NUTRITIVE VALUE, TANNIN BIO ASSAY AND PROCESSING EFFECTS OF ACACIA PODS AS SUPPLEMENTS TO GOATS IN MOGOTIO SUB-COUNTY, KENYA

BY

MUTAI PAUL ARAP

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL PHYSIOLOGY IN THE SCHOOL OF SCIENCE

UNIVERSITY OF ELDORET, KENYA

SEPTEMBER, 2022

DECLARATION

Declaration by the Candidate

I declare that my thesis is my original work and that it has not been submitted to any other institution for examination or award of degree and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret

……... ……

MUTAI PAUL ARAP **DATE**

SCI/BIO/P/02/18

Declaration by Supervisors

This thesis has been submitted with our approval as university supervisors

Sign ..... **Date …….**

DR. ANASTASIA NANDWA (PhD)

Lecturer, Department of Biological Sciences

University of Eldoret.

Sign ..... **Date …….**

DR. SALINAH RONO (PhD)

Lecturer, Department of Biological Sciences

University of Eldoret.

DEDICATION

This research thesis is dedicated to my wife, Helen Mutai, my children, Amos, Joyce, mercy, Bethwel and Caleb for relentless support while writing this research project.

ABSTRACT

Tannins are anti-nutritional factors in forages. This research was conducted in Emining ward, Baringo County- Kenya. The objective was to evaluate nutritive value, tannin bioassay and processing effects of mature green pods of *Acacia sp*ecies as supplements to goats and characterize rumen cellulolytic bacteria. A total of 20 bucks aged 4-5 months were randomly sourced from Radat market, sprayed against ectoparasites and dewormed against endoparasites. Each buck was housed individually in a goat pen raised 1 m high with slatted floor measuring 1.5 by 1.5m. All the bucks were fed on a basal diet of Rhodes grass hay (*Chloris gayana*) mixed with wheat bran at 3:1 ratio. They were divided into five treatments groups of four each and allocated to five treatments. T1- (control-untreated) pods of identified mature *Acacia brevispica, A. mellifera and A. tortilis, (*T2-shade –dried pods for 48hours, *T3-(*sun dried pods for 48 hours, and T4- pods soaked in wood ash-alkali solution (10-12 Ph.) mixed at 200g ash per/liter of water for 48 hours respectively while T5 was basal diet only. Nutrient composition was determined by proximate analysis and Van-Soest laboratory procedures (AOAC-1995) and tannin bioassay by Cio- calteu/makkar procedures (2005). Organic matter digestibility was assessed by *invitro* gas production technique and food conversion efficiency determined as a percentage of the total weight gain by the total feed taken. At end of 3-months, 3 bucks were randomly selected from each treatment group and killed humanely. Hot, cold carcass and organ weights were recorded and the effects of tannins on gut rumen cellulolytic bacteria were isolated, quantified using serial dilution, enumeration of colony forming units and characterized morphologically by Gram staining. Analysis of variance in Stratigraphic Centurion XII was used to test for significant difference between dependent variables. Mean differences were separated using Least Significant Difference. *Acacia mellifera* (9.03±0.57) had the highest percentage ($p=0.0011$) of moisture and crude protein. *A. tortilis* (4.73±0.77) and *A. brevispica* (4.13±0.14) had the lowest percentage of ash ($p<0.05$) from that of *A. brevispica* (0.0244). In all *Acacia sp*ecies, methane gas produced increased per day with more produced in alkali treated pods ($p<0.05$). Among the *Acacia sp*ecies, all supplements processed in alkali were ingested in large amounts ($p<0.05$) with *A. tortilis* pods taken in large amounts (416.50±6.50) compared with the others ($p=0.0012$). For control treatment 1, more of *A. tortilis* supplement was consumed in as compared with the others ($p=0.0011$). Initial weight of the Small East African Goat (SEAG) did not differ prior to feeding with supplements ($p>0.05$). Feeding with *A. tortilis* resulted to the highest final weight (14.20±0.36g). Bucks fed on *Acacia tortilis*- alkali-treated-pods resulted in best average daily gain of 15.03±2.01g followed by *A. brevispica* (14.13±1.94g) with a significant difference ($p<0.05$) with that of *A. mellifera*. Low food conversion ratio was recorded in *Acacia tortilis* treated in alkali as compared to other treatments. Pre- and post- slaughter weights as and kill out percentage did not differ among and within treatment. In conclusion, *A. tortilis* pods showed high nutritional components, alkali processing resulting in highest tannin reduction and highest rumen bacterial counts. Organ weights differed among and within treatments with the largest liver weight recorded in goats fed with *Acacia. brevispica* and *A. mellifera* pods. Alkali treated *A. tortilis* pods resulted in significantly high rumen cellulolytic bacteria. Alkali processing is recommended for tannin reduction and *A. tortilis* met most of the nutritional requirements and was recommended as goat supplement.

Key words: Acacia species, nutritive value, tannin bioassay, processing effects, goats.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS AND ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid Detergent Lignin
ANF	Anti-Nutritional Factors
ANOVA	Analysis Of Variance
ASAL	arid and semi-arid land
C_4H_9OH	Butanol
$Ca(OH)_2$	Calcium hydroxide
Ca	Calcium
$CaCl_2.6H_2O$	Calcium chloride
CFUs	Colony Forming Units
CO_{2-}	Carbon Dioxide
$COCl_2.6H_2O$	Cobalt chloride
CP	Crude protein
CTAB	Cetyl trimethlammoniumbromide

DM	Dry matter
DNA	Deoxyribonucleic Acid
EE	Ether Extract
FAO	Food and Agriculture Organization
$FeCl_3.6H_2O$)	Iron chloride
GCT	Gram Condensed Tannins
GDP	gross domestic products
GIT	Gastro-Intestinal Tract
GLM	General Linear Model
GODAN	Global Open Data on Agriculture and Nutrition
H_2S0_4	Sulphuric Acid
HCL	Hydrochloric Acid
HT	Hydrolysable Tannin
IOMD	in-vitro organic matter digestibility
KALRO	Kenya Agricultural and Livestock Research Organization
Kgs	Kilograms

KH_2PO_4	Potassium Dihydrogen Phosphate
LSD	Least Significant Difference
MALF	Ministry of Agriculture, Livestock and Fisheries
MPN	Most Probable Number
NAN	Non-Ammonia Nitrogen
NAOH	Sodium hydroxide
NaOH	Sodium Hydroxide
NARL	National Agricultural Research Laboratories Kabete
NDF	Neutral Detergent Fiber
NDIN	Neutral Detergent Insoluble Nitrogen
NH_4HCO_3	Ammonia Hydrogen Carbonate
^oC	Degree Centrigrade
OM	Organic Matter
OMD	Organic Matter Digestibility
PEG	Poly Ethylene Glycol
pH	Potential of hydrogen

PVPP	*polyvinylpolypyrrolidone*
RCBD	Randomized Complete Block design
RNA	Ribonucleic acid
SCFA	Short Chain Fatty Acids
SEAG	Small East African Goat
SEM	Standard error of mean
TA	tannic acid
TCT	Total condensed tannins
TEPH	Total Extractable Phenolic
TET	Total extractable tannins
VFAs	volatile fatty acids
VFI	voluntary feed intake

ACKNOWLEDGEMENT

I acknowledge the Almighty God for enabling me to come up with this thesis report. In addition, acknowledge my supervisors Dr. Anastasia Nandwa and Dr. Salinah Rono for their guidance, constructive comments and time sacrificed to read through my work since I started my research project.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Livestock sector is an important global player with enormous economic, social and environmental impact (Steinfeld *et al*., 2006). Worldwide, livestock production contributes 40% of global agricultural GDP, employs 1.3 billion people, providing livelihoods for 1 billion of the world's poor people (Alders *et al*., 2021). It is also the single largest anthropogenic land user accounting for 70% of all agricultural land and 30% of the land surface on the planet. The impacts of livestock subsector are being increasingly felt due to demographic increase and income growth. Global meat demand is projected to more than double from 229 metric tons in 1999- 2001 to 465 metric tons in 2050 and the minimum per capita protein intake recommended for maximum human physical and mental development is about 56g (Robinson *et al*., 2015). It is also estimated that in the developing countries like Kenya, 48% of protein food and 20% of food energy is obtained from livestock (Steinfeld *et al*., 2006).

Current productivity is unsustainable especially in Africa where the livestock sector is growing very fast driven by escalating demand, rising population and per capita income (Li *et al*., 2014). In Kenya the livestock subsector contributes about 12% of $4.5 billion per year which is 40% of agricultural Gross domestic products (GDP), employing over 50% of agricultural labor force and the beef sector is ranked as the fastest rising economic sector (Okello *et al*., 2021). The per capita meat consumption was 10.8 kg in

the year 2003. Meat consumption has increased by about 10% in the last six years and production rose from 287000 metric tons in 2001 per year to almost 300000 metric tons in the year 2008 (Belay *et al*., 2013). There is an escalating demand not only for a bigger scope of quality attributes from livestock but also of the practices used to produce (Behnke & Nakirya, 2012).

The livestock population in Kenya is estimated at 60 million units, made of 10 million beef cattle, 3 million dairy and dairy crosses, 9 million goats, 7 million sheep, 29 million indigenous and exotic chicken, 0.8 million camels, 0.52 million donkeys and 0.3 million pigs (Engida *et al*., 2015). The pertinent principle in profitable livestock production includes genetic selection for growth and profound knowledge on livestock nutritional requirements which underpins cost-effective productivity. The genotypic diversity in the goats kept in Kenya's arid semi-arid lands (ASALs) zones and the contrasting habitats confounds the complexity of their nutrient's requirements. It is therefore imperative to carry out research on acacia trees mature pods nutritional potential to mitigate the effects of livestock malnutrition, environmental degradation, and climate change (Omoyo, Wakhungu, & Oteng'i, 2015).

Kenya is divided into seven Agro -Ecological Zones each of which has its unique physiognomic characteristics such as the moisture availability zones and temperature zone. There are over 1,342 *Acacia sp*ecies distributed throughout the world with Kenya having 52 species widely distributed across the varied agro- ecological zones (Mutai, 2022). In northern Kenya, there are over three million pastoralists who often experience severe drought, and in the past one hundred years have experienced twenty-eight major

droughts four which took place in the last ten years. The pastoralists depend solely or mostly on livestock. Droughts usually result in high livestock mortality rate rendering these pastoralists among the most vulnerable communities in Kenya (Omoyo, Wakhungu, & Oteng'i, 2015).

The mixed grasses and browse vegetation commonly found in the field is low in nutritional value and hardly support fast growth rate and early maturity and off take that meets the pastoralists' various socio – economic requirements (Oba, 2012). Usually, at the onset of the dry season, the quality of feed worsens quickly, quantity reduces due to increased grazing pressure. The average crude protein percentage during dry season grazing is less than 3%. In the tropics, fodder shrubs and fodder trees usually supplement natural grazing for domestic animals and they are recognized as critical components of livestock nutrition supplying protein and energy in critical periods like during drought. In Kenya, artificially established field legume pastures like Lucerne, desmodium, vetch and beans have high protein content of over 20%.

The acacia tree legumes, being members of the same family (Leguminiceae), are expected to contain reasonably good proportions of plant protein for maintenance and production to support livestock growth and development. The most common *Acacia sp*ecies in the ASAL used by pastoralists in Kenya are *Acacia brevispica, A. mellifera* and *A. tortilis* hence the reason why they are the subject of this study. ASALs constitute about 80% of Kenya's land mass, and providing over 80% of the total meat produced in Kenya (Dinnage *et al*., 2019).

Most *Acacia sp*ecies possess tannins which protects them from excessive feeding by herbivores and also protects dietary plant proteins from ruminal microbial digestion by forming tannin- protein complexes hence making them unavailable for degradation and consequently increasing their output in faeces (Wink, 2013). It is perceived that poor protein digestibility in sheep and goats is attributed to presence of tannins in *Acacia sp*. estimated at 4-11% on DM basis (Mutai, 2022). In plants cells, hydrolysable and condensed tannins molecules are situated in the vacuoles and are said to be discharged to the cytoplasm during cell damage like during mastication by ruminants, and they have an affinity to bind chemically not only with proteins but also with polysaccharides, nucleic acids, alkaloids, saponins and steroids (Dinnage *et al*., 2019).

In Kenya, *Acacia sp.* are commonly used by pastoralists as a non-conventional feed resource (Mutai, 2022). Irrespective of this, they are said to have high tannin levels of about 18.71%, but rich in protein and energy (13%-20%) Crude Protein but recommended as livestock substitute to replace cereal-based concentrates in conventional rations (Dinnage *et al*., 2019). The acacia tree species legumes have huge nutritional potential being members of the *leguminosae* family usually very rich in proteins necessary for growth and development of meat goats and other livestock species (Dinnage *et al*., 2019). *Acacia mearnsii* is a commercial wattle grown in some parts of Kenya is a tannin rich species with tannin-based adhesive (Mutai, 2022). Many range lands of Africa are inhabited by various *Acacia sp*ecies and other pod producing trees which produce pods high in protein. Such pods incidentally ripen at the start of the dry season hence can be used in livestock supplementation due to scarcity of pastures at such times (Wink, (2013).

The greatest challenge in efficient, effective and sustainable livestock productivity is the provision of adequate nutrition both in quantity and quality and that nutritional costs accounts for 70% of the total livestock production costs (Wu *et al*., 2014). This is usually exacerbated in the tropics by prolonged droughts resulting in inadequate and low-quality feed. The inadequacy of livestock feeds in most developing countries affects productivity and pressure on the utilization of unconventional livestock feed resources has been growing to develop least cost rations and minimize production costs for optimal profit maximization (Wu *et al*., 2014). The ruminant production systems in these regions have lagged behind in productivity since they are predominantly in the hands of the resource-poor small-scale farmers for example in Kenya's ASAL (Mlambo and Mapiye, 2015).

Acacia mellifera pods can be a good source of protein and energy in a concentrate mixture in ruminant nutrition and can improve energy utilization. To supplement natural pastures and crop residues, foliage and fruits (pods) from leguminous trees is becoming an important source of good quality livestock feed, which, however, has not been fully exploited (Wu *et al*., 2014. The Crude protein (CP) content of *Acacia mellifera* was reported to be 159g/kg of digestible material as compared with CP of 45.5g/kg of DM on native pasture hay which cannot meet the normal requirements of CP of 80g/kg of DM for optimal ruminal microbial functions (Mutai, 2022). This calls for the identification and value –addition of locally available non-conventional feed resources like acacia tree legumes which have nutritional potential, and with little human- animal nutritional conflict (Mlambo and Mapiye, 2015).

The ruminal microflora in the gastro-intestinal tract of ruminants plays a significant role in the conversion of feed into useful end products. The livestock sector is fast-growing in the developing countries and hence the need to comprehend these microbial processes for improved management and utilization of feed-based and related non-conventional natural resources that underpins the innovation of sustainable nutritional systems (McSweeney & Mackie, 2012).

Goats and sheep are the most widely kept livestock species by pastoralists world-wide, and goats are able to access more arid areas than sheep (Hermes *et al*., 2020). The nutritional management of goats in the range eco- system is mainly dependent on the implementation of grazing management strategies that maintain the biodiversity of plant species that are available as feed resource for goat production. The daily dry matter intake of goats depends on type of breed, stage of productivity, sex, required level of weight gain, expected birth weight of kids and growth potential (McGregor, 2016). In Kenya goats are kept under two production systems, namely the extensive (Agro-pastoralists) system practiced in the arid and semi-arid lands (ASALs), and the intensive production system specifically for dairy goat production in the medium and high potential areas of Kenya.

In the ASAL areas the small ruminant keepers usually depend on the Galla goat breed or the Small East Africa Goat (SEAG) for meat, milk and capital income, but the production per animal is very low (Fanzo, 2014). The dry season in these regions is usually around July to March while the rainy season is from April to June and in these areas, goats are reared under the extensive system where they are left to scavenge for feed and water the

whole day, in natural range lands with grasses and vegetation mainly dominated by browse plants only to come back to the homestead to be housed in temporary structures, with no feed supplementation. Goats when only fed on scanty and low-quality natural pastures and browse material and crop residues without concentrate supplementation perform poorly (Lamidi & Ologbose, 2014). Other workers have found that goats spend up to 60-70% of their feeding time browsing which can be in the form of fresh leaves, ripe pods or dried leaves, and that browse pods are high in nutritive value and can be used as supplements with low quality roughages (Fanzo, 2019).

The ruminal microbiota must be in the right microbial status quo for the fermentation of coarse and often low nutrient potential of dry land forages and it is imperative to interrogate the potential effects of acacia tannins on the rumen microbiota microbiome (Abd'Quadri-Abojukoro, 2021). The ruminal microflora in the gastro-intestinal tract of ruminants plays a significant role in the conversion of feed into useful end products. However, the colonization of the rumen microbiota in relation to tannin level in ruminants have not been fully investigated (Howieson *et al*., 2014). The livestock sector is fast-growing in the developing countries and hence the need to comprehend these microbial processes for improved management and utilization of feed-based and related non-conventional natural resources that underpins the innovation of sustainable nutritional systems (Abdel-Shafy & Mansour, 2018).

1.2 Statement of the problem

The nutritive value and effects of Kenya's *Acacia brevispica, A. mellifera* and *A. tortilis* have not been fully studied and documented yet they are widely distributed in Kenya's

arid and semi-arid lands (ASALs). They are utilized by the pastoralists to feed their livestock but they possess tannins which interfere with protein bio-availability and digestibility by ruminants (Samtiya, Aluko & Dhewa, 2020). Information on the levels of tannins in *Acacia sp*ecies and their effects on ruminant nutrition is essential for improved livestock productivity (Bayssa, Negesse, & Tolera, 2016). The levels of these compounds in the plants depends on the location and the aridity of the area.

Little effort has been made in Kenya to process the *Acacia sp*ecies pods to add value to their nutritional potential by reducing the anti-nutritional factors (tannins). Most fodder forages possess *lectins* e.g. r*obin* and *ricin* found in Robinia pseudo-acacia and have been implicated in causing clinical signs of malaise, posterior paralysis, and anorexia in cattle (Kamo *et al*., 2012). It is in this aspect that the study intends to assess the nutritive value, tannin bio assay and processing effects of acacia pods to reduce tannin levels as supplements to goats.

1.3 Broad objective

The aim of this study was to determine the nutritional value, tannin bioassay and effects of *Acacia brevispica, A. mellifera* and *Acacia tortilis* pods processed differently as supplements to growing Small East Africa Goats (SEAG) in Mogotio sub-County-Kenya.

1.3.1 Specific objectives

i. Tannin bioassay in nutrient composition of mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* pods.

ii. To establish effects of tannins in mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* when used as supplements on *invitro* organic matter digestibility (IOMD) and growth performance of growing Small East African Goats (SEAG).

iii. To determine feed intake and feed conversion efficiency (FCE) of the growing SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements.

iv. To establish the effects of tannins on organs (lungs, liver, kidney and spleen) weights and carcass yields of growing SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements.

v. To evaluate the effects of *Acacia brevispica, A. mellifera* and *A. tortilis* tannin used as feed supplements to growing SEAG on quantitative and qualitative characteristics of rumen cellulolytic bacteria.

1.3.2 Research Hypotheses

i. H_{o1}: There is no significant difference in nutrient composition bio assay of *A. mellifera, Acacia brevispica* and *Acacia tortilis* pods.

ii. H_{o2}: There is no significant effect of tannins levels in mature *A. mellifera, A. brevispica* and *Acacia tortilis* pods processed differently used as supplements on growing SEAG on in-vitro protein digestibility and growth performance.

iii. H_{o3}: There is no significant difference in feed intake and feed conversion efficiency of mature *A. mellifera, A. brevispica* and *Acacia tortilis* pods processed differently as supplements to growing SEAG.

iv. H_{o4}: There is no significant difference in organs (lungs, liver, kidney and spleen) weights, and carcass yields of *A. mellifera, A. brevispica* and *Acacia tortilis* pods processed differently as supplements to growing SEAG.

v. H_{o5}: There are no significant effects on quantitative and qualitative characteristics of cellulolytic rumen bacteria in goats fed on, *A. mellifera, A. brevispica* and *Acacia tortilis* pods' tannins processed differently as supplements to growing SEAG.

1.4 Justification

Despite the nutritive potential of acacia tree legumes in animal feed, the research results are inconsistent to enhance the ruminant nutritional value chain. Use of these plants as food will improve resilience, and sustainability and help to mitigate the vagaries of climate change, hence improve ruminant productivity and farmers' income.

There is need to determine the nutrient and tannin composition in mature green pods of *Acacia brevispica, A. mellifera* and *A.tortilis* with the aim of checking how to improve organic matter digestibility from these potentially nutritious, locally available and non-conventional sustainable protein sources in livestock feeding. This will enhance goat productivity and improve on goat keeper's income and standard of living.

There is need to determine the effects of mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* pods*'* tannins processed differently on the SEAG internal organs

(lungs, liver, kidney and spleen) weights and carcass yields when used as supplements. This will improve on the overall carcass yield and boost the goat keeper's income.

There is need to determine the effects of mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* pods*'* tannins processed differently on rumen cellulolytic bacteria quantification, isolation and characterization when used as supplements on growing SEAG. This will reduce the negative effects of tannins on specific bacteria and hence improve on their cellulolytic functions, notwithstanding the identification of novel tannin-resistant cellulolytic bacteria which can be further developed and incorporated in to ruminant nutrition value chain so as to improve on ruminant animal performance.

