmers in the highlands should feed their broilers diet in the form of mash coupled with improved management practices that are known to influence metabolic disorders such as temperature control, oxygen, dust percentage in air, microorganism toxins, nitric oxide metabolism, vitamin E and selenium supplementation. Future research should evaluate the economic advantage of feeding mash versus pelleted feed through partial budget analysis.

DECLARATIONS

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Competing interests

The authors declared that they did not have a conflict of interest with respect to the research.

Author's contribution

Nchele Kuleile designed the experiment, supervised data collection, analyzed data and compiled the manuscript. Ncheche, Kamoho, Macheli, Jobo and Phororo collected data, conducted post mortem for dead chickens and inserted data in statistical analysis tool. All authors have proof read the final manuscript.

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EPIDEMIOLOGY, DIAGNOSIS AND PUBLIC HEALTH IMPORTANCE OF TRICHINELLOSIS

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Supporting Information

REVIEW ARTICLE

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ABSTRACT: Trichinellosis is a parasitic zoonosis caused by Trichinella following ingestion of raw or under cooked meat containing *Trichinella* larvae. Nematode worms of the genus *Trichinella* are one of the most prevalent zoonotic pathogens in the world. The parasite infects domestic and wild animals and has a worldwide distribution. The life cycle of the parasite consists of a domestic cycle in mainly pigs and a sylvatic cycle in a wider range of animals such as bears and wild boar. Humans become infected after eating raw or undercooked meat from domestic pigs, horses or game containing *Trichinella* larvae. There are twelve genotypes within the genus *Trichinella*, eight of which have been designated as species from which *T.spiralis* is the most pathogenic one. Host animals ingesting even high numbers of *Trichinella* larvae from infectious meat will not develop clinical symptoms. In humans, the clinical picture is usually illustrated by an intestinal stage within the first or second week after infection and later muscular stage with periorbital oedema, myalgia or muscle weakness as the major symptoms. The severity of the clinical course depends firstly on parasitic factors, such as the species implicated and the number of infective larvae ingested, and secondly on host factors, such as sex, age, and immune status. In practice, treatment with anthelmintics and immunosuppressive drugs is used only with human patients, not with animals. *Trichinella* infection in humans is strongly associated with the consumption of raw or undercooked meat; thus, cultural factors such as traditional dishes based on raw or undercooked meat or meat-derived products.

Keyword: Human, Parasite, Pig, raw meat, *Trichinellosis;* Zoonosis

INTRODUCTION

Parasitic zoonosis includes both helminthic and protozoan infections. Amongst one thousand five hundred known infectious agents for human being, 287 are helminths (Chomel et al., 2008). Helminths are complex eukaryotic organisms with large genomes and complex multistage life cycles that involve numerous hosts (Hewitson et al., 2009). Nematode worms of the genus Trichinella are one of the most widespread zoonotic pathogens in the world. Infection by Trichinella species has been identified in domestic and/or wild animals of all continents, with the exception of Antarctica, where there is no record of the parasite (Murrell, 2006).

Clinical signs of trichinellosis are not generally recognised in animals, and its main importance is as a zoonosis. Trichinosis is a food-borne zoonotic disease caused by *Trichinella* species. Trichinellosis in humans is caused by eating raw or undercooked meat from *Trichinella*-infected food animals or game (Gajadhar et al., 2006). Until recently, all Trichinella infections occurring in animals and humans were attributed to *Trichinella spirals.* Today, eight species (*T.spiralis, T.nativa, T.britovi, T.pseudospiralis, T.murrelli, T.nelsoni, T.papuae,* and T.zimbabwensis and three genotypes (Trichinella T6, T8, T9) within two classes (encapsulated and non-encapsulated) are documented in this genus (Zarlenga et al., 2006).

It is a tissue-dwelling nematode acquired by the ingestion of raw or insufficiently cooked meat-products containing encapsulated larvae (La Rosa et al., 2000). The most important source of human infection worldwide is the domestic pig. In Europe, meats of horses and wild boars have played a significant role during outbreaks within the past three decades. Infection of humans occurs with the ingestion of *Trichinella* larvae that are encysted in muscle tissue of meat from domestic or wild animals (Bruschi et al., 2007).

Trichinella infection in the human host can be divided into two stages: an intestinal (or enteral) phase and a muscular (or parenteral or systemic) phase. Low-intensity infection can remain asymptomatic, but parasite burdens greater than a few hundred larvae can initially cause gastroenteritis associated with diarrhoea and abdominal pain approximately 2 days post infection (intestinal acute phase of disease). Subsequently, migrating larvae and their metabolites provoke an immediate reaction, with an inflammatory and allergic response, pyrexia, eyelid or facial oedema, and eosinophilia are the most prominent manifestations, occasionally complicated by myocarditis, thromboembolic disease, and encephalitis. Months or even years at the acute stage, chronic trichinellosis may yield persistent fornication,

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numbness, and excessive sweating as well as impaired muscle strength and conjunctivitis, which may continue up to 10 years post infection (Zarlenga et al., 2013).

The diagnosis of trichinellosis is based on history of consumption of potentially contaminated meat, the presence of compatible signs and symptoms, and identification of *Trichinella* larvae in biopsy muscle tissue or specific antibody in serum. These diagnostic methods in human host can be categorized two as direct and indirect. Under direct there are direct muscle biopsy while under indirect such as serology and molecular technique (Oivanen, 2005). Muscle biopsies are rarely performed, but they allow for the molecular identification of the *Trichinella* species or genotype, which is not possible with antibody testing (Oivanen, 2005).

Prompt treatment with anti-parasitic drugs can help prevent the development of trichinellosis by killing the adult worms and so preventing further release of larvae. Once the larvae have become established in skeletal muscle cells, treatment may not completely eliminate the infection and associated symptoms (Sun, 2015). Therefore, the administration of effective anthelmintic drugs at the stage of intestinal invasion or in the acute phase is critical for successful therapy. In addition, because of the predominantly zoonotic importance of infection, the main efforts in many countries have focused on the control or elimination of Trichinella from the food chain (Gottstein, 2009).

The increase in the report of Trichinellosis has been observed many eastern European countries, in Africa and Asia (Blaga et al., 2007; Azim et al., 2008). Human population growth and socioeconomic changes might have played a fundamental role in the disease emergence and spread in recent years *(*Macpherson, 2005). The increase in human population density, ecological change, and subsequent increased contact between humans and wild animals necessitates the importance having an update on potentially emerging diseases like trichnellosis. Therefore, the objective of this paper is to review the epidemiology and public health importance of trichinellosis.

Taxonomy and Morphology of the Parasites

Taxonomy and Aetiology

The taxonomy of the genus *Trichinella* has been presented with slightly varying details According to the traditional classification, the genus belongs to the phylum Nematode, roundworms, class *Adenophorea*, order *Trichinellida*, and superfamily *Trichinelloidea* (Oivanen, 2005). The taxonomy has recently been challenged. On the basis of results from ribosomal deoxyribonucleic acid (DNA) sequences, the present higher-level classification of Nematode will need change in to two classes, *Secernentea* and *Adenophorea* (Oivanen, 2005). Within the genus *Trichinella there are* twelve genotypes have been identified, eight of which have been designated as species (Gajadhar et al., 2006; Murrell et al., 2000; Pozio and Zarlenga, 2005). *Trichinella spiralis* was recognized in London in 1835s. The parasite being detected in an autopsy of an Italian male corpse (Oivanen, 2005).

Trichinella spiralis (T1) is distributed in temperate regions world-wide and is commonly associated with domestic pigs. It is highly infective for domestic and sylvatic swine, mice and rats, but it can also be detected in other mammalian, carnivores and horses (Pozio and Zarlenga, 2005). This species is also the most important etiological agent to cause disease in humans (Pozio, 2006). Trichinella native is the species that are very widespread in arctic and subarctic areas of the northern hemisphere (Pozio, 2000). *Trichinella britovi* species differs from *T. spiralis* with weak infectivity for rats, moderate resistance to freezing, moderate infectivity for swine, slow nurse cell development and low *in vitro* production of NBL (Malakauskas and Kapel, 2003). *Trichinella nelson* has occasionally been detected in pigs (*Suidae*) and humans, although it has very low infectivity for pigs and rats. The infectivity for humans has not been long-established (Pozio, 2001).