It is essential to establish the best method of processing tannins in tanniniferous *Acacia sp*ecies so as to reduce their negative effects on the utilization of these otherwise nutraceutical tree legumes species

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

2.1.1 Small East Africa Goat (SEAG)

The Small East Africa Goat (SEAG) (Plate 2.1) is an indigenous breed associated with pastoral communities in the drier parts of northern Kenya where meat and milk are the predominant dietary sources together with cereal-based food (Fanzo, 2014). In the ASAL zones of Kenya goats' play a role in the socio-economic status and cushions against the adverse effects of climate change since they don't easily succumb to such effects (Wangai *et al*., (2013). The SEAG breed is selected for this research due to its adaptability to arid and semi-arid conditions, versatility, hardiness, good meat conformation and its ability to utilize low quality forage. Rearing resilient small ruminants like goats in drought-stricken areas of Kenya can be of benefit to livestock keepers to mitigate the adverse effects of climate change.

Goats are effective browsers and have the ability to utilize woody species and low-quality forages better than sheep and cattle and can adopt to harsh environmental conditions (Needham *et al*., 2022). They are classified as grass/roughage eaters, and concentrate selectors, and they have to go for forbs and browse vegetation and possess a lesser capacity to digest cellulose in cell walls.

2.1.2 Ethnology and physical Characteristics of the SEAG

The body is usually of no definite and is docile. Have low prolificacy (twinning rate) compared to Gala goat. Bucks can weigh up to 40-50 kg live weight and can reach a height of 50cm and does can reach an average of 35 kg. They have lower carcass quantity and quality compared to the *Galla* Goats.

2.1.3 Distribution

This breed is mainly found in north eastern, eastern and other parts of Kenya's arid and semi- arid areas in found in all the three east African countries, hence its name.

Plate 2.1: Small east Africa goat buck (Source: Author 2020).

2.1.4 Goats digestive system

Goats are Ruminants, like sheep, cows, and deer; the goat digestive system is made up of four stomach chambers (Wangai, Muriithi & Koenig, 2013). Namely: The Reticulum

(honey comb), Rumen (towel), Omasum (the book) and Abomasum (true stomach). The small intestine is absorption digested the nutrients takes place and the nutrients are then assimilated into body cells. Undigested feed is consolidated and passes through the large intestine where water is reabsorbed and un-useful byproducts passed out as solid waste.

Ruminants differ from monogastrics by having the four chambers. Digestion begins with chewing after which food is swallowed into the Reticulum. In the reticulum, it undergoes microbial breakdown. The reticulum works in tandem with the Rumen. Food particles can be passed back and forth between the reticulum and the rumen during digestion. In the rumen, food undergoes microbial breakdown by the rumen cellulolytic bacteria, a phenomenon lacking in monogastrics (Villot *et al*., 2018). The first two chambers function as a microbial fermentation chamber that breaks down hard plant fibers like hay, grass, and leaves.

Goats often eat quickly by swallowing food in chunks up to almost 2 inches long and this allows them to "browse on the move". These large chunks of food are 'sorted' by the reticulum and smaller Particles are passed to the rumen, and larger particles are later regurgitated by their bi-directional esophagus and chewed more thoroughly (Katz, 2016).

Ruminants spend a significant amount of time chewing cud but they can't while they are active. Therefore, goats and other ruminants need down time to digest properly. Chewing cud breaks up larger particles into smaller pieces. This also increases the surface area of the fibrous material enabling the cellulolytic enzymes larger surface for degradation (Serhan & Mattar, 2017).

After the first two chambers, food is then passed into the Omasum. The omasum functions as a filter where water is reabsorbed and food is pushed along to the Abomasum. The Abomasum is the last compartment and is the true stomach. This compartment functions like that of monogastric animals by breaking down feed with acids and enzymes. Bicarbonate 'insulates' the lining of the stomach as a buffer to the low pH in the stomach. Finally, digested food passes to the small intestine where nutrients are absorbed. Remaining water is then reabsorbed in the large intestine before being passed from the body as solid waste (Xue *et al*., 2018).

Other benefits of the digestive system other than allowing goats to digest dense plant fibers are the ability to synthesize vitamins and proteins. This is done by the bacteria that colonizes the rumen, these microbes can synthesize all the essential B vitamins. Goats can also synthesize protein from nitrogen gas left over from the digestive process in addition to protein that's been ingested. Goat's digestive systems have some protective qualities such as the ability to detoxify certain levels of tannin found in browse and feed. However, high concentrations of tannins can have negative effects on goat health (Serhan & Mattar, 2017). Knowing the goat digestive system is important for every goat owner. Understanding this process helps in planning dietary plans and feeding strategies on overall nutrition. Understanding goat's digestive system also helps understand what is toxic, harmful, and beneficial for goat digestive health.

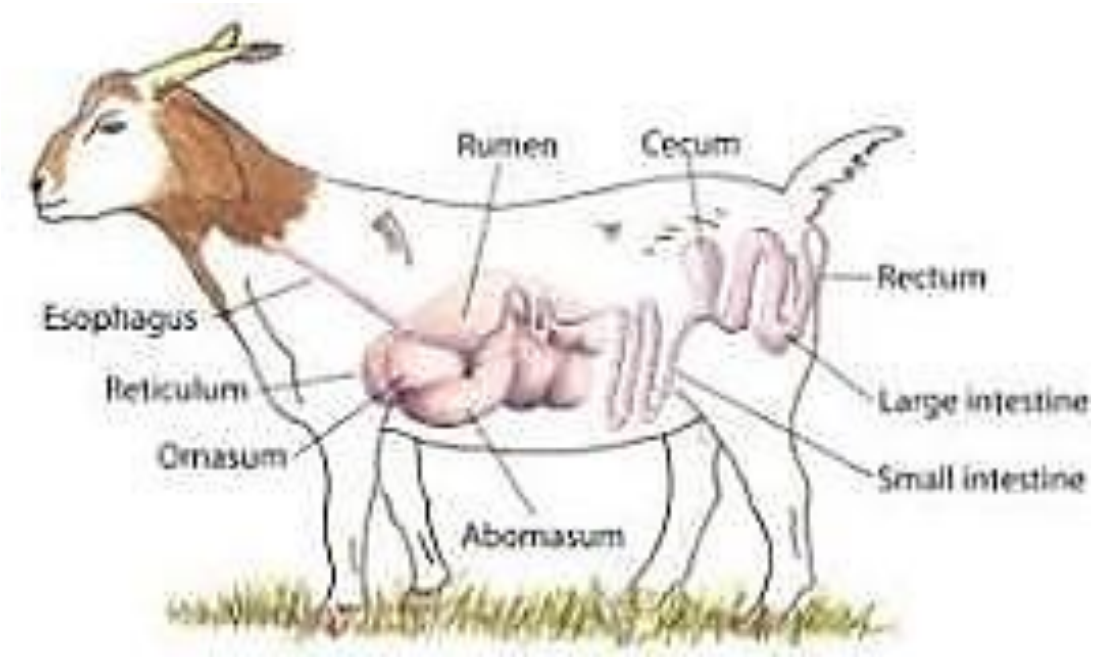


Figure 2.1: Goat digestive system

2.2 Description of *Acacia sp*ecies

2.2.1 *Acacia tortilis*

Acacia tortilis (Forssk.) Hayne is a thorny legume tree, usually about 4-8 m high, but it can reach 20 m. The crown is dense, umbrella-like and forms a flat-topped canopy supported by some well-developed branches occurring almost at the top of the tree plate. The trunk of the tree is rough and dark-brown and old barks have fissures and thick allowing the tree to store a lot of water making the tree evergreen. Partial removal of the bark induces exudates of gum – Arabic that exudates downwards (Heuze and Tran 2015). The young stems are smooth, while young branches have thorns with two types of thorns formation one with two pairs of thorns that are long, straight and white and the other pair is short, hooked and brownish. Leaves are compound and formed just above the base of the thorns and the leaflets (6-22 pairs) are very small (1-4 mm long x 0.6-1 mm broad),

glabrous to pubescent, the leaf stalks have 2-10 pairs of pinnae (raches) with each rachis having 4-2 pairs of leaflets depending on the length of the rachis. The leaves are greenish –grey in color and the color is retained when the harvested leaves are dried in a well-ventilated shade.

It is found between 15 and 30°N and between sea level and an altitude of 1000 m (Githae & Mutiga, 2021). It withstands wide temperature variations (0°C to 50°C). It grows well on alkaline sandy or rocky soils. It is tolerant to severe drought due to its deep taproot system. It is a phreatophyte, relying on aquifers as deep as 40-50 m. It tolerates strong salinity and seasonal waterlogging (Krishna *et al*., 2019).

In Australia, which is having a more or less similar Geo- ecological features as Kenya, *Acacia aneura* is cultivated in pure stands in a draught mitigation strategic forage reserve for grazing sheep. In North Africa, tanniniferous forage constitute 70% of productivity from range resources and making about 40% of the feed availability potential. The crude protein content of most tropical forages range from 20-50 g/kg DM which rarely supply the minimum crude protein required for maximum ruminal microbial activity, (Norton 2003). Some species of acacia like Acacia Karroo contain cp. Of 20-230 g/kg DM needed for gain in body weight and for maintenance and production of growing goats. The differences in nutrient components in *Acacia sp*. can be attributed to species variations, climate, soil types, stage of growth, browsing pressure, season and occurrence of ant nutritional factors (Bompadre *et al*., and 2014).

Flowers are white, cream or yellow, and highly aromatic and during flowering, a lot of these flowers fall to the ground due to strong winds and livestock picks them as feed

source. Fruit is a characteristic twisted browny pod, hence the epithet "*tortilis*" (Agassiz & Bidzilya, 2016). Young pods are green in color and get brownish as they mature and when mature they come off from the stem and fall off to the ground and split open to release seeds. In Mogotio sub-county, Radat sub- location, some of the trees produce flowers and pods in the month of April to July, while others flower in October and produce fruits in December to January. Pods and leaves are used as a fodder. Where leaves can be accessed by browsing goats when the trees are still young but older trees can be reached by goats through coppicing (Mutai, 2022). Camels can browse on the trees up to 4 m high, but sheep and goats feed on fallen pods and also livestock keepers during dry spells will shake the ripen pods which falls down for livestock to feed on. The pods are also collected and stored by livestock keepers to be used as supplements during dry season or even sold to other pastoralists. Fruits are more nutritious if ground and can be 8-10 seeds each constricted between the folds and which can be easily seen and counted. Trees can survive heavy browsing. *Acacia tortilis* pods are also used as a famine food in eastern Africa.

Plate 2.2: Mature *Acacia tortilis* with umbrella shape (Source: Author, 2020)

2.2.2 *Acacia brevispica*

Acacia brevispica is a shrub or slender tree, 1–7 m high, often scandent; twigs pubescent and with numerous reddish glands. Prickles scattered, ± recurved, arising from longitudinal bands which are usually paler than the intervening lenticellate bands. Leaves: petiole 0.4–1.5 cm long; pinnae up to 18 pairs; leaflets up to 50 or more pairs, 3–5 x 0.5–1 mm, ± ciliolate, midrib nearer one margin at the base. Flowers white to yellowish white, in heads which are arranged racemosely or in long panicles. Calyx 2–2.5 mm long. Corolla up to 3.5 mm long. Pods straight, dehiscent, 6–15 x 1.5–3.3 cm, glabrous or puberulous, with many minute reddish glands. Seeds elliptic, compressed, 8–12 x 6–8 mm; areole 6–8 x 3–5 mm (Daoub *et al*., 2018).

Acacia brevispica is widespread in dry and semi-humid parts of Africa; from Ethiopia, Sudan, Kenya and Southern Africa. It is found forming thickets together with other shrubs and trees in bushlands, usually at 50-1310 m above sea level. Propagation is done by use of seeds through direct sowing at the site of establishment. Seed yield is normally 7000-9000 seed in a kg pack and seed treatment-immersion in hot water and allowing to cool and soaking for 24 hours and the seeds can be stored well in a cool dry place. *Acacia brevispica* is a fairly fast-growing tree and coppicing helps is done to improve the stalk as a management strategy. It is largely used for livestock fodder (pods and leaves), fire wood, medicinal (roots) and live fence (Daoub *et al*., 2018).

Plate 2.3: Ripened *Acacia brevispica* pods with Galla goats feeding on pods.

(Source: Author, 2020)

Plate 2.4: Mature *Acacia brevispica* pod (Source: Author, 2020)

Plate 2.5: *Galla* goats picking *Acacia brevispica* pods (Source: Author, 2020)

2.2.3 Acacia meliffera

Local Names include Afrikaans (swarthook, swartaak); Arabic (*kitr, kedad, kitir*); English (wait-abit thorn, black thorn, hook thorn); *Swahili* (*kikwata*). *Acacia mellifera* is a low, branched tree with a more or less spherical crown. Black bark on stem becomes ash-grey to light brown on the branches, bearing small, short, sharply hooked spines in pairs. It has a shallow but extensive root system radiating from the crown, allowing the plant to exploit soil moisture and nutrients from a large volume of soil. The roots rarely penetrate more than 1 m. leaves characterized by 2 pairs of pinnulae, each with a single pair of leaflets. Leaflets elliptic 0.6-2 cm long and 0.6-1.2 cm wide, glabrous and highly colored beneath (Daoub *et al*., 2018).

Flowers sweetly scented, especially at night, in elongated spikes, cream to white in spiciform racemes, up to 3.5 cm long; pedicels 0.5-1.5 mm long; calyx up to 1 mm long; The papery pods with 2-3-seeds are reticulate, flat, elongated, 2.5-5.5 cm. The generic name ‘acacia’ comes from the Greek word ‘*akis*’, meaning point or barb. The specific name means ‘honey-bearing’.

Plate 2.6: *Acacia meliffera* tree with ripened pods (Source: Author, 2020)

Plate 2.7: Branch of *Acacia melliferra* with ripened pods (Source: Author, 2020)

Acacia melliferra: flowering and fruiting start 3 years after planting and generally occur twice a year. Flowers are borne on shoots produced the previous year, that is, old wood. The flowers open and shed before the leaves appear. In southern Africa, flowering occurs from September to November and fruiting from January to April.

Plate 2.8: Flowering *Acacia mellifera* (Source: Author, 2020)

Acacia mellifera is a commonly occurring shrub on rangelands throughout the savannah in western, eastern and southern Africa. The terrain preference is rocky hillsides with rainfall along seasonal watercourses, mixed with other trees. If left unattended, especially if grazing is heavy and no fires check its spread, it may form dense, impenetrable thickets, 2-3 m high and sometimes hundreds of meters across, slowly taking over good grazing land. This species is drought-tolerant. *A. mellifera* is normally found on hard-surfaced, sandy soils and rocky hillsides. It grows well in black cotton soils but prefers loamy soils.

Gum collected from injured stems is edible and relished by children, animals and birds. Camels and goats browse the leaves, which are rich in protein, taking them from the

shrubs or from the ground. The cream/white flowers produce excellent quality honey (‘*mellifera*’ = producing honey). Bees forage in the late morning to midafternoon when hot and dry. The honey is water color and granulates slowly. The wood is used for fuel and charcoal. The wood is taken for building huts and the branches for fencing. The poison with which Bushmen tip their arrows is often made from a powdered grub mixed with the sap of *A. mellifera.* The bark decoction is used for stomach-ache, sterility, pneumonia, malaria and syphilis. In Botswana, a decoction of the roots is a medicine for stomach pain. In Sudan, baskets made of the roots serve for collecting gum arabic.

Young trees are subject to heavy browsing by stock and game and must be protected for the first two seasons. *A. mellifera* has a moderate growth rate of up to 500 mm/year. It does not coppice well. Seed storage behavior is orthodox; viability can be maintained for several years in hermetic storage at 10 deg. C with 4.5-9% mc. There are approximately 20 000 seeds/kg (Heuze and Tran 2015).

2.3 Nutritional potential, tannin bio assay and effects of *Acacia brevispica, A. mellifera* and *A. tortilis* pods in goat nutrition in dry areas of Kenya

In Kenya, meat goats are mostly kept in the Arid and semi-arid areas (ASALs), and relies mainly on the natural pastures dominated by a wide range of grasses and browse vegetation including various *Acacia sp*ecies (Ratemo *et al*., 2020). However, this is normally possible for a short period of time since the rainy season is short. For the rest of the dry season, goat keepers rely on the use of *Acacia sp*ecies pods to supplement dry season grassing / browsing. Goats can utilize browse vegetation in form of fresh or dry leaves or ripe pods, said to have high nutritive value. Legume trees and foliage and agro-

industrial by-products plays an important role in livestock production due to absence of competition for food between man and animal, and they offer good protein supplementation especially during draught periods (Brewbaker & Hutton, 2019).

The acacia tree legumes in these areas like *A. brevispica, A.mellifera,* and *Acacia tortilis* are the common browse material for the goats where they consume their leaves and the pods which normally ripen during the dry season and sometimes even the barks, (Lengarite *et al*., 2012). This is due to heat and also acacia pods are known to have a short life span as a result of infestation by moulds and insect pests (Shorrocks & Bates, (2015). Many range lands of Africa are inhabited by various *Acacia sp*ecies and other pod producing trees which produce pods high in protein. Such pods incidentally ripen at the start of the dry season hence can be used in livestock supplementation due to scarcity of pastures at such times (Lengarite *et al*., 2012). Pods of other leguminous trees like *Acacia tortilis* are good protein sources with medium to high dry matter digestibility and rich in both macro and micro minerals (Derero & Kitaw, 2018). During the process of rumination, large amounts of undigested ingested seeds of *Acacia sp*ecies are lost through the faeces and the mouth hence nutrient loses occurs in spite of biting feed scarcity. Little research has been done in Kenya on the processing of *Acacia sp*. pods to exploit their full nutritional potential by reducing their tannin levels and there is dearth of information for the pastoralists on the potential effects and benefits of these tannins in *Acacia sp*ecies on growing goats (Shelton, 2021).

In order to improve on nutritional data availability to livestock keepers, livestock nutritionists and livestock feed manufactures, the government of Kenya through the

ministry of Agriculture, Livestock and Fisheries (MALF), hosted and adopted a global ministerial conference. The conference had thematic deliberations on Global Open Data on Agriculture and Nutrition (GODAN), Kenya-2017). Less available information on the nutritive potential of *Acacia sp*p.in the tropics limits the optimal utilization of tannin–rich browse fodders like Acacias (Kandie, 2022). There is need, therefore, to evaluate the inclusion of *Acacia sp*. Pods by reducing their tannin levels to suit ruminant nutrition without affecting palatability, digestibility and feed utilization efficiency (Su & Chen, 2020).

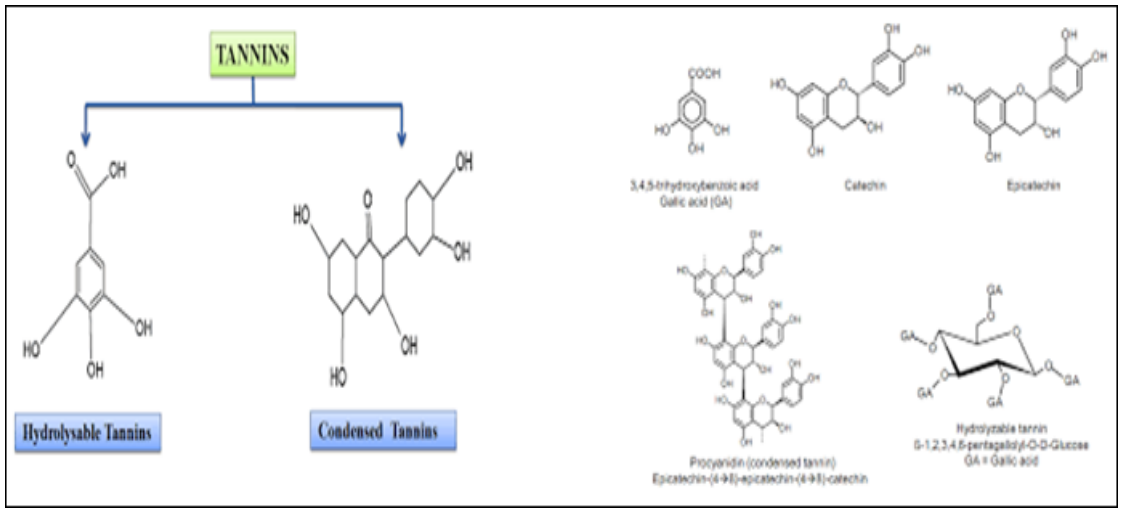


Figure 2.2: Hydrolysable and condensed tannins

Tannins can be hydrolysable (HT) or condensed (CT). The HT are composed of polyphenols-*gallic acid* and or *hexahydroxydiphenic acid, esters* joint to a hexose moiety and they undergo hydrolysis when heat with mild acids. However, the condensed tannins can only be degraded oxidatively by use of hot mineral acids. They are also formed from

precursors of the acetate and shikimic acid pathway just like lignin, (Feichtner & Gessner, 2018). There are great differences in CT monomers composition, size of polymer, stereochemistry, concentration and dynamics of their positions in the plant and these characteristics will affect the affinity of condensed tannins to complex with different molecules.

Due to the heterogeneity of their polymerization i.e. Linear and branched, and their bonding with different forage compounds, CT are usually hard to separate and quantify. For instance, the CT of two species of the forage –*Lotus* shows varying protein binding affinity attributed to result from variations in the stereochemistry of their respective monomers (Saminathan *et al*., 2014). Also, CT have been classified based on their hydroxylation behavioral patterns of their component ring i.e. *proanthocyanidines*. Condensed tannins (CT) are sub-divided into *procyanidines* and *prodelphinidines* as per the hydroxylation arrangement on the benzene ring, and that CT could also vary between species as per the molecular weight of between 1800-2100, and could have 6-7 monomers bound by interflavan bonds at the C-4 and C-8 or C-4 and C-6 locations.

The differences in the chemical structure of condensed tannins influences their capacity to complex with microbial, plant and mammalian proteins during herbivory, while their impacts on nutrition is a consequence of an intricate interaction chemistry and the ruminant physiology and grazing behavior. The astringency levels are attributed to CT on the context of the levels of proteins precipitated per unit weight and this protein complexation affinity forms the basis of one of the methods applied in the characterization of CT (Rosa Perez-Gregorio & Simal-Gandara, 2017). Astringency is

also a term used to describe the unpalatability of forages containing elevated amount of tannins, and this “puckering” factor causes the reduction of feed intake in tanniniferous forages and cushions plants from excessive herbivory (Bhat *et al*., 2013).

Astringency can also be defined as the unit protein bound per unit of CT and relative astringency expressed as μgCT required to precipitate 1000μg protein, and ranged from 435.5 for CT from *L. corniculatus* L) to 262.5 for CT from *Lespedeza Cuneata* for *Bovine serum albumin* (BSA) protein test. On the contrary, astringency for the same weight of Fraction1 protein (*ribulose1,5-bis-phosphate carboxylase oxygenase* EC4.1.1.39) ranged from108.2 for CT from Lucerne seed to 49.6 for CT from *Sainfoin* forage. The perceived roles of tannins in plants is explained by the allelochemical hypothesis of tannins which posits that CT derived from a rotting plant will have an effect on soil nutrients quantities available for the future plant generations, hence reducing the growth of the competitor plant species (Bhat *et al*., 2013).

The tannin levels in acacia tree legumes can be reduced by different processing methods like soaking the pods in *poly ethylene glycol* (PEG) solution, boiling pods in water, adding charcoal to pods, crushing and soaking pods in wood ash (Naoh), or ammonia solution, or by sun drying (Samtiya, Aluko & Dhewa, 2020). However, some of these methods of processing acacia pods are too technical for the pastoralists or even hazardous to man or livestock or pollutants to the environment, or too expensive hence beyond the reach of the pastoralists goat farmers.

Biological treatment in some forages involves the use of live organisms (*probiotics*) like fungi has been used to improve the quality of crop residues to improve digestibility

through delignification and increase of protein content (Asmare, 2014). Most research studies have concentrated on the use of chemical treatment to improve feed value of crop residues and forages by increasing feed intake by 50% and digestibility by 10% (Samtiya, Aluko & Dhewa, 2020).

The common ones used are acids, alkalis like sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonia and urea (Randall *et al*., 2016). Livestock which eats diets having >5% w/v tannin level normally develop negative nitrogen balance, reduced feed digestibility and animal performance (Samtiya, Aluko, R& Dhewa, 2020). There are proposals that the temperate Legume -*Lotus* species having condensed tannins level 20-40gm per kg^{-1} DM is the CT level suggested to enhance the protein utilization efficiency without reducing ruminal fiber degradation and the voluntary feed intake, and that it could be even at a reduced amount for the CT levels in many of the tropical legumes (Ronaldo *et al*., 2016).

However, in ruminants like in goat feeding, goat keepers do not process the acacia pods before feeding as supplements especially during dry season resulting in poor animal performance and low annual off-take. In plants tannins acts as the first line of defense against over consumption by animals (anti-herbivory) and hence threat of extinction but some animals like deer, mule and mice have been known to secrete *proline* –rich proteins in their saliva which counteracts the effects of tannins.

The presence of tannins in most forages have been reported to have negative effects on organs weights. There was significant difference on the organs weights of rabbits fed on different field legumes containing different amounts of anti-nutritional factors called

saponins (Mutai, 2017). Anti-nutritional factors in *Acacia sp*ecies should therefore be reduced so as to optimize animal productivity and farmer's income.

The significance of ruminal microbial ecology and their diversity in the rumen environment has elicited attention with respect to current trends in livestock production systems. Tannins have been said to have negative effects on the rumen microbiota in the rumen microbiome. Ruminal bacteria, fungi, protozoa and yeasts are the major players in the fermentation of forages to yield the various nutritional components like the volatile fatty acids (VFAs) e.g. *propionic acid, butyric acid, acetic acid* and a number of essential and non-essential amino acids. The ruminal microbes work in an intricate and symbiotic relationship and any disruption of this coexistence through tannins activity will lead to adverse nutritional imbalances not good for the ruminants and to the microbes (Gruninger *et al*., 2019).

A major limiting factor on the use of some browse vegetation is the perceived presence of phenolic substances like tannins, saponins, lectins and alkaloids occurring in most plants and with the ability to bind proteins hence lowering protein bio-availability and digestibility by forming protein-tannin complexes (Paul, 2020). Some animal browsers have their saliva rich in a small glycol-protein with a lot of *proline, glutamine*, and *glycine* which are tannin –binders but cattle, sheep and goats lucks these proteins (Gruninger *et al*., 2019).

Tannins are phenolic compounds naturally found in most plants and whose function is to protect plants from animal herbivory since they are said to be astringent and also play growth regulatory function when digested by the herbivores animals, but when digested

in greater levels could lower the activity and numbers of ruminal microbes thereby reducing ruminal bio hydrogenation (Mutai, 2017; Gruninger *et al*., 2019).

*Acacia sp*ecies can be used as non-conventional feed resource and are said to have protein content of about 13%-20% and energy of 72% Total Digestible Nutrient (TDN) but perceived to have high tannin levels of about 18.71%, but can be a substitute as livestock feed to replace the expensive cereal-based grains in conventional rations (Owen, Smith & Makkar, 2012). In the tropics livestock keepers uses agricultural crop residues to supplement animals after grassing on pastures but they are poor in nutritive value due to more cell wall content hence low in Crude Protein (CP) and with low feed intake and digestibility (Gruninger *et al*., 2019). It is therefore imperative that appropriate and sustainable strategies should be developed targeting post-harvest technology, treatment with affordable biological or chemical methods so as to maximize on the nutritive value of tanniniferous forages.

The use of non-conventional forage resources especially in the developing nations constrained by nutritional fluctuation and scarcity is an appropriate venture that underpins the potential success of the livestock industry (Gruninger *et al*., 2019). In such areas, arable land is diminishing as a result of soil erosion, industrialization and urbanization. As the demand for food escalates similar to that of animal origin food, the non-conventional feed resources like the tanniniferous forages must be incorporated within the sustainable livestock feed resources especially for ruminants in the small holder farming systems in developing countries. The nutritive value of feeds is dependent

on the composition of nutritive compounds in the forage and their degradability, and their metabolism, and level of the intake feed by the animal (Castillo-Martínez *et al*., 2015).

Tree legumes found in the tropics possess huge protein levels of between14-29% of DM. but the occurrence of anti-nutritional factors (ANF) e.g. Phenolic and tannins limits their utilization due to the quantities of tannins they contain which vary widely and their effects on livestock could be beneficial, or even toxic and lethal to animals (Bhat *et al*., 2013). This is due to their affinity to reduce voluntary feed intake, nutrient degradability and, lower gain in weight, milk yield and other production parameters (Mlambo and Mapiye, 2015). The CP content of the most commonly used feeds in the tropics like cassava, *calliandra, Erithrina, leucaena*, and pigeon pea range from 22.2-25.8%, while that of *Acacia sp, Gliricidia* and *prosopis,* CP range from 14.0-15.1% (Castillo-Martínez *et al*., 2015).

Studies have demonstrated that the lowest crude protein level of tree legumes leaves on dry period was over twice the level in grasses during the rainy period (Mendieta-Araica *et al*., 2013). Also, it has been reported that the forages used at dry periods contain a range of 42-98 gkg^{-1} of DM, unlike that of 49-105$gkg^{-1}$of DM, as compared to those used at wet season. The crude protein content of *Acacia mellifera*, *A. tortilis* and *A. faidherbia albida* have a range of between 10-15%. Other studies have demonstrated that most *Acacia sp*. have crude protein ranging from 12-18% on DM basis (Assogbadjo *et al*., 2012).

Research have also shown that *Acacia mellifera* pulps (pods without seeds), have less proteins than leaves i.e. about 12% in the 10-14% DM basis range compared to 14% DM range in leaves in the 10-20% dry matter basis range. They also have more fiber (ADF)

of 21% at the 17-21% DM basis, but the seeds have more proteins at 19%, and crude fiber at 29% on DM basis respectively (Castillo-Martínez *et al*., 2015).

Studies on Awassi sheep supplemented with *Acacia sp*. showed increased milk yield by 16% as compared with those feed on the control diet and also better growth rate of lambs from birth to weaning, and reduced feeding costs by 38%. There are variations in nutritional composition and anti-nutritional factors of the leaves and the pods of *Acacia mellifera* and the comparative tannin levels in *A. nilotica* are 5.4%, 7.6, 13.55 and 15.8% for pods, leaves, bark, and twigs respectively (Helal, 2018).

Studies in northern part of Nigeria using the Red Sokoto goat fed on *Acacia mellifera* pods processed differently demonstrated that the nutritive value were 96.13%,96.47%, 97.52%, 97.21% and 98.12% dry matter, 12.69%, 12.13%, 15.06%, 13.25%, and 10.13% crude protein; 2.81%, 2.33%, 5.51%, 9.46%, and 4.74% hemicellulose, for sun dried, crushed, soaked and milled, sun dried and milled, and sun dried milled with charcoal respectively (Assogbadjo *et al*., 2012). It has been showed that heating and drying of tanniniferous, plants could result in the condensed tannins to complex covalently with forage compounds and lower the levels of free CTs which could bind ruminal proteins (). Studies have demonstrated that the crude protein levels in forages treated with alkali did not show much difference but the condensed tannins in leaves treated with Ca $(OH)_2$ at 3% (W/W) depicted significant ($p<0.005$) reduction of total phenolics, (TP), total tannins (TT), total condensed tannins (CT), ether extract (EE), Ash, acid detergent fibre (ADF), ADL (Acid Detergent Lignin), and Calcium (Ca) content significantly ($p<0.005$) increased in the diet treated with alkali. Also, studies in Ethiopia demonstrated that the

digestibility of dry matter, crude protein, and Neutral Detergent Fiber (NDF) as well as the feed intake and Nitrogen retention when *Acacia tortilis* leaves were treated with Ca $(OH)_2$ alkali (Bayssa 2016).

It has also been shown that the pods of *Acacia mellifera* have the least levels of tannins i.e. 5.4% as against 7.6%, 13.5%, and 15.8%respectively for leaves, bark and twigs, (Owen, Smith & Makkar, 2012). Condensed tannins are often present in leguminous tropical forages more so those found in arid zones and acidic soils (Quintero-Anzueta *et al*., 2021). The levels of phenolic compounds in leguminous plants differently affects animals fed on such plants as protein supplements and there is need to pinpoint the particular compounds that causes lowered palatability intake and digestibility. It has been reported that the section of diet, protective responses of animals and the interactions of fed diet components and the rumen microbes' digestive enzymes, will influence the degree of nutritional effects of tannins (Assogbadjo *et al*., 2012). Ultimately, with respect to forage research, animal productive performance and wellbeing should be among the most critical criteria by which to charge the nutritional impact of the presence of tannins in forages. Some of the compounds which have been attributed to such negative effects includes; *saponins, alkaloids, phytohaemagglutamins*, (*lecithin*), *cyanogens*, non-protein amino acids, polyphenolics and oxalic acids (Mutai, 2017).

The levels of the polyphenolic compounds in the pods (pulp and seeds), range from 32-34%, and in leaf from 30-36% (Assogbadjo *et al*., 2012). The leaves have more total extractable tannins at 25% and 5% condensed tannins (CT), >60g/kg DM and these

phenolics have the tendency to lower the palatability and nutritive value of the *Acacia mellifera* (Mlambo and Mapiye, 2015).

Tannins are non-protein amino acids and by-products of nutrients metabolism by plants, also referred to as *allelochemicals,* and are polymeric, phenolic compounds with numerous hydroxyl groups and diverse in chemical structure and they are synthesized by plants via the phenylpropanoid pathway and is one of the most abundant among polyphenolic compounds next to lignin (Marchiosi *et al*., 2020). The high affinity for the hydrogen bonding between condensed tannins and different compounds is facilitated by the numerous hydroxyl groups of CT polymers, while the hydro-phobic reactions between the phenol rings and parts of the protein and or amino acids will affect the strength of the interaction. Condensed tannins could also interact with carbohydrates like those in glycoproteins but with a lesser capacity unlike for proteins (Addisu, 2016).

It has been suggested that tannins and lignin are generated biosynthetically partially or wholly from products of the shikimic acid pathway hence have common intermediaries, (Chen, 2014). The initial enzyme (*leucoathocyanidine reductase*) in the condensed tannins specific pathway, is only traceable in the forage tissues containing the CT, alluding that their disappearance in some tissues could be attributed to the absence of may be a fraction of the flavonoid pathway (Boudet, 2012). The condensed tannins go through a locational shift in a way that their presence is mainly in the juvenile and uppermost portions of plant leaves and limited or not found in the mature leaves (Schwab *et al*., 2022).

The basic metabolism of autotrophic plants combines photosynthesis with respiration, leading from CO_2 via the sugars of the Calvin cycle, pyruvic acid and acetic acid either to the fatty acids of the Lynen spiral (a reversible process) or to the simple aliphatic acids of the Krebs cycle and then back to CO_2. Connected by mostly reversible pathways are some essential intermediates such as the Krebs-cycle-derived Mevalonic acid, the sugar-derived glycerol and the sugar –plus pyruvic acid-derived shikimic acid-acetate – malonate, the latter functioning as a precursor to the aromatic amino acids (Rosales, 2014). Tannins are said to possess astringency which is a dry sensation in the mouth due to the precipitation of salivary proteins by condensed tannins, which are said to be influenced by their molecular weight and chemical structures (Soares, Mateus & de Freitas, 2012).

Proline-rich tannin-binding proteins are absent in goats and analysis of the composition of amino acids in the ruminants proline possessing proteins, they had minimal contents of the essential amino acids (Schmitt, Ward & Shrader, 2020). It is therefore prudent to be cautious when interpreting such findings since protein-tannin-complexation may be specific, based on structure –activity (Boudet, 2012). There are arguments that if proline-rich proteins were solely for cushioning ruminants against tannins, then such proteins could possess higher binding capacity in the ruminants consuming them.

There are other strategies that enable herbivores to cope with tannin effects like the presence of enzymes by certain animals e.g. Voles and hares which detoxify tannins through complexation with *glucuronic* acid (hydrolysable tannin) and the enzyme polyphenol oxidase are said to cancel tannin effects (Mlambo, Marume & Gajana, 2015).

Condensed tannins should be only be located inside the plant vacuoles so as to control their inhibition of cellular forage enzymes although the vacuoles are destroyed during prehension and mastication by the ruminants, hence enabling their interaction with plant protein and microbial enzymes (Schmitt, Ward & Shrader, 2020). Also absorbed tannins by tree –locusts are non-toxic and are utilized to make the cuticle. The effects and the mechanisms of ruminant-tannins interactions are highly different such that definite projections of livestock performance in terms of meat, milk or wool, under the influence of tannin intake is difficult to elucidate by virtue of the great complexity of tannin-nutrients, tannin –animal and tannin- microbiota interactions (Boudet, 2012).

There are two main classifications of tannins namely-condensed tannins (CT), also referred to as *proanthocyanidines* PA and hydrolysable tannins (HTS) also referred to as *anthocyanidines* (AC) and the HTS are polymers of flavonol units usually linked at C4-C8 or C4-C6and the structure is derived from a hexose sugar mainly glucose linked to some Gallic acid or modified units and this accounts for their variability. They are polyesters of phenolic acids like *gallic acid, M-digallic* acid (*gallotannins*) D-glucose or *quinic* acid, or *hexahydroxydiphenic* acid (Boudet, 2012).

Each type of tannins complex with varying levels of proteins and it has been observed that in-vitro protein inhibition by digestive enzymes had a positive correlation with the degree of polymerization of CT as was seen in five forage species (Boudet, 2012; Schmitt, Ward & Shrader, 2020).

Condensed tannins are however, different from hydrolysable tannins in that (CT) flavan -3-01 oligomers with varying levels of oxidation on the C and A- rings of specific

monomer and the chirality of C-4, which provide linkage of monomers takes place and C-3 opens up the likelihood of structural variability.

It is said that condensed tannins stereochemistry at C-2 and C-3 was demonstrated to affect the astringency of CT and forage palatability by rabbits, they are also said to have better stability and less susceptible to hydrolysis than the (HT). Also studies have demonstrated that the molecular weight of condensed tannins besides their concentrations could be a determining factor in the biological action of condensed tannins in the rumen environment (Naumann at al., 2013). Hydrogen –bonding and hydrophobic interactions in a pH reversible action are said to be responsible for the formation of tannin-protein complexes and the precipitation of protein complex depends on the molecular size of tannins, pH and ionic strength. The hydrophobic bonding and the hydrogen bonding are critical in the formation of complexes with different compounds and the occurrence of hydrogen donors inform of phenolic hydroxyl groups in tannins themselves and of hydrogen acceptors in form of peptide linkages of proteins could definitely cause the creation of hydrogen bonds (Zhou *et al*., 2020).

The biosynthesis of tannins in forage legumes can be transformed through nutritional, chemical and environmental manipulation or even genetically to lower or increase tannins in tannin-containing legumes, However, such methods are currently beyond the capacity of most of the developing countries and institutions hence for the time being, cheaper and sustainable methods of reducing tannins in forages remains the option as is the target of this research (Coburn & Bronner, 2014).

Both protein precipitation and incorporation of tannin phenolic into a precipitate increases with the increase of molecular weight of size of tannins and where the size is > 500 the tannins become insoluble and loses their protein precipitating capacity (Thakur, Sharma, & Thakur, 2019). Differences in CT) chemistry changes the protein - complexation affinity between polymers from varied forages at contrasting stages of development. Condensed tannins form complexes with salivary proteins, dietary proteins, microbial exoenzymes, endogenous proteins etc.as opposed to diets void of CT (inha *et al*., 2012). It has been suggested that the negative effects of condensed tannins on tanniniferous plants digestibility was optimized when the levels and average molecular weight was near 4900 Daltons and there seems to be an optimum molecular weight above and below which will result in a lowered affinity of condensed tannins to complex with proteins and different molecules (Coburn & Bronner, 2014).

There are suggestions that molecular weight of tannins should be 500-3000, to allow the tannin molecule to be able to orient itself among the protein chains, and to have enough phenolic groups to form cross-linkages effectively with proteins (Cirkovic Velickovic & Stanic-Vucinic, 2018). This protein-tannin complexation is key to the biological action of tannins open-ended protein structures together with proline-rich proteins seems to have a higher complexation capacity as compared to globular proteins, glycoproteins and others with less molecular weight which have less affinities (Habchi *et al*., 2014). Research findings have showed that there is some positive correlation in enzyme action and tannin molecular weight and this seems to allude to the presence of a limit for concentration of tannins below which tannin affinity to block digestive enzymes cannot easily have estimated with respect to the molecular structure of tannins. Research have demonstrated

that the molecular weight of condensed tannins in the legume *Desmodium ovalifolium* was positively correlated with gas production rate, suggesting that as tannin size surpasses the optimum amount, tannins will lose their affinity to form insoluble substances with different molecules (Cirkovic Velickovic & Stanic-Vucinic, 2018).

Tannin production and protein precipitation differs with plant species, parts of the plant, different time on the same species and different environments. The carbon-nutrient hypothesis posits that the formation of tannins is due to the hunting of the primary metabolites like carbon –skeletons, during unfavorable periods like when there is poor nutrient levels and growth is hampered. It has also been observed that some tannins depict characteristics of both CT and HT, like the family of *catechin* tannins (Habchi *et al*., 2014). Condensed tannins can be either *cis* or *Trans* based on the orientation of the functional group located on the C-3 and C-4 positions relative to Benzene ring, and this affects their capacity to form complexes with proteins, carbohydrates, lipids and minerals depending on the ph. of the rumen environment (Thakur, Sharma & Thakur, 2019).

The conformational open proline-rich proteins (PRPs) have higher affinity for condensed tannin- protein complexation than the globular tightly coiled proteins and this has been attributed to the latter's better accessibility of the phenolic groups of condensed tannins and the protein –carboxyl groups to complex due to more tannin-protein hydrogen bonding (Cirkovic Velickovic & Stanic-Vucinic, 2018). There are three different fractions in which condensed tannins occurs in tissues of plants namely: protein –bound, fiber-bound and extractable condensed tannins. The level of concentration of each depends on anatomical part of the plant tissue, climate, nutrient induced levels of stress,

age and varies both among and within species (Wolfe *et al.,* 2008). Research have showed that tannins in some forages have an effect in terms of degradability of cellulose usually through complexing with cellulose, cellulases, microbiota or to symbiotic ruminal enzymes (Habchi *et al*., 2014).

The presence of tannins in feeds reduces the nutritional value by binding with proteins rendering proteins unavailable for microbial degradation. However, this largely depends on the type of tannins and it is perceived that hydrolysable tannins (HT) are more hazardous than condensed tannins (CT) and it is said that CT have less harm when less than 5% of the dry matter in the feed, and that there is a negative correlation between CT levels in forages >50GCT/kg DM and their palatability, voluntary feed intake, digestibility and N retention in ruminants, (Mueller-Harvey 2005),.Also, goats and sheep fed on diets of *Desmodium intortum* and *Calliandra callothyrsus* legumes possessing 9.5g/kg and 22.5g/kg of condensed tannins respectively resulted in over 21% more Nitrogen reaching the abomasum when compared to the ones fed on tannin-free diet, Cirkovic Velickovic & Stanic-Vucinic, 2018).