Trichinella murrelli this species has very low reproductive capacity in pigs and rats, low NBL production *in vitro*, slow nurse cell development, and low resistance to freezing (Malakauskas and Kapel, 2003). *Trichinella pseudospiralis* strains three genotypic isolates were identified by multiplex polymerase chain reaction from different parts of the world (PCR) test (Zerlenga et al., 1999; La Rosa et al., 2001). *Trichinella papuae are where Muscle* larvae are non-encapsulated and lack freezing tolerance but can survive in +5^{o}C storage for four weeks (Webster et al., 2002). *Trichinella zimbabwensis* is the first *Trichinella* strain isolated in reptiles in nature. In the laboratory, it can also infect rats, mice, pigs, baboons (*Papio* sp.), turtles, pythons, varans, and caimans. Its muscle larvae are non-encapsulated. It is not infective for birds, nor can it resist freezing (Pozio et al., 2004).

Morphology of the Parasite

Trichinella worms are the smallest nematode parasite of humans, they are the largest intracellular parasite and have been described as “the worm that would be virus” (Foreyt, 2013). The morphology of the parasite’s oesophagus is characteristic of the *Trichinellidae* family, and it occupies approximately one-third of the body length and is surrounded by large cells. Adult males are 1.4 to 1.6 mm in length and do not have spicules, but a pair of lateral flaps is found on each side of the cloacal opening and two pairs of papillae are between them. Females’ are 3 to 4 mm in length, and the vulva opens in the middle of the oesophageal region (Foreyt, 2013). Adult the length of *T. spiralis* NBL is 80-120 µm and the diameter 5-6 µm. The larvae do not increase in size until they enter the muscle cells. The larvae begin to grow in their nurse cells, reaching a length of 900-1280 µm and a diameter of 35-40 µm by 30days p.i. *Trichinella* adult females are a little longer and thicker than the males. Their length and diameter are 2460-3390 µm and 35-70 µm, respectively, while the resulting figures for males are 1040-1300 µm and 29-32 µm (Oivanen, 2005).

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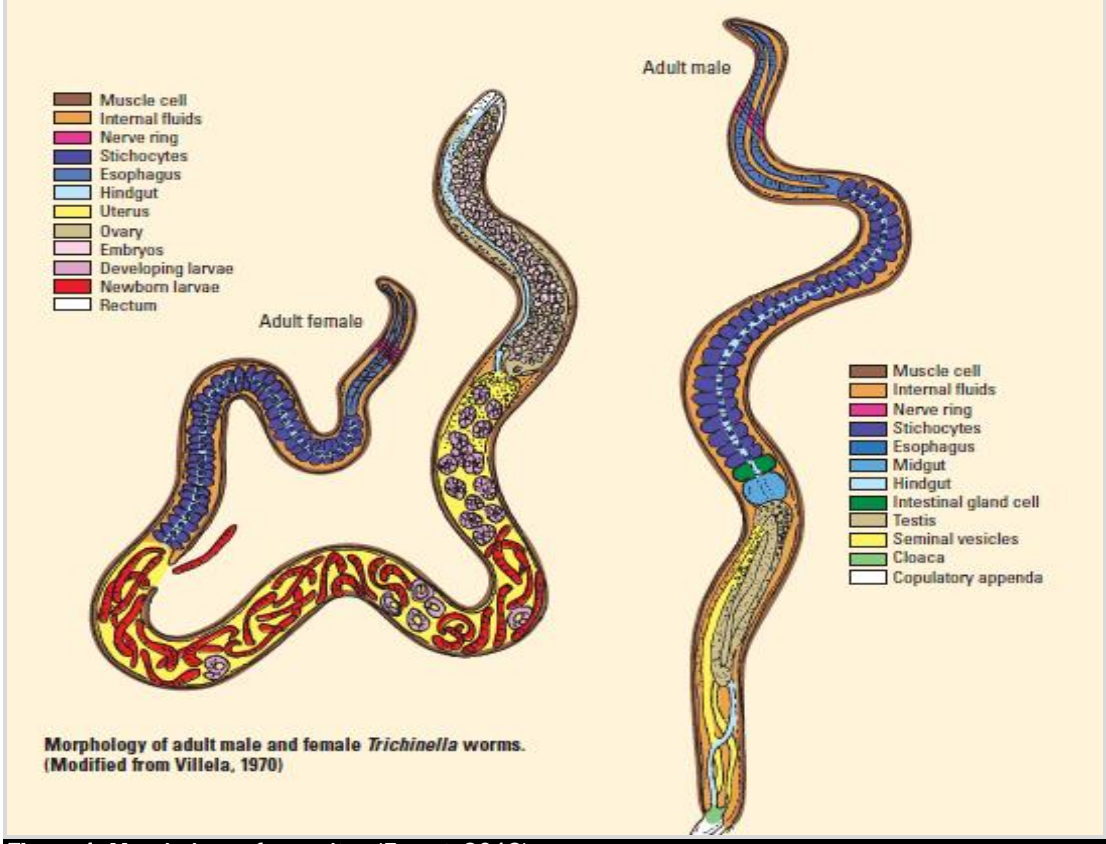

Figure 1- Morphology of parasites (Foreyt, 2013)

The life cycle of the parasites

The basic life cycle of *Trichinella* has been recognized since the middle of the 19th century. This genus is unique among parasitic nematodes in that all stages of the life cycle occur within a single host. In nature, the cycle is repeated when another host animal ingests the flesh of another host containing viable muscle-stage larvae (Oivanen, 2005). The generalized life cycle of *Trichinella* is described in figure 2. Enteral phase; 1: muscle tissues are digested in the stomach and larvae are released; 2: larvae penetrate the intestinal mucosa of the small intestine, reach the adult stage within 48 h post infection, male and female mate; 3: female worm releases new born larvae in the lymphatic vessels (from the fifth day post infection onwards; the length of New born production, from one to several weeks, is under the influence of the host immunity). Parenteral phase; 4: the new born larva reach the striated muscle and actively penetrate in the muscle cell; 5: the larva grows to the infective stage in the nurse ceil (the former muscle cell); 6: after a period of time (weeks, months or years) a calcification process occurs (Pozio and Murrell, 2006).

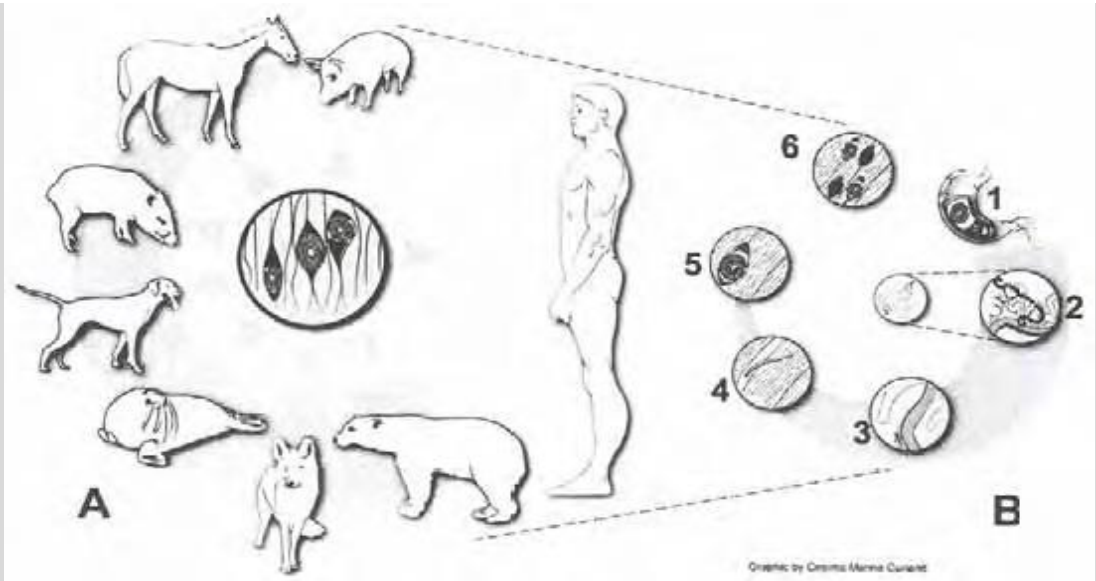

Figure 2- Trichinella sp. life cycle (Pozio and Murrell, 2006). A: main sources of Trichinella spp. infections for humans; B: Trichinella spp. cycle in the host body.