Current research has demonstrated that tannin-binding proline-rich salivary proteins are not generated by goats and that the analysis of amino acid composition of a ruminants' proline protein levels, was found that they have low quantities of the essential amino acids. It has also been argued that if the release of high proline proteins is originally aimed at protecting ruminants from the adverse tannin effects, then it could be concluded that such proteins should have higher tannin-binding capacity for the animals which usually consumes them, and other findings have suggested that the original purpose for

these proteins was for oral homeostasis maintenance and that the dietary tannin-binding was a derived function (Naumann *et al*., 2017). There are other strategies that enable the herbivores to counteract tannin effects like the presence of enzymes systems by some animals such as Voles and snowshoe hares which detoxify tannins by conjugation with *glucuronic* acid and also the enzyme *polyphenol oxidase* is said to cancel the effects of tannins, that tannins in tree-locusts, are non- toxic and are utilized by locusts to make their cuticles (Sinha *et al*., 2012).

Other studies have demonstrated that the use of a tannin chemical binder *polyethylene glycol* (PEG) incorporated into tannin containing feed resulted in better crude protein digestibility, feed intake, hemoglobin and urea N levels and growth rate when feed to kids at 5 g per day as assessed with the controls (Hlatini *et al*., 2018). Protein –tannin dissociation occurs post-ruminally in the true stomach (abomasum) and that sheep and goats fed on Hydrolysable tannins (HTs) causes diverse clinical manifestations in animals as a result of their hydrolysis in the rumen and the condensed tannins (CT) are said to cause more profound effects on forage digestibility. It has also been reported that CT in *A. seyal* pods when fed to sheep resulted in reduced NDF digestibility (Bhatta 2002).

Hydrolysable tannins are easily absorbed in the gut of ruminants and it has been observed that a deer that eats leaves and flowers with HT developed *haematuria* (Emire, Jha, & Mekam, 2013). Tannins can change the protein digestion site thereby improving the absorption of amino acids from the lower gut (abomasum) and from the small intestines hence a low level of tannins will improve nitrogen utilization by ruminants. This

phenomenon has been referred to as “by-pass proteins” (escape proteins) and results in improved growth rate, fertility and milk yield (Coburn & Bronner, 2014).

Studies on varying the type and amount of *proanthocyanidines* in tanniniferous plants is appropriate since they are linked to non-bloating legumes increased protein utilization and reduced soluble non-protein nitrogen in silage. Studies on molecular genetics, cell biology and biosynthesis in legume plants should be tied together with studies on nutrition and toxicology. Livestock which eats diets having >5% w/v tannin level normally develop negative nitrogen balance, reduced feed digestibility and animal performance (Wu *et al*., 2016). There is need to investigate the inefficient Nitrogen (protein) utilization by the ruminants through use of non-conventional forages containing condensed tannins (CT).

In order to improve the nutritive value of tanniniferous forages, various methods can be used to deactivate tannins based on the theory that tannins are hydro soluble polymers forming complexes with proteins and such complexes can be destroyed in conditions of low pH. (<3.5) or high alkalinity (pH.>7.5). Tannins also complexes with carbohydrates, steroids saponins alkaloids, nucleic acids and minerals (Saxena *et al*., 2013). The inclusion of dietary condensed tannins at 2-3% level is said to cause beneficial effects by lowering the wasteful protein degradation by forming protein- tannin complexes which eventually dissociates post ruminally in the abomasum at a low ph. where the proteins now forms complexes with the enzymes like pepsin (Wu *et al*., 2016).

In a study, a 10 percent solution of Oak wood ash and pine wood ash lowered the levels of total extractable phenolic (TEPH), CT and formation of tannin- protein complexes in

their leaves by 66.80, 75% and 69, 85, 80%, respectively (Saxena *et al*., 2013). The use of wood ash to reduce tannins in *Acacia cyanophylla* resulted in better digestibility of the forage in sheep and that it is an economical method as opposed to polyethylene glycol (PEG). Some communities have used wood ash to alleviate excess tannins in sorghum and millet used by man and the use of wood ash being a less expensive option has the potential in the reduction of tannins in forages, but requires more validation by animal experiments (Emire, Jha, & Mekam, 2013).

Other methods of reducing tannin levels in forages includes the use of polyethylene glycol (PEG) which is an inert and unabsorbed molecule which forms a stable complex with tannins hence interfering with the binding of tannins and proteins thereby releasing forage proteins hence enhancing nutritive value resulting in improved performance of goats and sheep. The major disadvantage of this method is the high cost of PEG that limits its practical use especially by the pastoralists and also their potential to cause environmental pollution. Studies have demonstrated that there exist differences in tropical legumes with tannins with respect to condensed tannins extraction using aqueous solvents and that these differences are due to forage maturity, environment-genotype interactions and how the forages are processed after harvest (Hlatini *et al*., 2018).

Humans for a long time have used activated charcoal (crushed in powder form) or as tablets to relieve cases of toxicity, similarly in veterinary medicine for the same purpose, (Emire, Jha, & Mekam, 2013). Also the Zanzibar red colombus monkey (*procolobus kirkii)* is said to eat a lot of wood charcoal since it feeds on a lot of foliage with high

phenolic levels to reduce toxicity by binding of the compounds to the charcoal (Bhat *et al*., 2013).

2.4 Effects of tannins on digestibility and growth performance of small east African goats (SEAG)

Digestibility can be defined as the amount of feed that does not appear in faeces and is therefore assumed to have been digested, absorbed and utilized by the animal. Digestibility is an important parameter for quality and various methods have been developed to measure in-vitro digestibility (Coburn & Bronner, 2014). Tannin renders ingested nutrients unavailable leading to deficiencies of essential nutrients that results in malfunctioning of physiological systems and may cause pathophysiological conditions in goats. They may also lead to poor growth and reproductive challenges in goats and meat quality issues.

The production of gas in digestibility experiments has proven to be accurate in estimating the ruminant's performance when fed on certain forages. The choice of in-vitro method of digestibility is because it is versatile and best mimics the in-vivo technique and assists in understanding certain critical mechanistic issues confounding the effects of tannins in some plants in animal nutrition (Naumann *et al*., 2017). In the rumen, plant material comes in contacts with numerous microbial colonies which starts the degradation of forage cell walls into the end products of carbohydrates digestion- the simple sugars which are utilized by ruminal microbes to make their own proteins for growth and development. They do these through fermentation of the sugars to yield volatile fatty acids (VFAs)-acetate, propionate and butyrate, and the release of gases like –ammonia,

methane, hydrogen sulfide, and carbon dioxide. The VFAs also known as short-chain fatty acids are products of microbial degradation of carbohydrates in the gastro-intestinal tract (GIT), and from the endogenous substrates like mucus, and there are no digestive enzymes which degrade cellulose or other complex carbohydrates (Bhat *et al*., 2013).

The production of gas from various materials of feeds is dependent on the technique used, nature of feed, species of animal used, the amount of measurements and accuracy of the digestion curve, and the mathematical model used (Soares, Mateus & de Freitas, 2012). Studies on gas production using sorghum Stover at 3, 6, 12, 24, 48, 72 and 96 hours respectively produced the following results: 3.16, 5.50, 11.00, 19.40, 28.77, 33.43 and 37.20 mls. Respectively, and in another study, gas production from eight forage plants observed for 24-48 hours was different and ranged from14.8-35.1mls in 24 hours and 26.60 to 38.70 mls in 48 hours., and that a strong relationship (R^2=0.84: $p<0.01$) occurred with the raise in (%) in gas produced and the amount of total (VFAs). The gas production curve on dry matter degradability has been termed as a sigmoid curve having 3 phases i.e. slow phase (involving hydration), microbial attachment and colonization and that the second phase (exponential phase) is attributed to enzymatic digestion and the third phase (Asymptotic phase) represents decline in gas yield and reaches zero. However, some researchers did not observe a similar curve in either gas yield or in dry matter digestibility, but instead observed a linear and exponential functions, a correlation between dry matter digestibility and gas production at 48 hours (Mlambo, Marume & Gajana, 2015).

Protein is of paramount importance in animal nutrition and is an expensive component for livestock (Mutai, 2017). It is necessary to strike a balance between protein and energy bioavailability for better feed utilization efficiency. It has been proposed that in *Lotus sp.* A 3-4 % concentration of tannins on DM basis could be enough to enhance protein digestibility without affecting the digestion of carbohydrates.

2.4.1 Plant factors affecting digestibility

Some of the plant factors affecting digestibility include the stage of growth of the plant, (age of plant). Younger plants are less fibrous and are more digestible due to less dry matter content than older plants with more dry matter. Similarly, the part of the plant being utilized. Leaves and pods are usually more digestible than the stems and the barks. Chemical composition of the plant is another factor. The higher the cellulose, hemicellulose and lignin in a plant, the lower the digestibility. In addition, the presence of anti-nutritional factors. Most plants with ANFs like tannins binds with dietary proteins hence rendering the proteins unavailable for digestion by the ruminal micro-flora (Gardarin *et al*., 2014).

2.4.2 Animal factors affecting digestibility

Some of the factors affecting digestibility of tannins include **f**requency of feeding the animal. Whereby, the more frequent the less the digestibility due to increased rate of passage of feed along the gut. Health status of the animals' gastro intestinal tract is another factor such that if the animal has gastritis, enteritis, diarrhea etc., digestibility will be impaired. The rate of removal of waste products of digestion. Research has demonstrated that there was significant ($p<0.05$) difference in digestibility of CP, NDF,

EE and HC on Red Sokoto goats in Northern Nigeria when they were fed on *Acacia mellifera* pods processed differently (Mertens & Grant, 2020).

Other studies have shown that Nitrogen retention, rumen ammonia, and apparent nitrogen digestibility was found to be persistently lower on animals given *Acacia seyal* compared with the ones fed on *Acacia nilotica* and *Sesbania sesban* (Krebs 2007). The pods of *Acacia tortilis* have crude protein and Dry matter digestibility of 18% and 46% respectively (Avornyo *et al*., 2020). Tannins have the affinity to reduce Dry Matter degradability in the rumen and also react with the external cellular lining of the intestinal wall hence reducing the permeability of the intestinal wall (Skenjana, 2012).

Also it has been demonstrated that enzymatic and other endogenous proteins constitute a sizable part of nitrogen excreted by animals given tanniniferous diet and that animals could also experience some amino acids losses (Mertens & Grant, 2020). Lowered digestion of ruminal proteins is attributed to tannins which raises the quality of non-ammonia nitrogen (NAN) and amino acids getting to the lower gut and that the forage – *L.corniculatus* having 20 gram condensed tannins (GCT) Kg^{-1} without incorporating PEG resulted in 50% extra essential amino acids and 14% extra non-essential amino acids in the small intestines, compared to when the same forage was offered to sheep and incorporated to PEG, and this change was attributed to the more apparent absorption i.e. 59 vs $36gd^{-1}$ in sheep where the forage CT were not inhibited by the addition of PEG (Skenjana, 2012).

The same reactions were demonstrated when sheep were offered *L.pendiculatus* forage having over 50g CT Kg^{-1}Dm given with and without PEG (Avornyo *et al*., 2020).

Condensed tannins have the affinity to raise the flow and assimilation of non- ammonia nitrogen like dietary, endogenous and microbial proteins, amino acids and peptides into the small intestine (Mertens & Grant, 2020). Inside the rumen, about 70% of soluble proteins could be digested resulting in ammonia due to the deamination of amino acids. Excess ammonia from the amount used in the synthesis of microbial proteins permeates the rumen wall into the blood stream. This is converted into urea in the urea cycle in the liver where energy is dispensed, and condensed tannins are said to reduce soluble proteins and Ammonia-Nitrogen amounts in the rumen fluids. It also enhances nitrogen retention by lowering urea elimination and /or through raising urea recycling to the rumen (Mertens & Grant 2020; Soares, Mateus, & de Freitas, 2012).

Condensed tannins when taken by animals' raises the level of dietary proteins reaching the duodenum of ruminants and it has also been demonstrated that in non-ruminants, tannins lower the absorptions of the essential amino acids like methionine. The degree of condensed tannin-mediated dried decrease in in-vitro protein digestibility is directly related to the content of tannins (Marchiosi *et al*., 2020). Ruminal by-pass protein escape levels derived from in-vitro protein digestibility values for some forages were-22% (Lucerne), 18% *L. corniculatus* 38% *L. pedinculatus*, 54% *Sainfoin* and 60% *Lespedeza* indicating that digestibility levels are different from species to species having similar content of tannins. This suggests that chemical properties of tannins and their amounts influence protein digestibility and that reduced amounts of condensed tannins raises the levels of proteins and amino acids getting to the lower gut because fewer proteins are digested in the rumen. Organic matter in-vitro digestibility (OMD) of *Acacia mellifera* treated with PEG and Sodium hydroxide (NaOH) and incubated for 24-hour period

showed significant increase in digestibility. Also, studies done in north Sinai- Egypt where *Acacia saligna* has the nutritional potential to provide both energy and protein to supplement ruminants fed on low quality roughage especially during dry season demonstrated that digestibility coefficients for OM, DM, CF, NFE and EE was reduced as the *Acacia saligna* consumption was raising (Addisu, 2016). The replacement of 50% of daily DM alfalfa intake for Barki sheep with Acacia foliage lowered the digestible proteins.

The pods and leaves of *Acacia mellifera* have been utilized by livestock keepers and researchers to supplement low quality roughages resulting in appreciable live-weight gains. However, it appears that protein in *Acacia sp*ecies is not fully digested by goats and sheep and is attributed to occurrence of tannins in these forages of between 4-11% on DM basis (Marchiosi *et al*., 2020).

During the chewing action by ruminants the disintegration of plant material enables tannins to interact with salivary glycoprotein to form insoluble tannin-protein complexes which tend to promote further binding to protein in the rumen at the ph. of 5.5-7.2, A ruminal pH. of 6.5 has been found to be ideal for the formation of tannin-protein complexes but at lower ph. of 3.0 which is next to the iso-electric point of the protein and this occurs in the true stomach-abomasum and the small intestines causing the dissociations of the complexes thus enabling enzymatic digestion to take place yielding amino acids required by the ruminants (Helal, 2018).

Condensed tannins also possess the ability to complex with minerals like iron, aluminum, at a low pH of 3.70 and can result in iron deficiency. Condensed tannins have the affinity

to form complexes with many digestive enzymes like proteases, amylases, pectinases, lipases and cellulases (Kandie, 2022). Condensed tannins could also bind to beyond one site of a protein and could alter the morphology of the substrate protein within the ruminant digestive system through induction of steric disturbance and inhibition of enzymatic hydrolysis, or by direct complexation to hydrolytic enzymes, making them inert catalytically (Helal, 2018).

This tannins configuration with digestive enzymes is facilitated by the numerous hydroxyl groups making the enzymes non-functional through disruption of the enzymes active sites in terms of their mode of action by the lock-and key or the induced-fit mechanisms on their substrates. They also complex with carbohydrates and lipids besides forming tannin enzyme binding which distorts enzymatic action and the digestive processes (Helal, 2018).

It has been demonstrated that tannins lower organic matter and crude fiber digestibility and in-vivo studies have shown that protein degradability is significantly reduced when tannin rich forages are part of the diet. It has been proposed that in-vitro carbohydrate and Dry Matter degradability has a negative correlation with hydrolysable and the condensed tannins levels (Chingala *et al*., 2019). It has been reported that in-vivo gas production is lowered by tannins and also the short chain fatty acids (SCFA) and the volatile fatty acids (VFA) production is lowered and that the named parameters could be used to determine the rate of feed degradability.

It has also been reported that there is negative digestion co-efficient for neutral detergent insoluble nitrogen (NDIN) and also acid detergent lignin (ADL), due to the complexation

of soluble tannins with the plant cell wall components and as a result the complexes are rendered indigestible or inaccessible by bacterial digestive enzymes (Helal, 2018). Also it has been shown that sheep which consumed silage with 18.7g tannins/kg had lowered digestion of CF and reduced ruminal activity as compared with the ones fed on maize silage with6.6gtannins/kg (Chingala *et al*., 2019).

Studies have shown that reduced weight gains of up to 68g/day to 16g/day was observed on bullocks fed 45% oil extracted seeds of *Acacia mellifera* and that average daily gain (ADG) was lowered by 18% when inclusion levels of tannins was raised to 10% (Chingala *et al*., 2019).

Also, reduced growth rate was reported on sheep fed on *A. seyal* pods and roughage at 204 and 347g/day respectively as compared to the controls by Helal (2018). Studies have also shown that there is improved growth rate and milk yield in ruminants fed with forage legumes containing tannins at less than 4%. One experiment on field grazing of nursing ewes with double lambs, milk production and components of the ewes fed on *L. corniculatus* having 4.5% tannins twice per day with or without PEG supplementation, the results indicated that as the lactation period continued, milk yield reduced together with that of the milk proteins and milk lactose on the ewes not supplemented with PEG (Chingala *et al*., 2019).

However, tannins are said to have beneficial effects in that *Acacia mellifera* tannins is reported to have been used in the pre-treatment of cotton seed cake before feeding to livestock to prevent ruminal digestion of the highly degradable plant proteins in cotton seed cake. This is because tannins may form complexes with proteins at a pH of 6-7

hence protecting proteins from digestion by microbial enzymes but the tannin-protein complexes are eventually digested in the abomasum where the pH is conducive for the enzymatic digestion for the now unstable tannin-protein complexes.

2.5 Effects of Tannins on Feed Intake and Feed Conversion Efficiency (FCE)

Feed intake is a very important parameter that can determine the production potential of livestock and any factor that hinders adequate feed intake will directly influence the expected production from the animal sp. leading to higher maintenance requirements and hence poor feed conversion efficiency (Kandie, 2022). Factors affecting feed intake includes animal factors e.g. body reserves, animal health and homeostasis, feed quality and physical characteristics such as dry matter content, fiber, size of particle and resistance to fracture have been known to affect the ease of prehension and hence rate of intake (Fterich, Mahdhi, & Mars, 2012).

Studies have demonstrated that the availability of protein in *Acacia sp*. to ruminal microbes is interfered with by tannins hence affecting dry matter intake (DM), Alam; *et ;al* (2005).Other findings have demonstrated that *Desmodium ovalifolium* and *Flemingia macrophylla* species containing same content of extractable condensed tannin of 90g/kg DM were chopped and added to (PEG) at 35g /kg DM for the binding of extractable (CT). The same diet was given to sheep with ruminal and duodenal cannulas, at a rate of 26g/kg body weight and there was no significant difference, but feed intake went up by 10% when the extractable (CT) was lowered from90- 50g/kg DM using (PEG) treatment. Also, the overall OM, ADF, and NDF digestibility improved (Krishna *et al*., 2019).

When *Acacia cyanophylla* forage was fed when fresh i.e. with 5.1% tannins, or Air-tried with 4.3% tannins, it showed that air –drying increased the voluntary feed intake (VFI) on DM basis in sheep by 8%, while forage from *Acacia aneura* fed with PEG at 24G/day/DM, resulted in a rise of 50% of voluntary feed intake (VFI) (Wink, 2013). Temperate legumes with high tannins contents have demonstrated reduced feed intake, protein and fiber digestion and nitrogen utilization when fed to sheep and lower tannin levels may raise nitrogen absorption (Smith 2005). It has been reported that a 5% decrease in feed intake was observed on bullocks fed 45% oil extracted seeds of *Acacia mellifera*, (Tufarelli & Laudadio, 2011). Dry matter intake (DM) and N- retention was reported to decline when goats were fed *A. nilotica* pods as supplements (Wangai, Muriithi, & Koenig, 2013).

Forages containing over 55g CT/kg DM usually lowers voluntary feed intake (VFI) and degradability and reduces wool growth in sheep and gain in weight by ruminants, and it has been proposed that moderate tannin levels may be utilized to boost protein degradability and enhance livestock production through use of non-conventional livestock feeding regimes (Wangai, Muriithi, & Koenig, 2013). Other reports have shown that lowered palatability and feed intake on browse plants by ruminants is associated with compounds like saponins,non-protein amino acids, *polyphenolics, cyanogen, alkaloids*, *phytohaemagglutamins*, *lecitin*, and oxalic acid, commonly referred to as anti-nutritional factors, (Mutai *et; al* 2017).

Acacia mellifera supplements when used at a rate of 200g/day results in relatively reduced feed intake and nitrogen retention associated with adverse tannins effects.

Chemical compounds like polyethylene glycol (PEG) can be used to increase feed intake in tannin-rich feed because PEG has a higher affinity for tannins than proteins but its major limitation is its expense and technical applicability especially by the pastoralists, and its effects on environmental pollution (Omoyo, Wakhungu, & Oteng'i, 2015).

2.6 Effects of tannins on internal organs weights and carcass yield of small east African goats (SEAG)

Anti-nutritional factors such as saponins in most field legumes like vetch, Lucerne and beans have been found to exert phato-physiological effects on organsof animals where overt signs of *anorexia, pica, unthriftyness* (*cachexia*), *dermatitis, haematuria* etc. were observed on live rabbits fed on beans, vetch, and Lucerne and *desmodium*in order of severity. Also post-mortem signs of *ecchymotic* and *petechial* (pin-point) hemorrhages were observed in the livers and lungs of sacrificed rabbits, and gastro-intestinal necrosis. There was also significant ($p<0.05$) difference in organ/systems weights of kidneys, livers and GIT (Mutai, 2017).

Tannins are known to affect palatability of feed, digestibility thereby affecting growth and carcass weight. It has been reported that bucks supplemented on acacia pods at 1.5% on body weight basis resulted in an increase in the killing-out % (Omoyo, Wakhungu, & Oteng'i, 2015). Also, the killing out % was reported at 50.13-52.43% on fat tailed Awassi ram lambs and the average weight at slaughter, weight of carcass and killing –out % was 42 kg, .57 and 51.35% respectively (Wink, 2013). Other findings have recorded up to 43.49% in carcass yield on male Awassi sheep, and that there was no significant difference in the weights of lungs, liver, heart and kidneys and that all organs looked

normal when the sheep were feed on acacia at 40% inclusion (Bayssa, Negesse & Tolera, 2016). Also, reports have demonstrated that the killing-out % inclusive of fat from tail, was 52.51 and 50.28% when diets were incorporated with forages with tannins unlike the control group which had 48.28% although there was no significant difference. The *Acacia saligna* shrub has been reported to have immense potential as a forage for growing sheep in the arid and saline soils of the Egyptian desert. It has been reported that weaner rabbits can tolerate dietary inclusion rate of 40% of *Acacia alibido* pods with no effect on digestibility of nutrients and blood parameters (Amadi *et al*., 2018). Studies have showed that there is lowered carcass fat in livestock fed on tanniniferous forages and this can be attributed to alterations in the molar proportions of volatile fatty acids generated on degradation of tannin forages (Abdel-Shafy & Mansour, 2018).

The daily supplementation on sheep fed on *lotus pedunculatus* forage lead to better gain in live weight by 41-61 g/d and with better growth of wool. Studies done on the fat-tailed Awassi ram lambs in North Sinai, fed on a ration with 40% Acacia indicated higher averages of fasting weights, hot carcass and cold-dressed weights as compared to other rations but with no significant difference among them. The dressing out % -inclusive of tail fat, were 52.51 and 50.28% in lambs fed ration with 40% Acacia (Amadi *et al*., 2018).

2.7 Effects of *Acacia sp*. tannins on rumen cellulolytic microbiota

Ruminants utilizes the resident ruminal microbiota to degrade cellulose converting them into palatable and nutritious livestock products which are finally utilized by human beings as food (Mlambo and Mapiye, 2015). Excessive digestibility of intake proteins by

the ruminal microbiota has long been seen as a negative in ruminant nutrition and that ruminants utilizes proteins more effectively if the proteins are protected from bacterial deamination in the rumen by the bacterial enzyme-*deaminase (*Behnke & Nakirya, 2012).

Roughages are leading source of feed for ruminants, and they are composed of polysaccharides like *hemicellulose,* cellulose, lignin, and pectin in their descending order of degradability. Ruminants derive their energy predominantly from forages which are composed of plant nutrients and other minor compounds e.g. *Saponins* and *silica,* and polyphenols and cellulose is the most abundant polysaccharide found in the cell wall and contributes to 20-30 % of the dry weight of most plant cell wall, The reticulo - rumen microbiota digests roughages into volatile fatty acids (VFAs) i.e. acetate for synthesis of fats, propionate for glucose synthesis and butyrate for carbohydrates, to be utilized by the host to generate energy (Amadi *et al*., 2018; Behnke & Nakirya, 2012).

Ruminant animals cannot fully digest fibrous forages e.g. consumed cell walls of polysaccharides due to both physical and chemical agents found in the ingested forage, and also by the retention time limits on the intake material within the rumen, notwithstanding other feed compounds e.g. *phenolics, saponins, silica* and *lignin* which possess inhibitory effects on cellulolytic action (Amadi *et al*., 2018). The bigger the size of feed particle the more the time the feed particles are retained in the rumen hence the better the digestibility. The predominant constraint to cell wall digestibility in the rumen is attributed to the cross linkages in the celluloses, hemicelluloses, lignin and some portions of plant cell walls on the surface of feed particles could hinder microbial attachment and hence their efficiency of digestion of such forages (Amadi *et al*., 2018).

It has been observed that the plant cuticle resistance to microbial digestive enzymes could be improved by forage mastication and pre-treatment of forages so as to reduce their negative effects on digestion (Belay *et al*., 2013; Amadi *et al*., 2018). The efficacy of ruminal microbial digestion could be improved by the physical and chemical nature of the roughage, provision of microbial proteins and through processing of the forages into usable forms. Utilization of forages can also be improved through acclimatization to animals slowly to enable them to accrue the relevant microbes for their degradation of forages, and it is necessary to check the protozoal colonization of the rumen since they have predatory behavior against the bacteria and other organisms, especially if they are outnumbering the other beneficial microbes (Amadi *et al*., (2018). The major rumen microbes responsible for plant cell wall degradation are the Fibro lytic bacteria and the ruminal fungi which generates a lot of enzymes responsible for the digestion of a varied amount of substrates and have a capacity to penetrate the plant cuticle surface and cell walls of lignified tissues (Akin, 2009).The protozoa in the rumen are responsible for the digestion of plant cell wall polymers and if not found in the rumen there could be a decrease in fiber degradability (Abd'Quadri-Abojukoro, 2021).

The ruminal microbial enzymes should function in synergy to enhance the degradation of the structural carbohydrates in most forages to enable efficient utilization by the animals, and if roughages hydrolysis is to take place in the rumen, the enzymatic activities should be in tandem with that of feed substrate. Microbes enhance their stay in the rumen through attachment to the feed particles hence getting their enzymes in proximity with the feed substrate (Dambe *et al*., 2015).

The use of condensed tannins to shield proteins from digestion by ruminal microbes sounds novel if the CT causes no disturbance to other ruminal activities and the rumen per see have no direct release of enzymes for degradation of forages but is a chamber for fermentation whereby bacteria, fungi and protozoa digests forage feeds. In mixed ruminal microorganisms' cultures, the degree of protein breakdown of purified Lucerne Fraction 1 protein bound to *Sainfoin*, CT, was lowered when the CT: protein ratio was 1:2, indicating that CT were lowering protein availability to the digestive bacteria (Dinnage *et al*., 2019). Fungi and bacteria have affinity to insoluble proteins, while protozoa swallow certain bacteria and proteins, instead of using extracellular soluble proteins (Engida *et al*., 2015). Protein digestion products in the rumen like amino acids, peptides, or ammonia – N are utilized for ruminal microbe's growth, or converted to (VFAs) in energy producing metabolic reactions. Also, the protozoa multiplied more in the rumen of sheep fed on *L. corniculatus* forage mixed with PEG to cancel tannins effects, while -condensed tannins in the forage -*Quebracho lowered* the quantity of protozoa in rumen simulation experiments (Rusitec device) and that ruminal fungi were not capable of degrading CT from *L.corniculatus*, (Makkar 2005). The rumen wall absorbs the soluble end products of microbial digestion, but the parts of residues of feed and the microbial cells are degraded in the small intestines.

Condensed tannins can also complex with cell-coated proteins, microbial cell-associated enzymes and co-enzymes. Also, CT are strong chelating compounds with the potential of lowering the availability of metal ions needed for metabolism by microbes and could also interfere with metalloenzymes, and that oxidized tannins have been demonstrated to possess toxicity on methanogenic bacteria (Fanzo, 2014). It happens that dietary protein

becomes initially available to the ruminal microbiota than to the ruminant itself. Tannin-protein –complexation could cause improvement of protein utilization efficiency, otherwise a lot of high-quality forage proteins could be used by ruminal microbes to make their own proteins which might not be of the immediate benefit to the ruminant (Hermes *et al*., 2020).

The protection of the proteins in tannin-protein complexes against the deamination by the bacterial de-aminase enzymes is actually a potentially beneficial attribute of tannins in forages. The protein precipitation through tannin-protein complexation is suggested to protect proteins from ruminal bacterial degradation occurring during mastication. In an experiment with the forage *L. corniculatus* having 10-50gCT/ KG^{-1} DM, in-vitro incubation using rumen fluids, gas production and volatile fatty acids and DM degradability were reduced with the high tannin content in the forage, and the decrease in dry matter degradability was followed by alterations in colonization by ruminal microbes on the stems and leaves. Also, the electron microscopy study showed that the bacteria developed glycocalyx-enclosed micro-colonies which had reduced attack on the stem tissues of high tannin birds' foot trefoil forage as compared with the lesser tannin variety, (Chiquette 2008). Subsequent research demonstrated that CT did not only influence cellulolytic enzymes but also inhibits bacterial cells (Hermes *et al*., 2020). The bacteria *prevotella ruminocola* had the least effects even with raising amounts of cell-associated condensed tannins in the culture, and that the bacteria *streptococcus bovis* was very sensitive to condensed tannins and showed lowered cell multiplication and changed cellular morphology in the presence of 500ugmL^{-1}, and it complexed with the most CT per unit weight of cells (Herremans *et al*., 2020). The bacteria *Fibrobacter succinogenes*

and *S. bovis* were found to be the most affected by CT from the forage *Quebracho, myrle* and desmodium where their bacterial growth was inhibited by as low as 100ugCTML, (Dambe *et al*., 2015). However, the bacteria *prevotella ruminocola* and a newly identified ruminal streptococcus species were more resistant to CT but the bacteria *Ruminococus albus* was average while with growth inhibition studies, the bacteria *F. succinogens* and *P .ruminocola* complexed with the highest and the least amount of tannins respectively to their cell surfaces.

It has been reported that there was a remarkable reduction of solvent extractable condensed tannins in a plant material after mastication which is associated with a rise in protein-bound and fiber –bound tannins (Dinnage *et al*., 2019). It has been said that the occurrence of some condensed tannins in forages resulted in reduced protein digestibility hence lowered free amino acids and reduced conversion of this acids into the branched –chain Volatile fatty acids (VFAs) preferred by bacteria as growth-promotors and microbial protein synthesis (Bayssa, Negesse & Tolera, 2016).

Majority of the crude –fiber digesting bacteria needs the branched –chained fatty acids for survival and fibro lytic action. Tannins have other benefits to ruminants since they can control bloat and have anthelminthic properties and can be integrated in worm control programs reducing excessive use of conventional dewormers (Chingala, 2018).

The diversity of bacteriophage, protozoa, bacteria, archae and fungi is estimated to be 8,500 species and technologies which are culture –based like isolation, enumeration and nutritional characterization have identified beyond 200 bacterial species and not less than 100 species of fungi and protozoa in the rumen (Crawford *et al*., 2020). Estimates of

microbiota population shifts offers a glimpse of the effects of tannins on ruminal microbes and other findings have indicated a positive relationship between ruminal protozoal levels and tannins content.

The isolation and identification of novel gut microbes, their physiological characterization and genomic sequence analysis will transform the utilization and interpretation of the preceding nucleic acid technologies and sets a bench mark for elucidating the diversity of the rumen microbiota (Dambe *et al*., 2015). Some of the current technologies used in the manipulation and modification of ruminal function which are on contemporary research studies includes: use of probiotics, antimicrobial compounds, plant extracts and inoculants consisting of natural or genetically modified ruminal organisms (GMOs) and vaccines.

Microbial ecology investigation in the rumen microbiome encompasses determination of their abundance, activity and diversity mainly through in-vitro and in-vivo studies and their association with one another and the host animal. This includes the need to manipulate the ruminal microbiota to improve ruminal digestion (Fanzo, 2014). Typical culture –based approaches have been used to understand the ruminant gut microbiology and it has been suggested that the gut microbial population and its total genomes referred to as the microbiome is approximately having one hundred times more genes compared to the host animal, and empowers the ruminant with metabolic and genetic capabilities which the host animal did not evolve on its own like the capacity to hydrolyze and degrade latent nutrients and detoxify toxins (Hermes *et al*., 2020).

Detoxification of toxins like those found in tanniniferous forages can be done by fore-gut animal fermenters through ruminal microbial degradation which has evolved to detoxify phytotoxins and mycotoxins which are found in various feeds, grains, protein supplements and some forages and can be in the form of *tannins, saponins, glucosinolates*, *alkaloids*, *goitrogens, gossypol, mimosine, cyanogens,* nitrates and *oxalate*. The capacity of the ruminal microbes to adapt and increase its ability to detoxify plant toxin with respect to the level consumed is a significant factor that dictates the pathogenesis of the toxicity in plants in the fore gut fermenters (Kamo *et al*., 2012). The degree of expression of enzymatic action that digests or alters plant toxins affects the initial rate of metabolism of specific toxin in the rumen and the level of ruminal microbes releasing the enzymes (Katunga, Mushagalusa & Kambale, 2020). It has been suggested that the most appropriate explanation of the ruminant microbial response to a plant secondary metabolite like tannins involves the animal feeding on the tanniniferous diet. The most abundant group of tannins are the condensed tannins (CT) and are said to be non-degradable by the anaerobic microbes and current research is centered on the inhibitory effects of CT on microbes, their activity and adaptive responses of the ruminal populations to these substances. Comparatively, condensed tannins have more complexity in their structure than hydrolysable tannins and not easily degradable and also needs more specific environments, microbes and enzymes (Addisu, 2016; Hermes *et al*., 2020).

Likewise, it has been demonstrated that the incorporation of tannin-rich cotton seed hulls reduced the digestion of cotton seed proteins under in-vitro tests and that the incorporation of the tannin-binding (PEG) was not fully able to remove the binding effect

(Heuzé *et al*., 2016). The legume *L. corniculatus* have been demonstrated to possess condensed tannins which inhibits the extracellular *endoglucanase* activity of *Fibrobacter succinogens* bacteria and that condensed tannins extracts from the forage *O. viciifolia* inhibited the proteolytic activity and growth of the bacterias-*Ruminobacter amylophilus, Butyrivibrio fibrisolvens* and *Streptococcus bovis* (Addisu, 2016). There are findings that condensed tannins extracts from Conifer seed cones have been said to interfere with the growth of fungal cultures of *ceratocystis coerulescens* and *Schizophyllum-commune* had no effect on *Trametes versicolor.* It was noted that the extent of microbial inhibition as a result of tannins is correlated to the types and number of tannins and to the susceptibility of the related microbes (Addisu, 2016; Hermes *et al*., 2020).

Enzymatic inhibition is said to be as a result of non-specific tannin-enzyme protein complexes, which can also take place with the substrate. In–Sacco experiments have indicated that the action of the enzymes-*urease*, *carboxymethyl cellulase*, *glutamateedehydrogenase* and *alanineamino transfarase* were reduced in the treated leaves of *Quercusincana*. Also it has been found that condensed tannins from the plant-sainfoinleaves curtailed the growth of the ruminal bacteria *Butyrivibrio-fibrosolvens* - A38 and Streptococcus bovis 45S, and had less effect on *prevotella ruminicola,* B,4 or *Ruminobacter amylophilus WP* 225 (Fanzo, 2014). The pre-disposing conditions which can influence the degree of digestive enzyme inhibition by tannins includes; the level of protein in the diet, relative enzyme quantities and types in the diet and the order of activity, the formation of tannin-protein complexes before and after ingestion of feed and the extrinsic factors such as ph. And intrinsic factors including type of animal and age (Addisu, 2016; Hermes *et al*., 2020).

The analysis of global data sets found in public data bases shows that 90% of the methanogenic bacteria (*Rumenarchaea*) are associated with the genera *Methano brevibacter* and that they differ with species of animal and in the varied agro ecological zones and this controls the acquisition, colonization and finally the structure of the microbiome in adult animal and ultimately the potential of protein digestion by the ruminant (Fanzo, 2014; Katunga, Mushagalusa & Kambale, 2020).

Genetic diversity exists among ruminal bacterial species which are of economic importance like the rumen bacteria *Synergistes jonesii* whose role is the detoxification of the economically important forage tree legumes, has genetic diversity based on geographical locations. There exist variations in rumen microflora among animal species and even within breeds when given same feed and assessed with respect to nutrient utilization (Gemede & Ratta, 2014).

The most remarkable finding in ruminal modification using a natural bacterium was found in the capacity of the bacteria *Synergistes jonesii*to detoxify the toxin mimosine found in the tropical browse legume *Leucaena leucocephala* and that this bacterium can be transferred from sheep, and goats and can colonize the rumen after being cultured in-vitro. Studies have discovered that the Bacteria-*Streptococcus-caprinus* isolated from the ruminal content of feral goats feeding on tannin-rich *Acacia sp*. and the bacteria is a Gm+ve, facultative anaerobic with the ability to clear tannic acid-protein complexes and said to be resistant to tannins and with the ability to grow in a media with 2.5% tannic acid or condensed tannins. It has also been demonstrated that the animal sp. (Koalas) harbors an *enterobacteria* which degrade the protein-tannin complexes associated with

hydrolysable tannins (tannic acids) but was incapable of dislodging proteins bound to condensed tannins present in *quebracho* plant. Subsequent research studies indicated that these bacterial strains degraded *gallic* acid to a substance called *pyrogallol* which did not undergo any further degradation and did not appear in the faeces of the *Koala*, and could not be established if it was absorbed by the Koala and if it had disastrous effects on the *Koala* (Fanzo, 2014; Gemede & Ratta, 2014; Katunga, Mushagalusa & Kambale, 2020).

Also, another bacterial strain –*Selenomonas ruminantium*., sub-species *ruminantium* isolated from feral goats browsing on tanniniferous *Acacia sp*. was also found to be able to grow on tannic acid or on condensed tannins (Katunga, Mushagalusa & Kambale, 2020). In a unique study, ruminal microbes from feral goats which resists tannins were inoculated to domesticated sheep and the results was that sheep fed on tannin –rich *Acacia –aneura* showed higher feed intake and Nitrogen retention on being administered with inoculum from feral goats' ruminal fluid, which also lead to improved weight gain (Katunga, Mushagalusa & Kambale, 2020). However, another study had contrasting results where the inoculation of sheep using the bacteria *streptococcus caprinus* from goats did not lead to better digestibility in sheep (Fanzo, 2014; Gemede & Ratta, 2014; Katunga, Mushagalusa & Kambale, 2020).

Toxicity of tannins affects ruminal cellulolytic microbes including the majority of the bacteria of the genera: *Megaspbaera, Eubacterium, Streptococcus* and *Synergistes jonesii*, known to have the capacity to degrade the *pyridinedisol* toxin and can solely use amino acids as its carbon source, and other members are e.g. *Butyvibriofibrosolvens, Streptococcus bovis, Streptococcus caprinus (Streptococcus gallolyticus), Ruminobacter*

amylophilis, Prevotella ruminicola and *Fibrobacter succinogens*. Findings from molecular ecology studies have found out that the Gram-negative group of bacteria *(Enterobacteriaceae* and *bacteroides sp*. are the most commonly present ones in diets with tannins, and a decline in the levels of the Gram +positive bacteria like the *Clostridium leptum* group., and that the leguminous forage *(Calliandra* and *Lotus)* condensed tannins lowers the numbers of the cellulolytic and proteolytic bacteria in the rumen (Katunga, Mushagalusa & Kambale, 2020).

Tannins can also cause enzyme inhibition and induce changes in the morphology of ruminal microbes (Dambe *et al*., 2015). The ruminal methanogenic microflora usually degrades the cellulosic material converting them to beneficial products to the ruminant and in the process ruminal methane gas is released. Proteins are degraded to amino acids and peptides and peptides can be acted upon further by peptidolytic bacteria like *Prevotella ruminocola* and others to amino acids prior to absorption by the rumen microbes. The ruminal protozoa tend to engulf the fungi, bacteria plus smaller protozoa, a phenomenon which has an influence on intra-ruminal nitrogen re-cycling and protein synthesis efficiency through controlling digestibility of proteins because they also possess protease enzymes like the bacteria (Katunga, Mushagalusa & Kambale, 2020).

Under specific enzyme –tannin ratio the reduction of enzyme action seen *in-vitro* is as a result of the features of the condensed tannins and the protein, and the less molecular weight of CT from the legume *L. leucocephala*,has showed constant and least inhibition of enzyme action produced by the anaerobic fungus- *Neocallimastix hurleyensis*,but contrary to the higher CT from *Desmodium ovalifolium* and from *Flemingia macrophylla*

which demonstrated high enzyme inhibition (Bayssa *et al*., 2021). There exist differences in bacteria with respect to the levels of tannins needed to hinder their growth and also among the tannins in their ability to inhibit the growth of the microbes, for example the fungi *Neocallimastyx frontaliss* have been found to be the most resistant while the fungi *Orpinomyces joyonii*, as the most vulnerable to tannin occurrence (Bayssa, Negesse & Tolera, 2016).

The reducing equivalents which are not utilized in the synthesis of volatile fatty acids can be changed into methane gas which can lead to a gross energy loss of 15% by the animal, (Hermes *et al*., 2020). However, the mode of action on methanogenic organisms by tannins is not completely comprehended and has been hypothesized that the dosage and the type of the condensed tannins could influence the growth of the rumen microflora since condensed tannins are hydrogen acceptors and lowers the volume of H_2 present in the rumen for use to synthesize methane gas (Dambe *et al*., 2015).

Both in-vivo and in-vitro studies have demonstrated that condensed tannins can lower the generation of methane gas by the ruminants (Katz, 2016). Hydrolysable tannins are said to be toxic since microbial metabolism and gastric degradation changes them to absorbable low molecular weight metabolites which are toxic with manifestations of liver necrosis, hemorrhagic gastroenteritis, and kidney damage, and lowered absorption of amino acids like *methionine* and *lysine* (Fanzo, 2019).