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Epidemiology

Geographic distribution

Trichinella species are present throughout most of the world in over 150 different hosts (Dick et al., 2001). In addition to *T. spiralis*, 7 other species in 4 genotypes, all of which are more commonly found in wild animals than in domestic pigs . *T. spirals* is cosmopolitan, this species is also the most important etiological agent to cause disease in humans (Pozio et al., 2006). In the domestic cycle, pork scraps from *T. spiralis*-infected pigs are the main source of infection for synanthropic *animals (e.g., rats, horses, stray cats, and dogs). Conversely to the* domestic cycle, the sylvatic cycle of *T. spiralis* includes a broad range of wild carnivores, which may, however, become the origin of a life cycle beginning into a domestic host population (Dick, 2001). *Trichinella native* is found in Arctic and subarctic areas of America, Asia, Europe. *Trichinella* genotype T6 is also found in Canada, Alaska, Rocky Mountains, and Appalachian Mountains in the United States (Pozio, 2001). *Trichinella britovi* is found in the temperate areas of Europe and Asia, Northern and Western Africa, *Trichinella* T8 is found in South Africa and Namibia. *Trichinella murrelli is found in the* United States and Southern Canada, *Trichinella* genotype T9 Japan, *T. nelsoni* Eastern-Southern Africa, and *Trichinella* genotype T12 is found in Argentina, and all the above are encapsulated while the below are non-capsulated. Those are *T. pseudospiralis* which is cosmopolitan in Palearctic, Nearctic, Ocean land (Pozio, 2001) Cosmopolitan , *T. papuae* is found in the Papua New Guinea, Thailand and *T. zimbabwensis* which is found in Zimbabwe, Mozambique, Ethiopia, South Africa (Gottstein, 2009).

Host range

The epidemiology and systematics (i.e., the study of the diversification) of this zoonosis are now recognized to involve in addition to *T. spiralis*, 7 other species in 4 genotypes, all of which are more commonly found in wild animals than in domestic pigs (Pozio, 2009). *T. spiralis* is found in the Domestic and sylvatic mammal, while *Trichinella* T8, *T. murrelli, Trichinela* genotype T9. *Trichinella native and Trichinella* genotype T6 is found in the sylvatic carnivores. Another species, *T. britovi* is found in the Sylvatic mammals and seldom domestic pigs, while *T. nelson* in the Sylvatic mammals. *Trichinella* genotype T12, *T. Pseudo spiralis* Sylvatic mammals and birds, domestic pigs, *T. papuae* Wild pigs, salt water crocodiles and *T.zimbabwensis* which is found in the Nile crocodiles, monitor lizards (Gottstein, 2009).

Main source of infection in human

Domestic pigs and wild boars were the major sources of *Trichinella* spp. infection for humans, but in recent years new infection sources, particularly from exotic hosts, have emerged (Boireau, 2000). The main source of infection in human*, T. spiralis* is found in the Domestic and sylvatic swine horses, while *T. native* is found in Bears, walruses. Others like *Trichinella* genotype T6 is found in Carnivores *T. britovi* is found in the Wild boars, domestic pigs horses, foxes, jackals *T. zimbabwensis, Trichinella* genotype T12, *Trichinella* genotyp T8 and *Trichinella* genotype T9. *Trichinella murrelli in Bears*, especially horses while *T. nelson* is found in the Warthogs, bush pigs Warthogs, bush pigs, T. *papuae* is found in the Wild pigs and *T. pseudospiralis* wild and domestic pigs (Gottstein, 2009).

Resistance of larvae in frozen muscle

Most of them are not resistance to the frozen muscle. *Trichinella* T8, *T. murrelli, T.pseud ospiralis T. papuae and T.zimbabwensis,* while others are *T. spiralis* resistance in horse muscles. *T. native* are resistance in carnivore muscles. *Trichinella* genotype T6 is resistance in carnivore muscles, *T. britovi* are resistance in carnivore and horse muscles and *Trichinella* genotype T12 Unknown (Gottstein, 2009). The epidemiology of trichinellosis is summarized as below in table 1.

Disease ecology

The usual source of trichinosis in humans is from eating pork products or meat from horses, dogs, or a variety of wildlife species, including wild pig, bear, walrus, and seal. *Trichinella* spp. is transmitted by two specific cycles, the domestic cycle and the sylvatic cycle (Dick and Pozio, 2001).

Domestic cycle

The domestic cycle is prevalent on small farms where disease control is not a primary objective in food production. Areas where infection is endemic are found throughout the world (Dupouy- Camet, 2000). The domestic cycle of transmission is primarily involves *T. spiralis* in a cycle of pig-to-pig transmission, and humans enter the cycle through eating pork. The infection can be highly pathogenic in humans. Synanthropic rats, mice, cats, dogs, and horses, as well as many wildlife species, also contribute to the cycle in many areas. Pigs maintain the cycle by eating pieces of infected meat scraps, eating infected rats or mice, biting the tails of infected pigs, cannibalizing dead pigs, ingesting feces from pigs that have recently eaten infected meat, or eating other species of infected mammals (Ortega-Pierres and others et al., 2000).

Sylvatic cycle

The sylvatic cycle of transmission predominantly involves predation, cannibalism, or scavenging behaviours of species of carnivorous wildlife. *Trichinella* spp. are transmitted when fresh, frozen, or decomposing carcasses or meat scraps are eaten (Dupouy-Camet, 2000). The species of *Trichinella* associated with the sylvatic cycle are *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*. *T. spiralis* can also affect wildlife in temperate and tropical regions, but it does not survive in arctic and subarctic regions because larvae do not survive in a frozen carcass (Ortega-Pierres et al., 2000).

Clinical signs

The severity of the clinical course depends firstly on parasitic factors, such as the species involved and the number of infective larvae ingested, and secondly on host factors, such as sex, age, and immune status (Bruschi and Murrell,

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2002). The chief clinical of trichinellosis were compatible in type and frequency with the classical trichinellosis syndrome, i.e., myalgia, diarrhoea, fever, facial oedema and headaches that, after treatment, disappeared within 2–8 weeks (Dupouy-Camet and Bruschi, 2007). The clinical signs of acute trichinellosis are characterized by two phases: an enteral and a parenteral phase, corresponding to the presence of parasites in the intestine and in the circulation and/or musculature, respectively (Oivanen, 2005). The most common signs during the enteral phase of a mild infection are transient diarrhoea and nausea. However, in moderate to severe infections, the first signs are upper abdominal pain, diarrhoea or constipation, vomiting, malaise, and mild fever. The enteral phase lasts for six weeks (Kocięcka, 2000). From the second to the sixth week post infection, the enteral phase is still present, but the dominating signs arise from the parenteral phase due to the migrating larvae and their indiscriminate penetration of different tissues. During the third week post infection the symptoms intensify due to invasion of muscle cells. Characteristic signs include weakness, pain, paralysis, and photo phobia. Edema is prominent and patients may have shortness of breath. Endocarditis, myocarditis, and cardiac failure have been reported. The signs of acute illness usually diminish from the fifth or sixth week post infection onwards (Kocięcka, 2000; Oivanen, 2005).