Studies have demonstrated that there could be a decline in ruminal VFA, microbial deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) production due to the increase of tannins concentration in animal diet and that the decrease in the digestion could be

attributed to either the effect of tannin on bacterial cell wall or the direct interaction between the tannins and the bacterial cell wall (Bayssa *et al*., 2016).. The hydrolysis of plant cell wall is effected by specific bacteria usually of the genera *Ruminococus* and *fibrobacter,* Ciliate protozoa and anaerobic fungi.

It has been found that a tannase enzyme responsible for the catalyzing the hydrolysis of the ester bonds linking the phenolic acids and alcohols was found to be produced by *Aspergillus* sp. of fungi and the same enzyme produced by the fungi *Aspergillus Niger ,A .oryzae, A. flavus, A. japonicus, penicillium Chrysogenum ,p. nottatum* and *p. isladicum*, and also yeasts-*candida* sp. *pichia* sp., *Debaryomyces hansenii*, and bacteria-*Achromobacter* sp. *Bacillus pumilis*, B.*polymyxa*, *Corynebacterium* sp.,*Klebsiella planticola* and *pseudomonas solanacearum*. The *sporotrichum pulvurelentum* fungus digests condensed tannins in the Oak leaves (*Quercus incana*) while the white rot fungus (*ceriporiopsis subvermispora*) and *cyathus stercorens* also digests condensed tannins (Bayssa *et al*., 2016).

The degradation of protein by ruminal proteolytic bacteria is a very efficient process that gives the cellulolytic bacteria a nitrogenous source for protein synthesis and the release of ammonia gas. Most ruminal bacteria use ammonia, urea or other non-protein nitrogen substances as an important nitrogen source and >60-80% of bacterial protein is synthesized from ammonia as the precursor (Addisu, 2016). However, too much conversion of dietary protein into ammonia gas can deny the host animal of the expected nutritional value of the generated amino acids. This elicits an interest in the control of ruminal microbial metabolism of proteins so as to optimize ruminal nutrients supply

(Crawford *et al*., 2020). Other studies have demonstrated that condensed tannins extract from *L. corniculatus* and *L. pedunculatus* evidently protected ribulose and Rubisco; fraction-1-leaf protein from degradation by mixed ruminal microorganisms (Aerts *et al.,* 2009). Concentrations of CT needed to produce maximum inhibition of proteolysis under in-vitro experiments is probably about400ug CT /ml or greater and the amount lowered microbial growth of certain strains of ruminal microbes.

In another experiment, when the diet was altered from perennial rye-grass/white clover pasture which does not contain CT, to *L. corniculatus* (32g CT/kg DM) in sheep, populations of the proteolytic rumen bacteria *clostridium proteoclasticum* B316, *Eubacterium sp*. C12b, *streptococcus bovis* B315 and *Butyrivibriofibrisolvens* C21 were reduced from 1.6x10r^{7}, 2.7x10r8, 2.7x10r6, and 1,2x10r^{6} to 5.1x10r^{7}, 1.5x10r8, 1.6x10r6 and 1.0x10r^{6} per ml, respectively (Gemede & Ratta, 2014).

Bacterial cell-associated proteinases on the rumen bacteria primarily affects the proteolysis of soluble proteins in the rumen and when CT-containing forage is masticated, insoluble CT-substrate complexes are formed and the CT in the rumen becomes bound to cell coat polymers of bacterial cells (Bayssa, Negesse & Tolera, 2016). The interactions between the bacterial cells and condensed tannins is not yet clearly comprehended unlike the interaction between CT and protein in forages. Latest findings indicate that the condensed tannins-bacterial interactions are stronger than condensed tannin-protein complexes, or that the interactions could be of a different nature. It is hypothesized that the condensed tannins binding to the bacterial cell surface like the cell-

bound extracellular enzymes thus inhibiting their action and that the degree of inhibition could depend on the different types of CT (Gemede & Ratta, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

3.1.1 Location and Size

The study was carried out in Radat sub-location in Eminimg ward within Mogotio Sub-County in Baringo County, Kenya (Figure 3.1**)**. Selection of the study was based on the fact that it falls within transitional zone IV which forms part of Kenya's ASAL regions and is the habitat of most of the *Acacia sp*ecies. This area is among the locations where SEAG are kept and form the live line of the pastoral community. The experimental site was located at latitude 0^0 15' 00'' N and Longitude 36^0 00' 00'E. The study site and its environs were visited and the three *Acacia sp*ecies under study i.e. *Acacia brevispica, A. mellifera* and *A. tortilis*, were positively identified with the help of the Government Kenya Forest Service (KFS) officers and the Range Management staff in the area.

3.1.2 Climate

The experimental site has an average unimodal rainfall pattern of 400-700 mm per year which usually starts from April to June and has day temperatures ranging from 29-32 ^oC (MOAlF, 2015).

3.1.3 Geology

Geologically, the study area consists of highly metamorphosed basement system formation, overlain by a sequence of tertiary volcanic strata. The basement system

consisted originally of fine-grained Precambrian sediments which have been highly metamorphosed by heat and pressure, in long series of orogenic events.

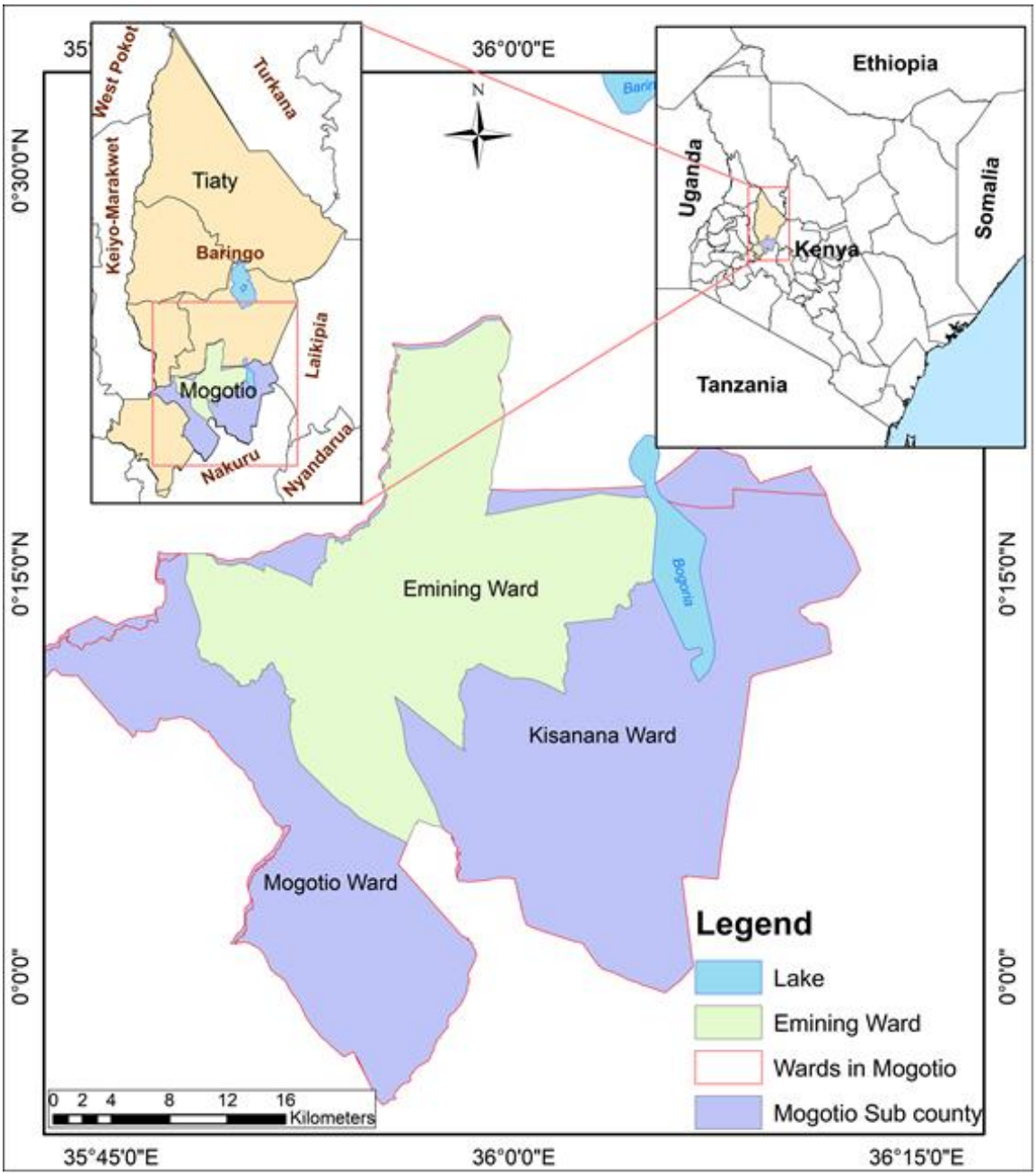


Figure 3.1: Map of the study area (Source: Author, 2018).

3.1.4 Flora

The area's vegetation is predominantly occupied by Acacia-grass species habitation like *Acacia sp-cynodon dactylon, or Acacia sp-tarconnanthus camphoratus* (tree legume – shrub) combinations. Common grass sp. includes: *Cenchrus ciliaris, Eragrostis* sp. And some tree species e.g. *Lantana camara, Balanites aegyptica*, *Andropogon sp.* and *Balanites glubra* (Amadi *et al*., 2018).

3.1.5 Fauna

Mammals include Dikdiks (*Madoqua kirkii*), Pangolin (*Smutsia temminckii*), Aardvarks (*Orycteropus afer*), Porcupines (*Hystrix cristata*), Bushbuck (*Trigelaphus scriptus*), Duiker (*Cephalophus natalensis*), Impala (*Aepyceros melampus*), Warthog (*Phacochoerus africanus*), Waterbuck (*Kobus ellipspiprymnus*), Tree squirrels (*Sciurus carolinensis*), Ground squirrels (*Xerus inauris*), Olive baboons (*Papio Anubis*), Vervet Monkey (*Chlorocebus pygerythrus*), spotted Hyena *Crocuta crocuta*), Leopard (*Panthera pardus*), Serval cats (*Leptailurus serval*, Silver backed jackal (*Canisme somelas*), Civets *Civettictis civetta*) among others. Kenya wildlife service has done reintroduction of wildlife to the reserve which include Impala (*Aepyceros melampus*), Giraffes (*Giraffa camelopardalis rotschildi*), warthogs (*Phacochoerus africanus*), and Burchell's Zebras (*Equus quagga burchellii*) (Amadi *et al*., 2018).

Reptiles include White Crocodile (*Crocodyllus moreletii*), Python (*Python sabae*), Black mambas (*Dendroaspis polylepis*), Green mambas (*Dendroaspis angusticeps*), Puff adders (*Batisa rietans*), Rattle snakes (*Crotalus cerastes*), among others (Lelenguyah, 2013).

3.1.6 Human Activities

The human activities in and around the reserves include livestock rearing (sheep, goats; donkey, cattle and camels), human settlements, small scale cultivation fields, charcoal production, and artificial water provisions. Besides goat keeping (caprine -agriculture) other livestock kept includes, zebu cattle (indigenous sp.), Sahiwal breed (dual purpose), the Small East Africa sheep, donkeys and indigenous chicken and apiculture (Muriithi, 2018).

3.2 Methods

3.2.1 Evaluation of nutrient composition and tannin bio assay of mature *Acacia brevispica A. mellifera and A. tortilis* pods processed differently

3.2.1.1 Determination of Nutritive Value

One kg of ripened pods was collected from at least 7-10 trees from each of the three Acacia species and placed in cotton bags after being washed and rinsed to get rid of dust. They were oven-dried at 105^0C (CONIC 23 Litres Electric Oven-China) for 12 hours to eliminate moisture content. The samples were then ground using a laboratory grinder (ESM-2.2 Lab Basket Mill For UV Ink- China) to be pieces that could pass through 1mm sieve and then packaged into polythene bags which were air-tight and clearly labeled. 200 g samples in triplicates from each *Acacia sp*. were taken and subjected to proximate and Van Soest nutrient analysis as per AOAC procedures (Koerner *et al*., 2013). This was done at Egerton University nutritional laboratory after being subjected to the four treatments i.e. T1, (unprocessed pods) T2, (shade-dried pods) T3 (sun dried pods) and T4, (pods soaked in wood ash (alkali treatment) respectively for 48 hours. In the alkali

treatment the ratio of pods to water was 1:5 (W/V) and the ratio of wood ash to water during mixing was 200 g to 1 liter of water (W/V) respectively. After soaking, the supernatant water was decanted and samples washed twice and finally rinsed using clean water before being oven-dried (CONIC 23 Litres Electric Oven-China) in hot air oven (CONIC 23 Litres Electric Oven-China) at 50 ^{0}C for 24 hours in All the treatment samples were subjected to proximate analysis to provide results for % Organic Matter (OM), Crude protein (CP), Dry matter (DM), Neutral Detergent Fiber (NDF), Acid detergent fiber (ADF), Ether Extract (EE), Acid Detergent Lignin (ADL), ash among others.

3.2.1.2 Determination of Moisture Content

About 200 g sample in triplicates from each *Acacia sp*. were oven-dried overnight in marked dishes at 105^{0}C temperature to a constant weight and the loss in weight was recorded, then cooled to a room temperature of 24 ^{0}C in a desiccator for about 20 minutes. before the weight being recorded. About 2g of the sample (in duplicate) were placed in the dishes, and the weight of the dish sample was recorded (Y). The ceramic dishes containing the samples were placed in an oven (CONIC 23 Litres Electric Oven-China) set at 135^{0}C for 2 hours., then were removed and cooled a room temperature of 24 ^{0}C in a desiccator for about 20 minutes and weight recorded (Z). The loss in weight was calculated thus:

Dry matter% = (Z-X) -(Y-X)) ×100/wt. of sample.

3.2.1.3 Determination of Crude Protein

About 1-2g of air dry sample was weighed into Kjeldahl flasks and about2 spatula (approximately 5g) of anhydrous copper sulphate ($CuSO_4$) was added and washed down with some distilled water. 20ml of sulphuric acid (H_2SO_4) was carefully added and the flasks were placed on digestion racks. The flasks were swirled gently and heating continue for about 2 hour. The mixture was cooled to 24 ^{0}C and 200ml of distilled water added. 25 ml of boric acid was added into Erlenmeyer flask and 3-4 drops of methyl red indicator was added. The water tap to the cooling system was opened and the heaters of the distillation apparatus was switched on and 2-3 pieces of zinc mossy granules followed by 70ml of sodium hydroxide (NaOH) into Kjeldahl flasks was added. Then immediately the distillation apparatus was connected to the flask and complete mixing and distillation for about 20 minutes was run until about 100ml of the distillate collects and turn from red/pink to green, then the distillate was titrated with 0.1 ammonium sulphate (NH_4SO_4).

Calculation: The micro-Kjeldahl method was used to determine the crude protein content (CP): Nitrogen content (% N) in the sample was calculated by multiplying the % N by a conversion factor of 6.25 to obtain the % CP (Trikilidou *et al*., 2020).

3.2.1.4 Determination of Neutral Detergent Fiber

The total fiber in fibrous feed was determined using the neutral detergent fiber procedure. The NDF includes cellulose, hemicellulose and lignin as major components.

Procedure:

1-2g of air dried sample was weighed and ground to ensure they pass through 1mm mesh into a 600ml refluxing beaker. 70ml of NDS was added and the beakers placed on hot refluxing apparatus and the condenser was put in place. The mixture was heated to boiling (about 60 ^{0}C) and refluxed for 1h from the time of onset of boiling. The previously tarred crucibles (X) were placed on the filtering apparatus, and the beakers were swirled to suspend the solids and fill the crucibles, then were filtered using a low vacuum. The sample in the beaker were rinsed into the crucible with minimum hot water (100 ^{o}C) and the crucibles were filled twice with hot water and filtered again, and were repeated twice with acetone. The crucible was dried at 135^{0}C for 2h then cooled in Desiccator and weighed (Z).

%NDF=(Y-Z) ×100/wt. of sample x

3.2.1.5 Determination of Acid Detergent Fiber

The acid detergent fiber is a rapid method for determination of lignocellulose in feed stuffs and the method is also a preparatory step for lignin determination. Subsequently hemicellulose and cellulose may be determined by difference between NDF and ADF; and ADL respectively.

The reagents required included acid detergent solution (ADS), 72% sulphuric acid (H_2S0_4), Cetyl trimethlammoniumbromide (CTAB) and Acetone. A total volume of 84 ml of conc.H_2So_4 was measured and added to 2916 ml of water, then 60g of CTAB was added to the prepared H_2SO_4 solution.

Procedure:

1-2g of air-dried sample (in duplicate) was ground to enhance passing through 1mm screen into a 60ml refluxing beaker, then 100ml of acid detergent solution was added. The solution was then placed on hot Refluxing apparatus and the condenser was put in place. The mixture was heated to boiling for 5-10 minutes., then adjusted to gentle boiling, then refluxed for 1h at about 60^{0C}. The previously tarred crucible(X) was placed on the filtering apparatus and the beaker was swirled to suspend solids and to fill the crucible and low vacuum was used initially was gently increased. The sample was rinsed into crucible with minimum hot water (100^0C) The crucible was filled with twice with hot water, filtered and again filter dried with twice with acetone, then the crucible was dried at 105^0C for 2 hr. then cooled in a desiccator and weighed (Y).

Calculation:

ADF=(Y-X) ×100/wt. of sample (DM)

% Hemicellulose = NDF-ADF

3.2.1.6 Acid Detergent Lignin

Procedure:

This was a continuation of ADF analysis, where the ADF results were added to 72% H_2S0_4 and stirred with glass rode to smooth paste and left to stand for4h while being stirred every hour. The glass rode was then rinsed with 100^0C hot water together with the contents then filtered to fill the crucible to half with hot water then filtered as completely

as possible with vacuum. The contents were then dried at 105^0C for 3hr. in a muffle furnace and cooled in a Desiccator and the weight recorded (Z).

Calculation:

%ADL= (Y2-Z) ×100/wt. of sample (DM)

%Cellulose =ADF-ADL

3.2.1.7 Crude fibre

Crude fiber (CF) was determined by the use of acid detergent fiber method (ADF) and finally through alkali treatment and weighing the residue then incinerating in a muffle furnace then reweighing again and the loss in weight was the crude fiber content. EE, The Ether Extract (EE) was determined using the Soxhlet lipid extraction procedure (Koerner *et al*., 2013).

3.2.1.8 Ether Extract

In the determination of the lipid (ether extract) a sample was placed in a continuous extractor for about 16h and subjected to extraction using petroleum ether. The weight which was increased was the ether extract and was expressed as a percentage. The reagent used was the Diethyl ether and the apparatus was the Soxhlet extraction apparatus.

Several clean numbered flasks were placed in an oven at 105^0C for 2hr, then cooled in a desiccator and weight recorded (X). Then 2g of sample was weighed and placed in a folded filter paper and put in to the extractor. About 150 ml of diethyl ether was put into

half the flask and placed on the heating system which was connected to the extractor and the condenser was covered with cotton wool and water temperature was set at 70^0C. The extraction continued for 16h and the flask was then removed, covered with cheese cloth and left overnight. The flask was then placed in an oven at 105^0C for 2hr, cooled in a desiccator and weight recoded as (Y).

% EE=(Y-X) ×100/wt. of sample DM.

3.2.1.9 Ash

Ash (minerals), the mineral content (ASH) was evaluated through the method of muffle furnace by burning the sample at 550^0C to remove organic matter and the residue was the mineral content and the organic matter (OM) was obtained through the difference ie-100%-mineral matter%. The burning of the sample in a muffle furnace which was set at 550^0C gave the total mineral content.

Procedure:

Marked porcelain crucibles were placed in an oven set at 105^0C for 2h then cooled in a desiccator and weight recorded as (X). Then 2g of the sample (in duplicate) was placed in the crucible and the weight of the crucible and the sample were recorded as (Y). The crucible and the samples were placed into a muffle furnace set at 550^0C for 3hr. The furnace temperature was set at 105^0C and the crucible was left to cool to room temperature. The crucibles were weighed immediately and weights recorded as (Z).

% Ash=(Y-X) - (Z-X) ×100/wt. of sample

3.2.2 Determination of Total Extractable Phenolics (TEPH) and Total Extractable Tannins (TETs)

Extraction of tannins was by use of aqueous acetone (70%) and tannins concentrations was determined indirectly by precipitation with *polyvinylpolypyrrolidone* (PVPP). The Calorimetric method was used in determining the condensed tannins. The procedure required Acetone (70%), 700ml of acetone+300ml distilled water was taken and used as solvent, Standard tannic acid solution, 25mg of tannic acid (TA) was dissolved in 50ml of distilled water and Folin Cio-calteu reagent (IN): The procedure involved dilution of Folin reagent (2N) with an equal (v/v) amount of distilled water and then kept in refrigerator at 4^0C, maintained in golden in color and was not used when it turned olive green

Determination of TET

100mg of PVP was weighed into test tubes and about 5ml of the original extract was added to each tube and vortexed and kept in tubes in ice for 5minutes. All the tubes were vortexed and kept in ice for 5min. then repeated for 7 min and repeated once more. The tubes were centrifuged at 3000g for 10 min and the supernatant collected and 0.1ml of the extract taken into separate tubes.

Procedure: Assayed by Butanol / HCl / Fe_3+ assay (Porter *et al*., 2006).