Table 1 - Epidemiology of Trichinellosis

Species or genotype	Geographical distribution	Host range	Main source of infection in human	Resistance of larvae in frozen muscle
Encapsulated				
T. spiralis	Cosmopolitan	Domestic and sylvatic mammals	Domestic and sylvatic swine horse	Yes in horse muscle
T. nativa	Arctic and subarctic areas of America, Asia ,Europe	Sylvatic carnivores	Bears, walruses	Yes in carnivores muscle
Trichinella genotype T6	Canada, Alaska, rocky mountains, and Appalachian Mountains in the united states	Sylvatic carnivores	Carnivores	Yes in carnivores muscle
T. britovi	Temperate areas of Europe and Asia, northern and western Africa	Sylvatic mammals and rarely domestic pigs	Wild boar, domestic pig ,horse, foxes, jackal	Yes in carnivores and horse muscle
Trichinella T8	South Africa and Namibia	Sylvatic carnivores	None documented	No
T. murrelli	United states and southern Canada	Sylvatic carnivores	Bears, horses	No
Trichinella genotype T9	Japan	Sylvatic carnivores	None documented	No
T. nelson	Eastern-southern Africa	Sylvatic mammals	Warthogs, bush pigs	No
Trichinella genotype T12	Argentina	Cougars	None documented	Unknown
Non encapsulated				
T. pseudo spiralis	Cosmopolitan	Sylvatic mammals and birds, domestic pig	Domestic and wild pigs	No
T. papuae	Papua new guinea ,Thailand	Wild pig , salt water crocodile	Wild pig	No
T. zimbabwensis	Zimbabwe,Mozambique, south Africa,Ethiopia	Nile crocodiles, monitor lizards	None documented	No

Source: Gottstein (2009).

Diagnosis

Direct method

Meat inspection for the detection of *Trichinella* larvae is designed to prevent clinical trichinellosis in humans but not to prevent infection. The identification of *Trichinella* larvae in muscle samples from pigs and other animal species intended for human consumption (e.g., horses, wild boars, and bears) is limited to post-mortem inspection of carcasses. Muscle biopsy is a traditional method applied to diagnose trichinellosis. Samples are usually taken from the *M. deltoideus*. Other possible sites are the *Musculus biceps brachii*, *Musculus gastrocnemius*, *M. pectoralis*, *M. gluteus maximus,* and *Musculi intercostali* (Gamble, 2000). Muscle biopsy is recommended only in cases where serological results are unclear. In autopsy, the sampling site is the diaphragm (Bruschi and Murrell, 2002). Direct detection is also applied in wildlife monitoring, where indicator animals (e.g., foxes or raccoon dogs) are examined to assess the prevalence of *Trichinella* infection among the wildlife reservoir and the risk of introduction into domestic animals. Methods to detect *Trichinella* larvae in muscle samples need to be highly sensitive, and performance is greatly influenced by the sample size, the muscle type selected for sampling, and the specific method used (Nockler, 2000). In order to identify predilection sites, in particular, animal species that optimal for diagnostic investigations, several experimental studies using doses that mimic natural infections have been performed. Thus, in domestic swine, the three main predilection sites for *T. spiralis* are the diaphragm crus, the tongue, and the masseter (Gamble, 2000), and analogous results were observed in experimental *T. britovi* and *T. pseudospiralis* infection in this host species (Nockler, 2005). Some of the sampling sites recommended by the International Commission on Trichinellosis for different domestic and wild animals subjected to meat examination or epidemiological studies are summarized in Table 2.

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Table 2 - Predilection site of *Trichinella* larvae in different animal species

Animal species	Predilection site	Aim of detection
Domestic pig	Diaphragm, Masseter, Tongue	Meat inspection (domestic animals)
Horse	Tongue masseter	Meat inspection (domestic animals)
Wild boar	Forearm, diaphragm ,tongue	Meat inspection (game)
Bear	Tongue, diaphragm, masseter	Meat inspection (game)
Water seal	Tongue,diaphragm flipper, masseter	Meat inspection (game)
Fox	Tongue, forearm, diaphragm, masseter	Epidemiological studies (reservoir animal)
Raccoon dog	Diaphragm ,forearm, muscle, tongue	Epidemiological studies (reservoir animal)

Source: Nockler, (2005).

Serology

Serology is considered to be appropriate for the surveillance and epidemiological investigations of *trichinellosis* in domestic animals and wildlife (Dworkin, 1996). The indirect serological diagnostic methods can be used at both anti-mortem and post-mortem examination for *Trichinella*-specific antibodies. Several conventional Sero diagnostic methods have been practiced in detecting *Trichinella* larvae. These include ELISA, immunofluorescence antibody test (IFAT), complement fixation test, and hemagglutination test and molecular technique (Oivanen, 2005).

Enzyme Linked Immunosarbant Assay (ELISA)

The ELISA method is relatively simple to apply, and it can be automated in *Trichinella* diagnostics. It is sufficiently sensitive to detect low-level infect ion (Nöckler et al., 2000). Traditionally ELISA has been applied to analyse antibodies in serum samples. According to some reports, samples of muscle juice can substitute for serum samples. This may be a practical solution if serum is unavailable. Results with muscle juice were hopeful in pigs but inconsistent in wild red foxes (Vercammen et al., 2002). However, this method cannot replace the direct methods at meat inspection because it can fail to detect early or very late stages of infections (Gamble et al. 2004). Infection levels as low as one larva/100 g of tissue is detectable by ELISA in pigs (Gamble et al., 2004). This high level of sensitivity makes serological testing by ELISA a useful method for detecting ongoing transmission of *Trichinella* infection at the farm or for more broadly based surveillance programmes. A disadvantage of serology for the detection of *trichinellosis* is the low rate of false-negative results observed in infected animals (OIE, 2012). For this reason, serological methods are not recommended for individual carcass testing. Serological responses in pigs persist for a long time after infection with no decline in titre; however, antibody has been reported to reject in horses within a few months following infection. The use of ELISA to detect the presence of parasite-specific antibodies provides a quick method that can be performed on serum, blood or tissue fluid collected before or after slaughter. The dilution used is different for serum than for tissue fluid (Nöckler et al., 2000). Antigens that are specifically secreted from the stichocytne cells of living L1 larvae and bear the TSL-1 carbohydrate epitope are recognised by *Trichiella*-infected animals. The specificity and sensitivity of ELISA is largely dependent on the quality of the antigen used in the test (Forbes et al., 2004; OIE, 2012).

Molecular technique. Since there are no morphological features to specify larvae, molecular diagnosis is used to yield the species or genotype diagnostically recovered. For this purpose, a multiplex PCR has been developed for the simple and unequivocal differentiation of *Trichinella* species and genotypes (Zarlenga, 1999). Polymerase chain reaction limited studies have shown that PCR can be used to detect the nucleic acid of larvae in the musculature of infected animals (Zarlenga et al., 2003). However, this method lacks sensitivity and is not practical for routine testing of food animals. Identification of the species or genotype of *Trichinella* recovered from muscle tissue is useful in understanding the epidemiology of the parasite in animals, in assessing the relative risk of human exposure and to trace back the infection to the farm of origin (OIE *Terrestrial Manual,* 2012). Specific primers have been developed that allow the identification of single larva collected from muscle tissues at the species and genotype level by PCR). This multiplex PCR is a sensitive, inexpensive, and rapid molecular approach that can unequivocally identify a single larva at the species and genotype levels (Pozio et al., 2003).

Status of Trichinellosis in Ethiopia

At least two confirmed outbreaks of trichnellosis had been reported in Ethiopia. One of the outbreaks was reported in Gojjam administrative region. The outbreak was associated with ingestion of meat from a wild boar. In this outbreak, from 30 soldiers, 20 who ate the raw meat became ill and 5 of them were admitted to Hospital with distinctive history and clinical features the disease. The diagnosis was confirmed by deltoid muscle biopsy in all the 5 cases. Similar outbreak had been reported from Central Arsi (Kefenie et al., 1988; Kefenie and Bero, 1992).

Public health importance of trichinella

In humans, trichinosis is an important food-borne disease that can cause acute and chronic illness. Humans are only infected with *Trichinella* larvae through the ingestion of meat that has not been appropriately cooked. All species of *Trichinella*, except for the none encapsulated species (*T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*), can be highly

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pathogenic in humans (Kociecka, 2000). *T. spiralis* is apparently more pathogenic in humans than other species because more larvae are produced by the female worms (Foreyt, 2013). Recently, *T. papuae* has been implicated in outbreaks of human trichinosis (Khumjui et al, 2008) .Clinical manifestations are often complex, and they depend on the age of the human host, the state of resistance, and the numbers of larvae ingested. Most clinical symptoms are present between 1 and 6 weeks after infection and the psychological effects of affected humans advance complicate the physical symptoms of the disease. Three stages of disease in humans have been described: the enteral or intestinal phase, the migratory or mucosal invasion phase, and the parenteral or convalescence phase (Foreyt, 2013).