Tannin extract (0.50ml) was pipetted in duplicate to the tubes and added to 3.0 ml of Butanol-Hcl reagent followed by 0.1ml of ferric reagent and vortexed in the tubes (Porter *et al*., 2006). The mouth of each test tube were covered with a glass Marble and the tubes

placed on a heating block adjusted at about 97^0C for 60 minutes. The tubes were then cooled and the absorbance recorded at 550nm. The condensed tannins were calculated as %DM as *leucocyanidine* equivalent using the formulae:

(A550nm ×78.26×dilution factor++) / (% DM of the sample)

3.2.3 Determination of In-vitro Organic Matter Digestibility (IVOMD)

All the samples were subjected to IVDMD test following the procedures of Tilley and Terry, and as modified by Menke and Steingas (2008). Samples were incubated in thermostatically controlled water circulating bath (Chibinga *et al*., 2016). *In-vitro* gas production procedure was used where samples were incubated in syringes containing rumen fluid for a period of time and the amount of gas produced as a result of incubation recorded. The calculated values of gas production were fitted into a model to determine the degradability of the *Acacia sp*ecies pods treated differently. The ruminal fluid was obtained by fitting a ruminal Cannula to a 3-year-old goat to obtain the rumen liquor for use in the in-vitro dry matter digestibility and gas production.

Procedure

About 200mg of the sample and its duplicates of the three *Acacia sp*ecies and from all the four treatments was weighed and transferred into the glass syringe (30 ml). The piston greased with Vaseline/pure oil was pushed down the cylinder and the rubber tube attached to the capillary were closed. Blank syringes were included with each test and standard syringes containing 200mg DM from each *Acacia sp*ecies and from each of the four treatments with known gas production. The glass syringes were placed in water bath

at 38-39^{0}C for 1h before incubation started. Five different solutions were prepared plus the required amount of media to be mixed with the rumen fluid then the solutions were stored. At about 15 minutes. Before the trial started, rumen fluid (about 250ml, as per the number of tubes to be incubated) were collected in equal proportions from the rumen fistulated donor, before the morning feed under the same feeding regime. The samples were filtered through two layers of cheese cloth into a warm flask (kept in a bucket of water at 37-38^{0}C and flushed with carbon dioxide (CO_2). The Rumen fluid and the buffer medium mixture were mixed at a ratio of 1:2, and the glass syringes was removed from water bath and the rubber tube firmly fixed on to the needle of the automatic syringe. About 30 ml of the rumen fluid/medium mixture was pipetted with an automatic syringe into each of the pre-warmed glass syringes and any air bubbles trapped in the syringes were brought to the surface by gently shaking and removing through the capillary attachment by careful upward movement of the piston, then the clip on the tube closed, then the initial volume was read and recorded as VO.

Then the syringe was placed back in the syringe rack for the incubation in the water at 38-39^{0}C and the temperature in the incubator was at 39 (+/-) 0.5^{0}C during the incubation period. Incubation continued and the immediate final reading was taken after the required period of time and the suggested incubation times was: 3, 6, 12, 24, 48, 72, and 96 hr. The position of the piston (t h) was read after incubation and recorded as intermediate volume (Vi) and the piston gently moved beforehand to make sure that it was sticking.

Calculation and result interpretation:

The gas volume recorded at different times was used to estimate the in-vitro gas production during incubation of the *Acacia sp*ecies pods. Gas production was defined as the total increase in volume minus the blank. The mean blank value was deducted from the recorded gas production of all the samples and was given the net gas production. The gas volume from the blank value deducted and related to a weight of exactly 200mg of the sample taken. As for the standards, it was determined by how far the recorded value deviates from the standard value and this was done by dividing the gas production by the recorded gas production of the standards. If the quotient was between 0.9 and 1.1, then the gas production of the samples was corrected accordingly by the factors.

The general formula for calculating the corrected gas production at time (T) was as follows:

ml/200mgDM = (XV1-30Vt final1-V0-GPO)-X200(CF)/Weight in mgDM

Where X= the number of times that the gas is released from the syringe and the volume is setback to 30ml

VO=the initial volume of the gas recorded before incubation starts

VI=the volume of the gas recorded before the gas is released from the syringe and the volume is set back to 30ml.

VT final=the final volume of the gas recorded at the end of incubation time, GPO=the mean blank value; CF=the correction factor for the standard/standards; DM=dry matter. The following model was fitted to the data:

$Y=a+b\ (1-e^{-ct})$ Where:

Y=the volume of gas produced with time (t)

A=the intercept of the gas production curve

B=the asymptote of the exponential $b\ (1-e^{-ct})$

C=the gas production curve

The (a+b) represents the potential extent of the gas production

3.2.4 Data analysis for chemical composition and tannin bioassay

The data obtained from the proximate laboratory procedures, Van Soest nutrient fractionation procedure and the Folin-Ciocalteu/ Makkar (2003) tannin bioassay laboratory procedures was fed to Microsoft Excel and subjected to ANOVA using the F-test.

The following digestibility parameters were determined:

(a) Digestibility factor (a+b)

(b) Organic matter digestibility (OMD%)

(c) Metabolizable Energy (ME) in Mcal/kg of dry matter (DM)

(d) Short Chain Fatty Acids (SCFA) composition of the forages.

Significant differences in means values from the rumen degradation experiment were analyzed using Analysis of Variance (ANOVA) and Turkeys test was used to separate the means.

3.3 Growth performance

3.3.1 Sourcing of the experimental Small East Africa Goat (SEAG)

A total of 20 bucks 4-5 months old weighing 12kg± 1.05 and of the Small East Africa Goat (SEAG) breed were purchased from goat keepers around the Radat sub location in Emining ward, Radat sub-location in Mogotio county. The bucks were blocked according to their live weights (lwt) and body conditions score (BC) and randomly allocated to five treatments (the 5^{th} treatment was a negative control), in a Randomized Complete Block design (RCBD). They were dewormed with a broad –spectrum anthelminthic-*albendazole* 2.5% according to individual animal live weight and allowed 7 days feed acclimatization period before data collection was done. There were five treatments each allocated with 4 bucks (replicates) and fed on: T1, (fresh mature green pods of *Acacia sp.* untreated (positive-control), T2- (fresh mature green *Acacia sp*. pods - shade dried for 48 hours), T3- (fresh mature green pods of *Acacia sp* pods- sun dried for 48 hours.) T4- (fresh mature green pods of *Acacia sp.* soaked in wood ash mixed with water for 48 hours, (prepared by burning each *Acacia sp*. pods and sieving to give a fine clean soluble powder (alkali treatment 3% W/W) sol. T5-(negative control-basal diet of ground Rhodes grass Hay-*Chloris gayana* mixed with wheat brand at a ratio of 3:1). All the bucks in all the treatments were fed on the same basal diet of 400grams (as supplement) to meet their nutritional requirements (Tufarelli & Laudadio, 2011).

All the bucks were given water and mineral salts *ad libidum*. The mature green *Acacia sp*ecies pods used as supplements were harvested daily by picking the pods from the fruited *Acacia sp*ecies and subjected to the five treatments then fed approximately 400 g (supplement) per buck per day in individual troughs and the refusals was collected and weighed in the next morning using a digital weigh scale before the next feed supplement was offered. Each *Acacia sp*. feeding trial experiment lasted for 3 months. Growth rate was monitored by weighing the bucks fortnightly using a digital weigh scale by hanging the well secured bucks on the scale. The data recorded was analyzed to give the average fortnight growth rate and the final weight gained (kg).

3.3.2 Data analysis for growth performance

Data obtained from feeding trials on growth performance was fed to Microsoft Excel and subjected to ANOVA test and Least Significant Difference (LSD) and Standard error of mean (SEM).

3.4 Feed intake and Feed Conversion Efficiency (FCE) of the Small East Africa Goat (SEAG) when supplemented with mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis processed* differently.

3.4.1 Feed intake of the Small East Africa Goat (SEAG)

Feed intake was obtained from daily measurements using a digital weigh scale in kiligrames of mature green pods of the *Acacia sp*ecies given to each buck as per *Acacia sp*ecies treatment and deducting from the next day's refusals in kilograms for a period of 3 months for each *Acacia sp*ecies. All goats were fed with half (0.4 kilogram) of acacia

ponds with respect to treatment. The data obtained was used to indicate average daily gain (ADG) (gm) and was calculated by dividing the average cumulative weight gained (gm) for each buck in each *Acacia sp*ecies treatment for the period of three months by the cumulative average daily feed intake (kg) for each buck for the period of 3 months as a supplement.

3.4.2 Feed Conversion Efficiency (FCE) of the Small East Africa Goat (SEAG)

Feed Conversion Efficiency (FCE) was calculated based on data collected for the period of 3 months on feed intake for each group of bucks and for each treatment as per the *Acacia sp*ecies. This was calculated by dividing the total average weight gained by bucks per treatment in (gm) by the total average feed consumed in (kg) by the bucks per treatment per each of the *Acacia sp*ecies.

Calculation:

$$\text{FCE} = \frac{\text{Total weight gained (kg)}}{\text{Total amount of feed taken (kg).}}$$

Data obtained from feeding trials on feed conversion efficiency (FCE) was fed to Microsoft Excel and subjected to analysis of variance (ANOVA) test, Least Significant Difference (LSD) and Standard error of mean SEM).

3.5 Tannin Effects in mature green pods of *Acacia brevispica, A. mellifera and A. tortilis* processed differently on organs weights of SEAG when fed as supplements

3.5.1 Procedure for slaughtering and carcass measurements

At the expiry of 3 months of the experimental period, three bucks from each treatment group and from each *Acacia sp*. pods were selected randomly and sacrificed after fasting them for a period of 12 hours and the weights were determined using a digital weigh scale in kg of the livers, kidneys, lungs with trachea and spleen then recorded and compared.

The bucks were first fasted for a period of 12 hours then their body weights recorded pre-slaughter in kg. Each buck underwent pre-slaughter examination done by a qualified meat inspector before being subjected to the normal good practices of humane killing and slaughter of meat animals as per the meat inspection ACT cap 356 No.5 of 2007 of laws of Kenya. This law requires that an animal to be slaughtered must first undergo stunning. Stunning is any mechanical, electrical, chemical or other procedure that causes immediate loss of consciousness when used. Pre slaughter stunning is aimed at reducing pain in an animal. In this study, stunning was achieved by a quick knocking of the back of the goat's head with a hammer causing the goats to fall down unconscious. The goat was then held down by two handlers and the moth was held tightly and turned backwards to stretch the neck. The slaughterer severed the throat transversely repeatedly halfway deep into the neck and bleeding was allowed until the goat was completely dead then the head was cut off.

3.5.2 Carcass yields of the SEAG fed on mature green pods of *A. brevispica, A. mellifera* and *A. tortilis* processed differently as supplements

After slaughtering, the bucks were subjected to pre-slaughter examination by a qualified meat inspector before being subjected to the normal good practices of humane killing and slaughter of meat animals as per the meat inspection ACT cap 356 no5 of 2007 of laws of Kenya. The carcasses were weighed and compared and carcass yield were calculated as follows:

$$\text{Carcass yield} = \frac{\text{weight after removal of head, limbs, skin, offal's, lungs with trachea, organs and testis}}{\text{The slaughter weight (kg) (pre-slaughter weight)}} \times 100$$

Data analysis for organs weights and carcass yield: The data obtained from the organs and systems weights and carcass yields was fed to Microsoft Excel and subjected to analysis of variance (ANOVA).

3.6 Isolation and quantification of rumen cellulolytic bacteria

3.6.1 Serial dilution and bacterial enumeration

The goat rumen contents were sampled aseptically using sterile universal bottles with use of a cannula and stored in an ice box under 4^{o}C before processing in the laboratory within 24 hours of collection. In the laboratory, 1gram of the homogenized (mixed content from each replicates) gut sample was transferred immediately to a conical flask containing 150 ml of normal saline (Needham *et al*., 2022). The mixture (approximately 200 g) was stirred with a Teflon-coated magnetic bar for 15 minutes. The gut suspension was serially

diluted with 1ml of the gut suspension added to 9 ml test tube of normal saline. Dilution ratios include: 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. These preparations were vortexed at approximately 150 g for 1 minute and 1ml of aliquots was rapidly transferred to other 9 ml tubes. For plate count experiments, 200 µl aliquots from the last two dilutions was transferred to petri dishes containing molten tryptone soy agar and swirled around to mix the media and sample in triplicates. This was followed by incubation at 37 ^{o}C for 24 hours and calculated using the formula below. Sub culturing was done on tryptone soy agar plates to isolate pure cultures.

$$\text{CFU ml}^{-1} = \frac{\text{CFU per plate} \times \text{dilution factor}}{\text{volume of sample taken (ml)}}$$

Experiment one – lay out for quantification of Rumen cellulolytic bacteria using Colony Forming Units (CFUs)

3.6.2 Data analysis for quantification of rumen cellulolytic bacteria

The data obtained from the rumen fluids for rumen cellulolytic bacteria isolation was quantified using colony forming units (CFU).

CHAPTER FOUR

RESULTS

4.1 Nutrient composition and tannin bioassay of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

4.1.1 Nutrient composition of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

Nutrient composition of mature green pods of *A. brevispica, A. mellifera*, and *A. tortilis* pods processed differently was assessed. For percentage moisture content, *A. mellifera* (9.03±0.57) had the highest percentage followed by *A. tortilis* (4.53±0.14) while *A. brevispica* (3.28±0.21) had the lowest with a significant difference among the species ($F_{0.05\ (2\ ,3)} = 141.83$, p=0.0011). Similarly, *A. mellifera* possessed the highest average crude fiber (30.08±0.16) followed by *A. brevispica* (28.88±0.69) with a significantly difference ($F_{0.05\ (2\ ,3)} = 126.83$, p=0.0013) from that of *A. tortilis* (23.78±0.18). *A. tortilis* (4.73±0.77) and *A. brevispica* (4.13±0.14) had the lowest percentage of ash significantly different from that of *A. brevispica* ($F_{0.05\ (2\ ,3)} = 16.33$, p=0.0244). *A. mellifera* had the highest percentage of crude protein (20.71±0.15) when compared with the other *Acacia sp*ecies ($F_{0.05\ (2\ ,3)} = 376.29$, p=0.0003). There was no significant difference in ADF (g/Kg) among all *Acacia sp*ecies studied ($F_{0.05\ (2\ ,3)} = 0.72$, p=0.5563) as illustrated in Table 4.1. Both ADL and NDF in g/Kg were significantly high in *A. brevispica* ($p<0.05$) while both *A. mellifera* and *A. tortilis* had significantly same amounts of ADL and NDF as illustrated in Table 4.1.

Table 4.1: Nutrient composition of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

	A. brevispica	*A. mellifera*	*A. tortilis*	F-Ratio	p-value
Moisture content (%)	3.28± 0.21a	9.03± 0.57b	4.53± 0.14a	141.83	0.0011
Crude fiber (g/Kg)	28.88± 0.69a	30.08± 0.16b	23.78± 0.18a	126.83	0.0013
Ash (%)	4.13± 0.14a	6.83± 0.35b	4.73± 0.77a	16.33	0.0244
Crude Protein (%)	9.36± 0.31a	20.71± 0.15b	9.88± 0.73a	376.29	0.0003
ADF (g/Kg)	34.66± 2.44a	38.17± 3.66a	35.50± 2.97a	0.72	0.5563
ADL (g/Kg)	32.40± 0.66b	28.63± 0.48a	29.81± 0.74a	18.37	0.0207
NDF (g/Kg)	57.00± 2.50b	47.57± 1.05a	44.50± 0.61a	32.86	0.0091

Means followed with the same letter in the same row are insignificantly different ($p<0.05$)

4.1.2 Tannin bioassay of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

Total Extractable Tannins (TETs) in mg/g DM did not differ within *Acacia sp*ecies for alkali, sun dried, and shade dried and control treatments. Similarly, condensed tannins (mg/g DM) as well as Total Extractable Tannins (TETs) (mg/g DM) did not differ within did not differ within *Acacia sp*ecies for alkali, sun dried, shade dried and control treatments as illustrated in Table 4.2. There was a significant difference in Hydrolysable Tannins levels in mg/g DM among and within the treatment as illustrated Table 4.2.

Total Extractable Tannins (TETs) (mg/g DM) for *A. brevis pica* and *A. mellifera* differed significantly with treatment ($p<0.05$) while *A. tortilis* did not ($p>0.05$) as illustrated in Table 4.2. Hydrolysable Tannins in mg/g DM differed significantly with treatments for all *Acacia sp*ecies ($p<0.05$).

Table 4.2: Tannin bioassay of mature green pods of *A. brevispica, A. mellifera* and *A. tortilis* pods processed differently

Bioassay	Species	Alkali	Sun dried	Shade dried	Control	F-Ratio	p-value
Total Extractable Tannins (TETs) (mg/g DM)	*A. brevis pica*	9.59± 0.23a*	11.47± 0.21a*	9.546± 0.13a*	18..32± 0.11a*	1.06	0.4592
	A. mellifera	8.22± 0.18a*	7.72± 0.10a*	6.51± 0.16a**	9.87± 0.08a**	1.15	0.4320
	A. tortilis	11.03± 0.04a*	11.05± 0.10a*	11.51± 0.04a*	12.24± 0.11a*	0.28	0.8397
Hydrolysable Tannins (mg/g DM)	*A. brevispica*	146.48± 16.30a*	154.83± 14.71a*	139.57± 12.03a*	173.09± 17.23b*	0.9	0.0221
	A. mellifera	53.91± 7.94a**	76.08± 9.32a**	61.76± 7.99a**	39.88± 8.80a**	1.02	0.0438
	A. tortilis	40.23± 6.90a**	39.71± 9.27a***	27.16± 3.19a***	44.45± 6.20a**	0.29	0.0381
Condensed tannins (mg/g DM)	*A. brevispica*	473.58± 46.30a*	454.50± 42.73a*	444.40± 31.03a*	445.05± 27.42a*	0.8	0.5399
	A. mellifera	454.83± 42.94a*	453.45± 29.55a*	462.65± 21.99a*	439.00± 25.80a*	1.07	0.4496
	A. tortilis	480.71± 22.90a*	439.85± 28.27a*	435.65± 32.19a*	436.45± 31.20a*	0.32	0.8221

Means with different alphabets in a row differ significantly ($p<0.05$). Means with different numbers of asterisks in a column differ significantly ($p<0.05$).

4.1.3 Tannin absorbance and transmission of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

Determination of the relative concentration of a tannin from mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently was assessed. All the assessed *Acacia sp*ecies had tannins with similar absorbance as well as transmission as illustrated in Table 4.3 ($p>0.05$). For alkali treatment, species did not differ in absorbency influenced by tannin as well as in transmission ($p>0.05$). Similarly, Sun dried ($p>0.05$), Shade dried ($p>0.05$) and Control processed species (*A. brevispica, A. mellifera,* and *A. tortilis* pods) ($p>0.05$) did not influence tannins absorbency and transmission among the *Acacia sp*ecies.

Table 4.3: Tannin bioassay of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

Bioassay	Species	Alkali	Sun dried	Shade dried	Control	F-Ratio	p-value
Absorbance	*A. brevispica*	0.39± 0.27a	0.64± 0.24a	0.36± 0.11a	0.35± 0.07a	1.06	0.4592
	A. mellifera	0.35± 0.12a	0.28± 0.08a	0.22± 0.15a	0.41± 0.07a	1.15	0.432
	A. tortilis	0.43± 0.03a	0.41± 0.09a	0.45± 0.03a	0.44± 0.01a	0.28	0.8397
Transmission	*A. brevispica*	45.00± 26.30a	24.50± 12.73a	44.40± 11.03a	45.05± 7.42a	0.8	0.5544
	A. mellifera	45.85± 12.94a	53.45± 9.55a	62.65± 21.99a	39.00± 5.80a	1.07	0.4572
	A. tortilis	37.65± 2.90a	39.85± 8.27a	35.65± 2.19a	36.45± 1.20a	0.32	0.8106

Means followed with the same letter in the same row are insignificantly different ($p<0.05$).

4.2 Effects of tannins in mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently on in-vitro organic matter digestibility (IOMD) and growth performance when used as supplements on growing SEAG

Effects of tannins in mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently on in-vitro organic matter digestibility (IOMD) and growth performance was assessed on the amount of gas produced per minutes. In *A. brevispica,* treatment with alkali resulted in better digestibility portraying significant difference in all time periods. The same is slightly reflected in *A. mellifera*. However, *A. brevispica* under the shade dried had the poorest performance. In *A. tortilis* there was no significant differences among the treatments except with the negative control.

In alkali processed species of plants, *Acacia brevispica*, *A. mellifera* and *A. tortilis* portrayed a significant increase in gas (CO_2) production in-vitro organic matter digestibility (IOMD) with time ($p<0.05$). In *Acacia. brevispica*, gas produced increased by 0.24 ml per week with an equation of; *A. brevispica* = 36.56 + 0.238*week with a significant difference ($\beta=0.23$, $R^2= 83.07\%$, $F_{0.05\,(1,\,7)} = 34.37$, $p=0.0006$). In *A. mellifera*, gas produced increased by 0.40 ml per week with an equation of *A. mellifera* = 44.9317 + 0.4041*week with a significant difference ($\beta=0.4041$, $R^2= 82.83\%$, $F_{0.05\,(1\,,7)} = 33.78$, $p=0.0007$). In *A. tortilis*, gas produced increased by 0.50 ml per week with an equation of; *A. tortilis* = 45.3889 + 0.5055*week with a significant difference ($\beta=0.5055$, $R^2= 80.72\%$, $F_{0.05\,(1,\,7)} = 29.31$, $p=0.0010$) as portrayed in Figure 4.1.