Recently, *T. papuae* has been implicated in outbreaks of human trichinosis. Twenty-eight people in Thailand became sick after eating wild boar and suffered symptoms of trichinosis, and *T. papuae* was identified in a muscle biopsy from one of the patients (Khumjui et al., 2008). *T. papuae* was also suspected as the cause of an outbreak of trichinosis in eight people who had eaten raw soft-shelled turtles in Taiwan (Lo et al., 2009).

CONCLUSION AND RECOMMENDATION

Trichinellosis (trichinosis) is caused by nematodes (roundworms) of the genus *Trichinella.* The disease has a significant public health importance. All mammals are susceptible to infection, but the number of larvae required for infection varies according to the genetic constitute of the parasite and the host species. Trichinellosis is acquired by eating raw or undercooked meat that contains *Trichinella* larvae. Domestic animals can be infected by the consumption of infected raw tissues. *Trichinella* has a direct life cycle, which means it completes all stages of maturity in one host. Transmission from one host to another host can only occur by ingestion of muscle tissue which is infected with the encysted larval stage of the parasite. When ingested, muscle larvae excyst and enter tissues of the small intestine, where they undergo development to the adult stage. Male and female adult parasites mate and produce newborn larvae which leave the intestine and migrate, through the circulatory system, to striated muscle tissue. The severity of human trichinellosis is dependent upon the number of infected larvae ingested, the species of *Trichinella*, and the immune status of the human host. Muscle biopsy, ELISA and PCR method is important tool for diagnosis of infection.

Based on above conclusion the following recommendations are forwarded: 1) Education of the consumer about the risk of consumption of raw or undercooked meat and meat products from both domestic and wild pigs should be emphasized; 2) Strict quarantine should be exercised to control the slaughter and meat distribution of potentially infected animals.

DECLARATIONS

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Consent to publish
Not applicable.

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

Authors' contributions
M. Yayeh and M. Birhan conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. M. Birhan participated in drafting and reviewing the manuscript. M. Yayeh and M. Birhan conceived the study, coordinated the overall activity, and reviewed the manuscript. M. Yayeh and M. Birhan participated in drafting and reviewing 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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PARTICIPATORY EVALUATION OF IMPROVED FEED TECHNOLOGIES TO ENHANCE SMALL RUMINANT FATTENING ON PASTORALIST RESEARCH GROUP (PRG) MEMBERS IN CHIFRA DISTRICT OF AFAR NATIONAL REGIONAL STATE

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ABSTRACT: The causes for low productivity of sheep and goat include poor veterinary services, inadequate quantity and quality of feed. Among these limiting factors, poor feed supply and feeding system is the most important. The objectives of this project were to demonstrate and evaluate Urea Molasses Multi-Nutrient Blocks (UMMNB) and concentrates mix feed technologies in participatory manner through Pastoralist Research Group (PRG) approach and look in to the perception and opinions of agro-pastoralist to the new feed and feeding techniques. The PRG has 25 members and was established a year ago. Among the PRG members, 6 trial agro-pastoralists were selected by the PRG members purposefully to implement the experiment. A total of 36 small ruminants (sheep and goat) were used for the trial. Training on UMMB preparation, formulation of concentrate feed, animal management and data collection were given for PRG members and Development Agents (DAs). The demonstration and evaluation trial were lasts for 4 months data collection period. Data were collected by the trial PRG agro-pastoralist throughout the trial period with close follow up of DAs and woreda experts. For data analysis purpose the researchers used descriptive statistics. The results showed that the final body weight and daily body weight gain was higher in grazing when supplemented with concentrates mix (Treatment 3) in compared to grazing + urea molasses block supplementation (T2) and control one/free grazing (T1). The partial budget analysis also indicated that small ruminant daily weight gain for 4 months with supplementation of concentrate mix supplemented groups (T3) was found to give on average economic return of 250 Ethiopian Birr (ETB) or 6.70 Euro per head. From this study, it can be concluded that the supplementation concentrate mix for small ruminants (sheep and goat) has better weight gain and economically feasible for the chifera district PRG established in 2017. It was also economical at PRG level, with average gross profit of 250 ETB per head in chifera area. Because of this, so money agro-pastoralists has been attracted by the business and engaged in small ruminant fattening practices by purchasing animals from market place without external advice and support.

Key words: Small ruminant, Urea molasses block, Feed technology, Pastoralist research group

INTRODUCTION

Sheep and goats, with their small body size, high reproductive capacity and rapid growth rates are ideally suited to production by resource-poor smallholders. They can be integrated into the overall production system, absorbing surplus labor and consuming small amounts of otherwise unused feed. Despite a large population and the contribution of the national and regional sheep and goat flock to the export earnings of the country Ethiopia in general and Afar region in particular as well as the livelihoods of households in rural and semi urban areas, their productivity is very limited. The causes for low productivity of sheep and goat are multifaceted and include poor veterinary services, inadequate quantity and quality of feed. Among these limiting factors, poor feed supply and feeding system is the most important (Alemayehu, 2002).

Extensive sheep and goat production under the traditional communal grazing/ browsing system is widely practiced in Afar region. Pasture, crop residues and browses are the main feed supply to sheep and goat in the region and such types of feeds rarely satisfy the maintenance requirements of animals. Most of the available feeds are noted for their poor feeding value in terms of protein and energy contents besides their low digestibility.) Some studies, reported that in semi-arid and tropical ecosystems, the quality of forages decreases greatly during the dry season, leading to substantial weight loss of animals (Pinkerton, 2005; Njidda, 2010; Njidda and Nasiru, 2010; Amiri and Mohamed Shariff, 2012; Njidda et al, 2012). This phenomenon requires the alleviation of nutrients deficiency in animals through implementing different feed utilization strategies. Even though many studies were taken place on small ruminants in Ethiopia most of the technologies were not transferred to end users and farmers. As a result, the small ruminant development in smallholder farms remains unchanged. Hence there is a need to search for alternative technologies, which could improve the nutritive value of the poor-quality feeds to enhance the production of small ruminant. There are different techniques that could improve the

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feeding value of basal diet among which urea treatment legume supplementation and concentrates supplementation are well known for their technical and economic feasibility under smallholder Agro-pastoralist especially in developing countries. Moreover, semi-intensified market-oriented fattening of small ruminant has the potential to make smallholder Agro-pastoralist/ pastoralist more profitable.

Objectives of the Study

General objective: The general objectives of this study were to demonstrate and evaluate Urea Molasses Multi-Nutrient Block/UMMNB/ and concentrates mix feed technology in participatory manner through PRG approach and look in to the perception and opinions of Agro-pastoralist to the new feed and feeding techniques.

Specific objectives: A) To evaluate the response of small ruminants (goat & sheep) to the new alternative feed source-based feeding under smallholder Agro-pastoralist during drought season; B) To see the perception of Agro-pastoralist to the new feed and feeding techniques.

METHODS AND MATERIALS

Description of the study area

The study was undertaken in Chifra district of zone one (Awsi Rasu) of the Afar Regional State. It is located south west of Semera on the main road of Mile to Woldiya, which is about 162 km from the regional capital city (Semera) and bordered on the south by Mille, on the west by Amhara Region, on the north by the Administrative Zone four (Fantena Rasu), and on the East by Dubti (Zone one). The total land area of the district is about 173,374 ha of which the largest area is rangeland (APARDB, 2006). The average temperature of the area is about 29oC, and the rainfall is bimodal with erratic distribution, with the long rainy season (Kerma) is between Mid-June to Mid-September and the short rainy season (Sugum) that occurs between March and April. The average annual rainfall is recorded to be between 400 and 600 mm (APARDB, 2006). The altitude range of the area is between >550-1,100 m above sea level and most of the rangelands of the study district falls below 850 m.a.s.l. The dominant soil types in these areas are black, sandy, vertosols and deposits of silt and fine sand particles occur in the plain flat areas where cultivation is practiced (APARDB, 2006). The study area consists of 19 pastoral associations of these 13 of the associations are pastoralists, which entirely depend on livestock production. The remaining 6 associations are agro-pastoralists. The district has an estimated total population of 91,078, of which 50,859 are males and 40,219 are females; 9,132 or 10.02% of its population are urban dwellers and the household numbers are 17,744 (CSA, 2007).

Sampling methods of the study

The PRG having 25 members were established in 2017. Among the PRG members, six trial agro-pastoralists were selected by the PRG members purposefully to implement the experiment. Each treatment was tested by each trial pastoralist. From all trial agro-pastoralists, 36 animals were contributed for the trial (fattening). A total of 36 small ruminants (sheep and goat) were used for the trial, thus each of the six pastoralists had six small ruminants. Training was a crucial component in introducing any new technology. Training on UMMB preparation, formulation of concentrate feed, animal management and data collection were given for PRG members and DAs. The demonstration and evaluation trial were lasts for 4 months data collection period. Data's were collected by the trial PRG Agro-pastoralist throughout the trial period with close follow up of DA's and woreda experts. Finally, data were analyzed using descriptive statistics such as (percentage, ranges, etc.).

Treatments

- Grazing (Traditional) (T1)
- Grazing (improved pasture) + Urea molasses block supplementation (T2)
- Grazing (improved pasture) + Concentrates mix supplementation (T3)

Table 1 - Ration Formulation

Concentrate mix		Urea Molasses Multi Nutrient Block/UMMNB/		Remarks
Items	Percentage (%)	Items	Percentage (%)	
Cottonseed cake	49	Molasses	40	
Wheat bran	50	Urea	10	
Salt	1	Cement	10	
		Salt	5	
		Wheat bran	25	
Total	100	Cotton seed cake	10	N.B:2.5% of their body weight level of supplement given to the animal.
		Total	100	

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RESULT AND DISCUSSION

In this section the findings/ results of the study are presented and discussed. Accordingly, the body weight gain of the experimental sheep & goat fed with grazing + Urea molasses block supplementation (T2) and grazing with supplemented with Concentrates mix (T3) and control one/ free grazing (T1) are presented in Table 1. The initial weight of each sheep and goat was 18.5kg and 15.5kg respectively. Number of animals was 12 in each of the three treatments (i.e., T1, T2, and T3). The final body weight and daily body weight gain was higher 26.5kg final weight in grazing with supplemented with Concentrates mix (T3) than grazing + Urea molasses block supplementation (T2) & control one/ free grazing (T1). In other words, the result shows, that concentrate feed supplementation (T3) results the highest body weight increment on the animals followed by UMMNB feed (T2). On the other hand, free grazing of small ruminants taken as control (T1), gives the lowest effect on animals to increase their body weight. The average daily weight gain range in sheep & goat concentrate mix supplemented group (T3) are 0.066kg/day & 0.066kg/day respectively. The higher live weight gains of concentrate mix supplemented groups(T3) may be due to adequate amount of nutrients in concentrate mixture the CP and energy are comparable than Urea molasses block supplementation (T2) & natural grass grazing without supplementation in (T1).

As table 2 below shows, economic return from goats is slightly higher than sheep under study. This could happen because of the community's food habits. In Afar region, goat is preferred than sheep in their diets. Likewise, goat population is higher than sheep in the region. In every households of the pastoral community of the region; goats are the dominant animals. This could be resulted from feed availability/browses, environmental, agro-climatic condition of the region, genetic make-up of the animals /goats/ adaptability to harsh climate, productivity as well as cultural practices of pastoral community in the region.

The result clearly shows that Urea molasses block supplementation (T2) and concentrate mix supplemented groups (T3) has a great influence on body weight gain of small ruminates relative to control one. Fattening of Afar sheep and goat with supplementation of cconcentrate feed for three months is highly profitable and brings better average daily weight gain as compare to animals feed on Urea molasses block supplementation (T2). The partial budget analysis indicated that small ruminant daily weight gain for 4 months with supplementation of concentrate mix supplemented groups (T3) was found to give on average economic return of 250 birr per head. For instant six agro-pastoralists in Chifra district has tried to fatten six goat & sheep at a time while after four months they sold all goats and they got net earnings of 9000birr. The remaining group members have been castrated their male goats for further fattening purpose. Because of this, so money agro-pastoralists has been attracted by the business and engaged in small ruminant fattening practices by purchasing some animals from market without external advice/support.

Table 2 - Body weight change and Economic Benefits of Afar Shoat fattening (Sheep & Goat) by feeding UMMNB and Concentrate Supplementation in Chifra district

Items		Grazing +UMMB	Grazing+ Concentrate feed(300gm/DM)
Initial weight(kg)	Shee	18.5	18.5
	Goat	15.5	15.5
Final weight(kg)	Shee	25	26.5
	Goat	22	23.5
Weight change with four months (kg)	Shee	6.5	8
	Goat	6.5	8
Daily weight gain (kg)	Shee	0.054	0.066
	Goat	0.054	0.066
Total cost per trial PRG		1600	1700
Gross return per trial PRG		3000	3200
Gross margin per trial PRG		1400	1500
Total gross output		18000	19200
Total costs		9600	10200
Total gross margin		8400	9000
Economic return		233 per animal	250 per animal

CONCLUSION

This study aims to evaluate different feed technologies on small ruminants in participatory manner on pastoralist context through pastoralist research group. Three-hundred-gram concentrate mix /head/day supplementation (49% Cottonseed cake mixed with 33% wheat bran and 1% salt) of small ruminants (sheep & goat) has better weight gain and economically feasible for the chifera woreda established PRG. It was also economical at PRG level, with average gross profit of 250 ETB per head in chifera area. Because of this, so money agro-pastoralists has been attracted by the business and engaged in small ruminant fattening practices by purchasing some animals from market place without external advice/support. In

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the future animal fattening will be played imperative role to improve the livelihood of the poor pastoralists and agro-pastoralists through enhancing the daily income. Besides, agro-pastoralists and extension workers had appreciated fattening practice with concentrate mix feed supplementation than conventional fattening practice.

Recommendations

Even though promising practices had been observed during the study; there are some critical activities has to be done in order to increase benefit and support pastoral livelihoods in this endeavor. The following are recommended to be done in the future.

- Extending on-farm research and increasing numbers of PRG and numbers of agro-pastoralists in each PRG
- Continuous awareness raising to the communities on how to fatten small ruminates
- Working closely with Agro-pastoralists
- Scale up of on-farm research result

DECLARATION

Authors' contribution

Mohammed Nuru contributed in all stages of implementation including review and developing the content.
Mohammed Yasin contributed mainly on data gathering and actual implementation of the trial.

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Conflict of interest

The authors declare they have no competing of interests.

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ISOLATION OF EXTRACELLULAR PHYTASE PRODUCING LACTIC ACID BACTERIA FROM THE GASTRO INTESTINAL TRACT OF POULTRY BIRDS

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ABSTRACT: Bacterial phytases and phytase-producing bacteria are of great industrial significance in the poultry industry and also in phosphorus pollution management. This study was designed to isolate and screen for phytase producing lactic acid bacteria from the duodenum, ileum and cecum of eight healthy cockerel samples. Standard microbiological procedures were followed to isolate phytase producing lactic acid bacteria using de Man Rogosa and Sharp (MRS) agar while extracellular phytase screening was done using phytase specific medium. The range of total microbial count obtain was highest at the cecum (2.85±0.11 to 4.34±0.12 log_{10} cfu/ml), lower at the duodenum (2.02±0.11 to 4.27±0.20 log_{10} cfu/ml) and lowest at the ileum (2.00±0.21 to 4.19±0.25 log_{10} cfu/ml). Nineteen bacterial isolates were identified as lactic acid bacteria on the basis of morphological, biochemical and physiological characterization and later identified as *Lactobacillus* species (78.94%), *Enterococcus* species (15.78%) and *Lactococcus* species (5.26%). Thirteen out of the nineteen lactic acid bacteria showed phytase activity. Low phytase activity was observed in eight of the lactic acid bacteria isolates while five of the isolates produced significant extracellular phytase activity (>6mm). The most predominant *Lactobacillus* species were also found to be the most potent phytase producers. This can be exploited for industrial production of phytase in upgrading the nutritional status of feed and combating phosphorus pollution from poultry waste.

Keywords: Phytase, Gastrointestinal tract, Lactic acid bacteria, Phosphorus pollution, Poultry industry.

INTRODUCTION

Phosphorus is an important nutrient stored in the form of phytic acid (*Myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate*) in cereals, legumes, and oilseed crops (Azeke et al., 2007). Phytic acid acts as antinutrient constituent in plant-derived food and feed as it forms complexes with proteins, amino acids, and various metal ions (Astley and Finglas, 2016; Nissar et al., 2017). The bound phosphorus is poorly available to monogastric animals such as pigs, poultry and fishes, due to lack of production of phytases in the gastrointestinal tract (Jacela et al., 2010; Abdel-Megeed and Tahir, 2015). Excretion of the undigested phytate poses a serious phosphorus pollution problem contributing to eutrophication in areas of intensive livestock production (Singh et al., 2011; Abdel-Megeed and Tahir, 2015). The enzyme phytase hydrolyzes the ester bond in phytic acid to liberate inositol and inorganic phosphate (Nissar et al., 2017). It can be sourced from some plants, animal tissues and microorganisms; microbial sources are however more promising for the commercial production of phytases (De Angelis et al., 2003).

Phytases have been obtained mainly from filamentous fungi (Maller et al., 2013); it has also been detected in various bacteria species such as *Bacillus*, *Pseudomonas*, *Escherichia coli, Enterobacter*, *Klebsiella*, *Lactobacillus sanfranciscensis* as well as anaerobic rumen bacteria, particularly in *Selenomonas ruminantium*, *Megasphaera elsdenii, Prevotella* sp., *Mitsuokella multiacidus* and *Mitsuokella jalaludinii* (Shim and Oh, 2012).

Species of lactic acid bacteria (LAB) belonging to numerous genus under the family of *Lactobacillaceae* have been widely applied in food fermentation worldwide due to their widely known status as generally recognized as safe (GRAS) microorganisms (Hayek and Ibrahim, 2013). They are also recognized for their fermentative ability which contributes to enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits (Sharma et al., 2012; Steele et al., 2013). There are only few reports of phytase producing lactic acid bacteria available in literature, therefore this present study was designed to isolate phytase producing lactic acid bacteria from the gastrointestinal tract of poultry. The addition of phytase to poultry feed will improve the nutritional quality of feed by increasing the amount of free phytate phosphorus in poultry diet and diminishing the necessity of addition of inorganic phosphate to animal feed, thereby combating phosphorus pollution associated with the feed and poultry industries.

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MATERIALS AND METHODS

Ethical Approval

The Ethics Unit of the Research and Innovation Committee of Samuel Adegboyega University approved the study protocol.

Study Area

The study was carried out at College of Basic and Applied Sciences, Samuel Adegboyega University, Ogwa in Esan West Local Government Area of Edo State, Nigeria.

Sample collection and preparation

Eight cockerels were purchased from Global Poultry, Uromi, Esan North East Local Government Area, Edo State, Nigeria. The gastrointestinal tracts of the eight chickens were aseptically collected in ten sterile plastic bags and transported to the laboratory in ice packs for microbiological analysis. The samples were represented with codes A-H. The duodenum, ileum and cecum represented with codes d, i and c for each of the eight samples were removed separately under sterile conditions to give a total of twenty-four samples.

Enumeration and isolation of bacteria

Ten grams of the duodenum, ileum and cecum respectively for each sample was weighed aseptically and transferred into a sterile beaker containing 100ml of normal saline. Six-fold serial dilution (10^{-1} to 10^{-6}) was made using normal saline. An aliquot of 1 ml of the appropriate six-fold serial dilution (10^{-2}) of the intestinal samples were inoculated into the de Man Rogosa and Sharp (MRS) agar plates using standard pour plate method and incubated anaerobically at 37°C for 36 hours. Visible discrete colonies on inoculated plates were counted using the colony counter and expressed in colony forming units per millilitre (cfu/ml) of the intestinal sample. Discrete colonies were selected and purified by subculturing in MRS broth. Further purification was carried out by repeated streaking on freshly prepared MRS agar plates. The pure isolates were stored at 4°C using MRS agar slants.

Characterization and identification of bacterial isolates

Pure cultures of all isolates were characterized and identified by means of their cultural, morphological, physiological and biochemical characteristics using Bergey's manual of systematic Bacteriology (Holt et al., 1994)

Phytase activity screening

The isolated pure strains were screened for the production of extracellular phytase using phytase specific medium (Chunshan et al., 2001). The phytase screening medium was prepared by dissolving 3g glucose; 1g Tryptone; 1g sodium phytate; 0.3g $Cacl_2$; 0.5g $MgSO_4$; 0.04g $MnCl_2$; 0.0025g $FeSO_4$; and 15g agar in 1 litre of distilled water. The pure cultures were streaked at the centre of the plate and the plates were incubated at 37^0C for 62 hours as described by Kumar et al. (2011). The plates were then observed for formation of clear zone around the colony. A clear zone around the colony indicates positive result. Only those with zones greater than 6mm in diameter were recorded as significant.

Data analysis

The mean, standard error of mean, one way ANOVA and Tukey's Post Hoc analysis were done using IBM SPSS Statistics 23 software for Windows. P value < 0.05 was statistically significant.

RESULTS

The total bacterial count from the duodenum, ileum and cecum of the eight chicken samples are presented in Table 1. A total of fifty-seven bacteria isolates were randomly selected based on distinct colony morphology and purified. The morphological, physiological and biochemical characteristics of the pure isolates revealed that 49.12% of the bacterial isolates were white, viscous, entire, glistering and raised. 10.53% were creamy, viscous, entire, glistering and flat. 26.32% were white, viscous, entire, glistering and raised. 12.28% were white, dry, entire, rough and raised. 1.75% were creamy, viscous, entire, glistering and raised. Nineteen out of the fifty-seven bacterial isolates were presumed as lactic acid bacteria on the basis of gram stain reaction, catalase production and oxidase activity. The isolates were gram positive short rods and cocci, catalase negative and oxidase negative. Further presumptive tests including growth at temperature 10^0C and 45^0C, growth at pH 4.5 and 6.5, gas production from glucose and ability to ferment various carbohydrates (lactose, maltose, sucrose and glucose) performed indicated that growth was recorded for all the isolates at pH 4.5 and pH 6.5 at 45^0C only. The isolates were identified as *Lactobacillus*, *Lactococcus* and *Enterococcus* species. The percentage occurrence of the lactic acid bacteria isolates is shown in Figure 1. Thirteen out of the nineteen lactic acid bacteria isolates showed phytase activity by hydrolyzing sodium phytate to form a clear zone around the colony (Table 2). Five bacterial isolates, all *Lactobacillus* species, (Dc2, Dd2, Dd4, Fd1 and Fc3) had a significantly different ($p<0.05$) ability to hydrolyze phytate by forming a clear zone > 6mm.

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Table 1 - Total bacterial counts (Log_{10} cfu/ml) from the gastrointestinal tracts of samples.

Sample	Duodenum	Ileum	Cecum
A	2.02 ± 0.11	-	2.85 ± 0.11
B	2.26 ± 0.17	2.00 ± 0.21	3.88 ± 0.14
C	3.77 ± 0.12	2.98 ± 0.25	-
D	4.27 ± 0.20	4.11 ± 0.20	3.37 ± 0.14
E	2.03 ± 0.15	3.23 ± 0.22	4.34 ± 0.11
F	2.57 ± 0.38	4.05 ± 0.13	3.53 ± 0.12
G	3.57 ± 0.37	4.19 ± 0.25	3.90 ± 0.18
H	3.85 ± 0.33	3.65 ± 0.25	3.69 ± 0.11

Values are mean ± standard error of mean of triplicate determinations. - = Absent, A-H = Isolation codes for the 8 chicken samples.

Table 2 - Phytase screening of lactic acid bacteria from the gastrointestinal tract of poultry samples

S/N	Isolation code	Hydrolysis of phytate	Clear zone (mm)	Probable bacterial species
1	Ad1	+	5.13 ± 1.02[a]	*Lactococcus*
2	Bc1	+	3.24 ± 0.86[b]	*Lactobacillus*
3	Dc2	+	9.26 ± 2.11[c]	*Lactobacillus*
4	Dd1	-	0	*Enterococcus*
5	Dd2	+	11.21 ± 1.32[d]	*Lactobacillus*
6	Dd3	+	5.42 ± 1.51[a]	*Enterococcus*
7	Dd4	+	8.25 ± 0.93[e]	*Lactobacillus*
8	Dd5	-	0	*Lactobacillus*
9	Dd6	-	0	*Lactobacillus*
10	Dd7	-	0	*Lactobacillus*
11	Di3	+	4.18 ± 1.44[f]	*Lactobacillus*
12	Ei1	-	0	*Lactobacillus*
13	Ei2	+	5.22 ± 0.76[a]	*Enterococcus*
14	Fd1	+	8.33 ± 0.43[eg]	*Lactobacillus*
15	Fd2	+	2.38 ± 1.63[h]	*Lactobacillus*
16	Fc3	+	7.44 ± 0.88[ei]	*Lactobacillus*
17	Fi2	-	0	*Lactobacillus*
18	Gi1	+	5.23 ± 1.05[a]	*Lactobacillus*
19	Gi3	+	6.00 ± 1.32[a]	*Lactobacillus*

KEYS: A-H= isolation codes for the 8 chicken samples, + = positive, - = negative, d= duodenum, i= ileum, and c=cecum. Values with different superscript are significantly different ($p<0.05$).

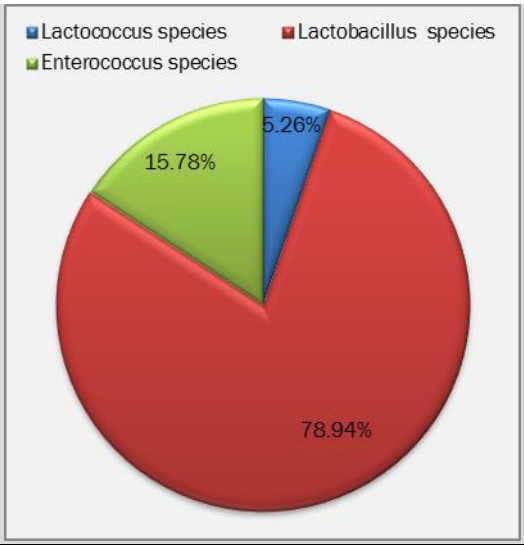

Figure 1 - Percentage occurrence of lactic acid bacteria isolates from gastrointestinal tract of poultry samples

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DISCUSSION

The bacteria growth recorded in the duodenum, ileum and cecum of all the chicken samples had different growth count range (Table 1). The variation in the microbial population suggests that each region developed its own unique bacterial community due to the pH of the stomach contents, the toxicity of bile salts, fermentative metabolism and the relatively swift flow of the digesta in the gastrointestinal tract (Walter, 2008). This result agrees with the findings of other researchers available in literature. Jiangrang et al. (2003) reported differences in the diversity of bacterial floras in the ilea and ceca of maturing broiler chickens; Bjerrum et al. (2006) investigated microbial communities in the ileum and cecum of broiler chickens, they reported that lactobacillus species dominated the chicken ileum while the cecum harbored more diverse microbial community and Abbas et al. (2007) identified various levels of abundance of different lactobacillus species from the crop of 1- and 5- week old broiler chickens using 16s rRNA gene sequence.

The isolates were identified as *Lactobacillus* species, *Lactococcus* species and *Enterococcus* species. Previous studies confirm the existence of these organisms in the gastrointestinal tract of chicken (Lan et al., 2003; Sonplang et al., 2007). The Lactobacillus species were more dominant because of their ability to adhere to the surface of the non-secretary epithelium lining of these sites, which enables the bacteria to form a biofilm-like structure that provides a bacterial inoculum of the digesta (Salas-Jara et al., 2016). Different studies on the microbiota of the gastrointestinal tract of poultry have pointed out the predominance of lactobacilli in chicken crops and intestine (Beasley et al., 2004; Bakari et al., 2011).

Thirteen out of the nineteen lactic acid bacteria showed phytase activity, suggesting that they could be a potential source of phytase to be used in improving the nutritional quality of poultry diet and decreasing the amount of phosphorus released to the environment (Hill et al., 2007; Abdel-Megeed and Tahir, 2015). Five of the *Lactobacillus* species were found to be the most potent phytase producers. Phytase producing ability of lactic acid bacteria has also been reported in some previous studies. Raghavendra and Halami (2009) isolated forty lactic acid bacterial strains with phytate degrading ability while Anastasio et al. (2010) reported the use of lactic acid bacteria to improve mineral solubilization during dough fermentation due to their production of phytate-degrading enzymes.

CONCLUSION

In this study, phytase producing lactic acid bacteria were isolated from the gastrointestinal tract of healthy cockerels. These findings can be further explored in the industrial production of phytase. It will be of immense benefit to the poultry industry. Feed supplementation with phytase will help to improve the nutritional status of the feed. This also has implication for environmental management as it would lead to a reduction in phosphorus pollution.

DECLARATIONS

Competing interest

The authors declare that they have no competing interest.

Ethics

The research was done following ethical procedures.

Authors' contribution

AAD, LE designed the experiment; AAD, GDO carried out data collection; LE performed data analysis; AAD, GDO and LE contributed in manuscript preparation and approval for publication.

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i. DISCUSSION
j. CONCLUSION
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The sections "RESULTS AND DISCUSSION" can be presented jointly.
The sections "DISCUSSION AND DISCUSSION" can be presented jointly.

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Following the abstract, about 3 to 7 **key words** should be listed.

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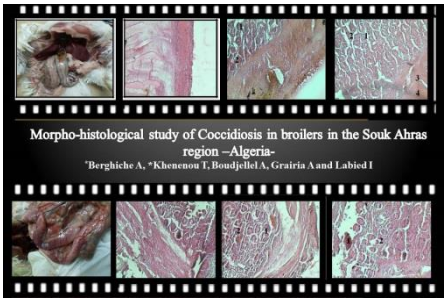

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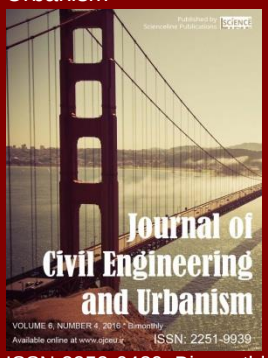

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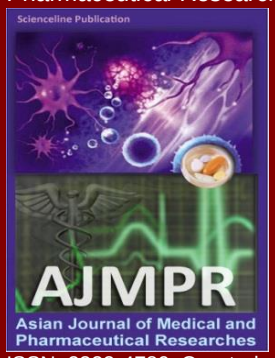

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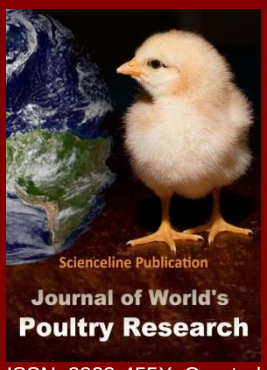

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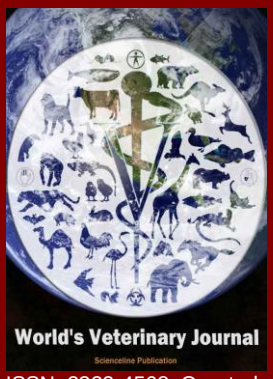

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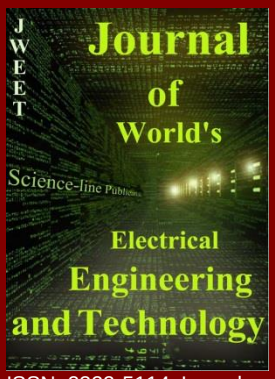

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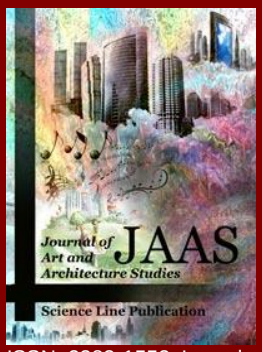

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