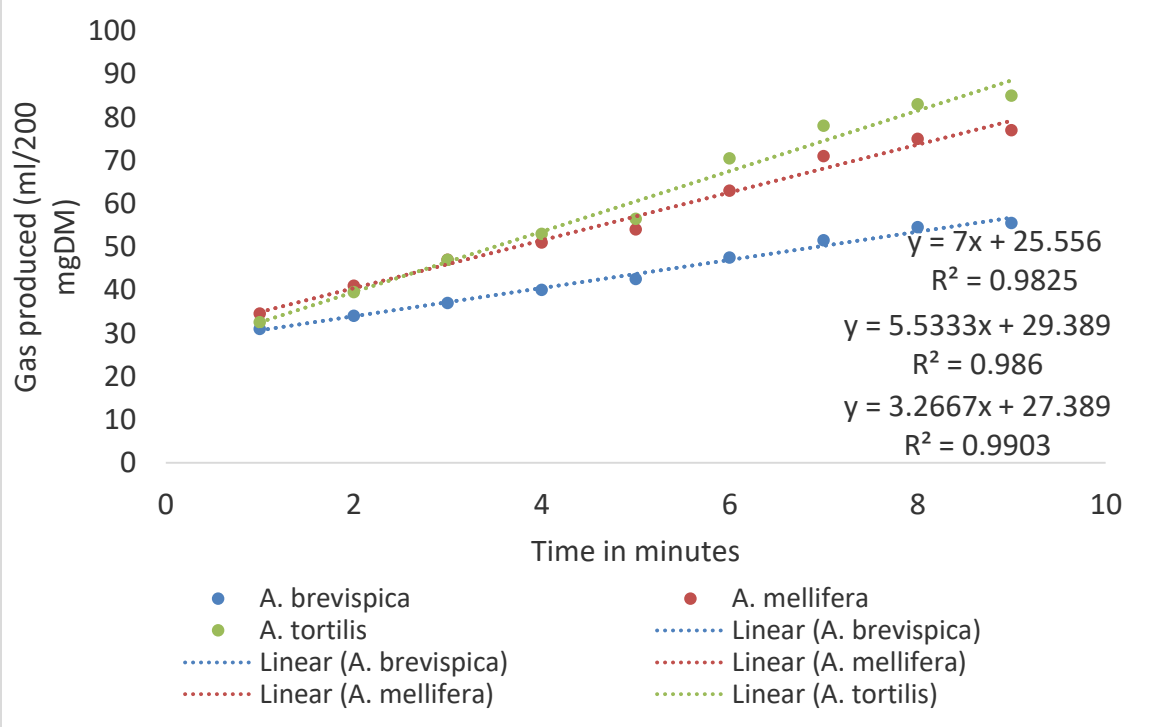


Figure 4.1: Graph illustrating gas production by *Acacia* species pods under alkali treatment

In sun dried, *Acacia brevispica, A. mellifera* and *A. tortilis* showed a significant increase in gas production in-vitro organic matter digestibility (IOMD) with time ($p<0.05$). In *Acacia. brevispica,* gas produced increased by 0.56 ml per week with an equation of; *A. brevispica* = 47.0603 + 0.560952*week with a significant difference ($\beta=0.5609$, $R^2=$ 81.53%, $F_{0.05\,(1\,,7)} = 30.92$, $p=0.0009$). In *A. mellifera,* gas produced increased by 0.45 ml per week with an equation of; *A. mellifera* = 50.2254 + 0.4462*week with a significant difference ($\beta=0.4462$, $R^2=$ 76.61%, $F_{0.05\,(1\,,7)} = 22.92$, $p=0.0020$). In *A. tortilis,* gas produced increased by 0.51 ml per week with an equation of; *A. tortilis* = 49.2683 + 0.5058*week with a significant difference ($\beta=0.5055$, $R^2=$ 81.57%, $F_{0.05\,(1\,,7)} = 30.98$, $p=0.0008$) as portrayed in Figure 4.2.

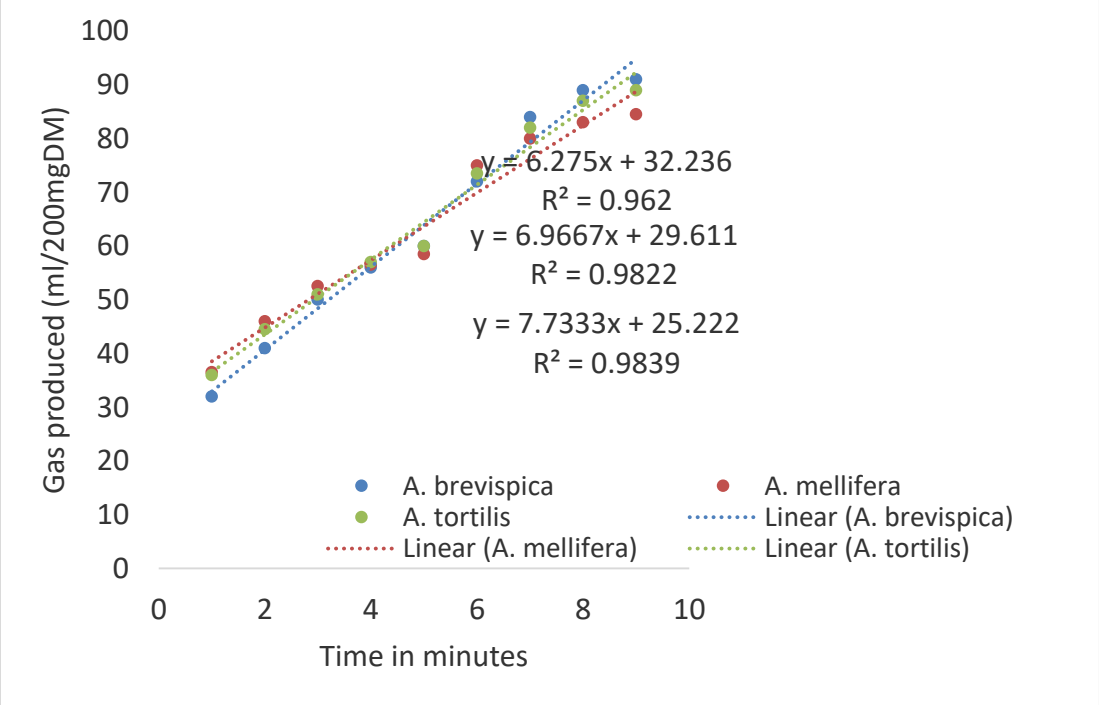


Figure 4.2: Graph illustrating gas production by sun dried *Acacia* species pods

In shade dried, *Acacia brevispica*, *A. mellifera* and *A. tortilis* showed a significant increase in gas production in-vitro organic matter digestibility (IOMD) with time ($p<0.05$). In *Acacia. brevispica,* gas produced increased by 0.58 ml per week with an equation of; *A. brevispica* = 42.9508 + 0.5757*week with a significant difference ($\beta=0.5757$, $R^2= 86.44\%$, $F_{0.05\ (1\ ,7)} = 44.62$, $p=0.0003$). In *A. mellifera*, gas produced increased by 0.37 ml per week with an equation of; *A. mellifera* = 42.7032 + 0.3729*week with a significant difference ($\beta=0.3729$, $R^2= 77.97\%$, $F_{0.05\ (1\ ,7)} = 24.78$, $p=0.0016$). In *A. tortilis,* gas produced increased by 0.40 ml per week with an equation of; *A. tortilis* = 50.3762 + 0.4041*week with a significant difference ($\beta=0.4041$, $R^2= 69.01\%$, $F_{0.05\ (1\ ,7)} = 24.78$, $p=0.0016$) as portrayed in Figure 4.3.

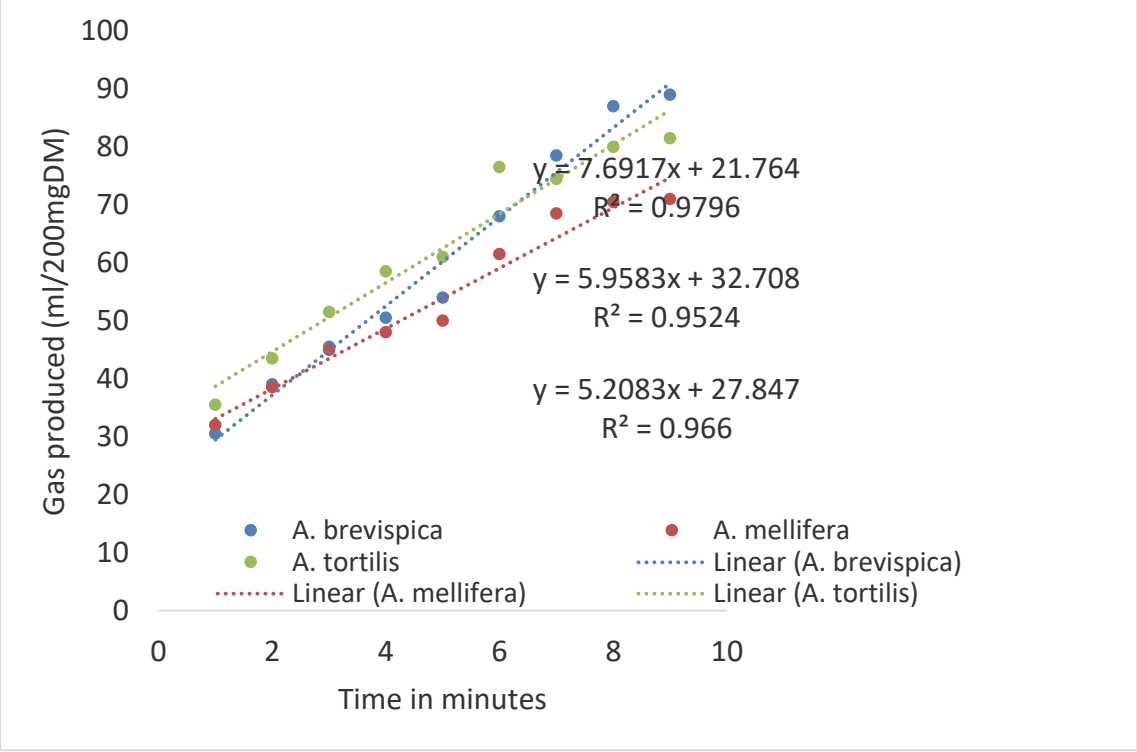


Figure 4.3: Graph illustrating gas production by shade-dried *Acacia* species pods

In control processed, *Acacia brevispica, A. mellifera* and *A. tortilis* showed a significant increase in gas production in-vitro organic matter digestibility (IOMD) with time ($p<0.05$). In *Acacia brevispica*, gas produced increased by 0.52 ml per week with an equation of; *A. brevispica* = 39.5286 + 0.5157*week with a significant difference ($\beta=0.5157$, $R^2= 86.57\%$, $F_{0.05\ (1\ ,7)} = 45.15$, $p=0.0003$). In *A. mellifera*, gas produced increased by 0.36 ml per week with an equation of; *A. mellifera* = 39.6794 + 0.3625*week with a significant difference ($\beta=0.3625$, $R^2= 77.97\%$, $F_{0.05\ (1,\ 7)} = 32.18$, $p=0.0080$). In *A. tortilis*, gas produced increased by 0.47 ml per week with an equation of; *A. tortilis* = 42.0175 + 0.4679*week with a significant difference ($\beta=0.4679$, $R^2= 82.19\%$, $F_{0.05\ (1,\ 7)} = 32.29$, $p=0.0007$) as portrayed in Figure 4.4.

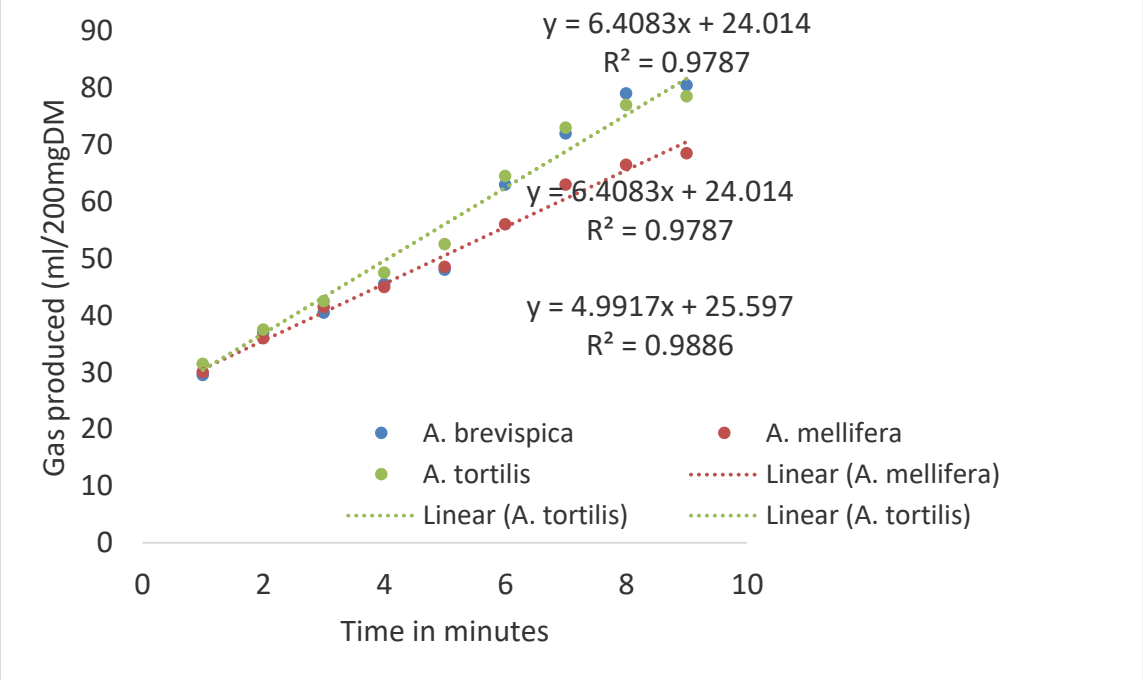


Figure 4.4: Graph illustrating gas production by *Acacia sp*ecies pods under control experiment

4.3 Feed intake and Feed Conversion Efficiency (FCE) of the SEAG fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

4.3.1 Mean daily Acacia pods (supplement) intake (g)

Average mean daily feed (supplements) intake for the entire period was assessed. Between the *Acacia sp*ecies, all supplements processed in alkaline were ingested in large amounts with a significant difference with other treatments ($p<0.05$). *A. tortilis* pods processed differently as supplements were taken in large amounts when processed in alkaline (416.50±6.50), sun dried (305.25±15.19), shade dried (259.96±4.23) and control 1 (194.42±6.17) compared with the others ($F_{0.05\,(2,\,15)}$ =11.04, p=0.0012). For *A. mellifera* pods high amount ingested were processed in alkaline (371.71±8.71) and sun dried (276.42±8.13) ($F_{0.05\,(2,\,15)}$ = 4.09, p=0.0302) while for *A. brevispica* pods processed in alkaline (397.46±3.46), sun dried (281.29±11.77) and shade dried (250.92±8.66) were taken in high amounts compared with the others ($F_{0.05\,(2,\,15)}$ =11.04, p=0.0012).

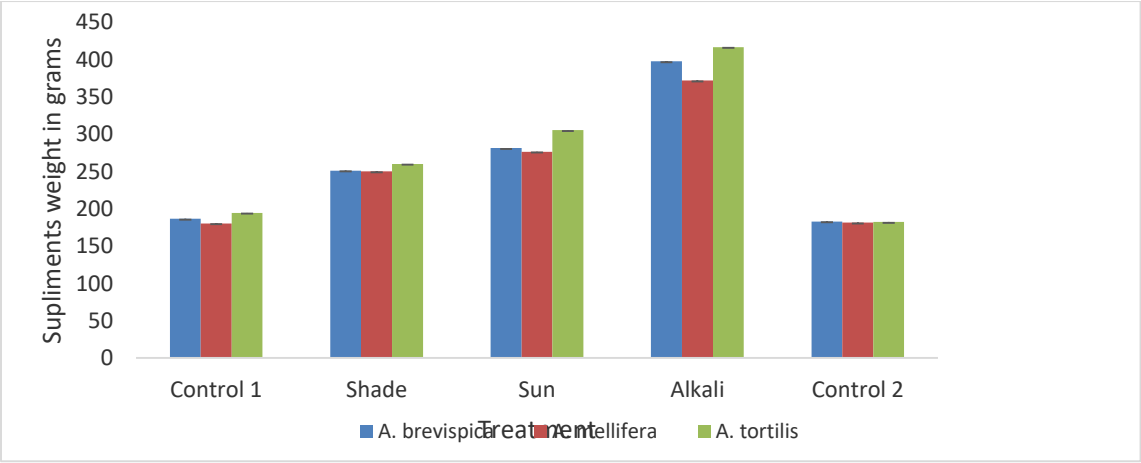


Figure 4.5: Mean daily feed (supplement) intake

4.3.2 Average feed taken for the entire period

Average feed intake in grams was computed for three months between the treatments. For control 1, more of *A. tortilis* supplement (17497.53±55.56) was taken in as compared with *A. brevispica* (16781.22±54.42) and *A. mellifera* (16211.25±26.65) with a significant difference ($F_{0.05\ (2,\ 15)} = 11.06$, p=0.0011). There was no significant difference between *A. brevispica* and *A. mellifera* supplements taken in (p>0.05). In shade dried, *A. tortilis* supplement (25396.22±38.06) was taken in high level as compared to others with a significant difference ($F_{0.05\ (2,\ 15)} = 4.43$, p=0.0008) with no significant difference between amount taken in *A. brevispica* and *A. mellifera*. Similarly, in sun dried treated *A. tortilis* were taken in in larger amounts as compared with the others with a significant difference ($F_{0.05\ (2,\ 15)} = 11.76$, p=0.0008). Within treatments, there was a significant difference between control (16781.22±54.42) and shade dried (23596.22±38.06), sun dried (25777.53±28.13) alkaline treated (35771.22±37.12) treated *A. brevispica* as well as between Alkali and all other treatments Similar trend was followed in different treatments of *A. mellifera* and *A. tortilis* as illustrated in Table 4.4.

Table 4.4: Average Feed taken for the entire period in grams

	A. brevispica	*A. mellifera*	*A. tortilis*	F-ratio	p-value
Control 1	16781.22± 54.42a*	16211.25± 26.65a*	17497.53± 55.56b*	11.06	0.0011
Shade dried	23596.22± 38.06a**	22582.53± 77.95a**	25396.22± 38.06b**	4.43	0.0008
Sun dried	25777.53± 28.13a**	25316.28± 15.94a**	27472.50± 136.70b**	11.76	0.0008
Alkali	35771.22± 37.12a***	34353.72± 33.37a***	36585.00± 38.50b***	12.78	0.0006

Means followed by different letters in the same row are significantly different. Means followed with the same number of asterisk (*) in the same column are insignificantly different (p<0.05).

4.3.3 Initial and final mean body weight(kg)

Initial weight of the SEAG did not differ prior to feeding with supplements ($p>0.05$). There was a great variation in final weights after feeding with supplements treated differently. For control, Shade dried, Sun dried and Alkali treatment, *A. tortilis* ponds resulted to the highest weight of 14.20±0.36 kg significantly different from the others *Acacia sp*ecies ($F_{0.05\ (2,\ 15)} = 6.28$, $p=0.0196$). There was a significant difference in treatment within a species as illustrated in Table 4.5.

Table 4.4: Initial and final mean body weight (kg)

	Treatment	*A. brevispica*	*A. mellifera*	*A. tortilis*	F-ratio	p-Value
Initial weight (kg)	Control 1	12.63± 0.48a	12.38± 0.95a	12.58± 0.56a	0.15	0.8658
	Shade dried	12.05± 0.50a	12.30± 1.29a	12.63± 0.85a	0.38	0.6963
	Sun dried	12.31± 0.48a	12.23± 0.48a	12.75± 0.65a	1.10	0.3746
	alkali	12.63± 0.48a	12.63± 0.48a	12.90± 0.64a	0.35	0.7141
	Control 2	12.00± 1.29a	12.14± 0.74a	12.10± 0.74a	0.02	0.9778
Final Weight (Kg)	Control 1	13.64± 0.71a	13.47± 1.30b	13.59± 0.36a*	6.28	0.0196
	Shade dried	13.07± 0.61a	13.41± 0.47b	13.78± 0.72c*	9.21	0.0066
	Sun dried	13.47± 0.47a	13.27± 0.48a	14.01± 0.22b	9.06	0.007
	Alkali	13.81± 0.47a*	13.72± 0.47a*	14.07± 0.34b*	9.22	0.0066
	Control 2	13.00± 1.30a*	13.15± 0.74a	13.18± 0.74a	0.93	0.429

Means followed by different letters in the same row are significantly different. Means followed with the same number of asterisk (*) in the same column are insignificantly different ($p<0.05$).

4.3.4 Average Daily Gain (ADG) in g

Average daily gain (ADG) (g/d) of the goats fed with differently processed mature green pods of *Acacia sp*. was assessed. Bucks fed on *Acacia tortilis*- alkali-treated-pods resulted in best Average Daily Gain (ADG) of 15.03 KG ±2.01 gm followed by on *A. brevispica* (14.13±1.94) with a significant difference with that of *A. mellifera* ($p<0.05$). Control 1, shade dried and control 2 treatments resulted in insignificant average daily gain among and within all *Acacia sp*ecies tested as illustrated in Figure 4.6.

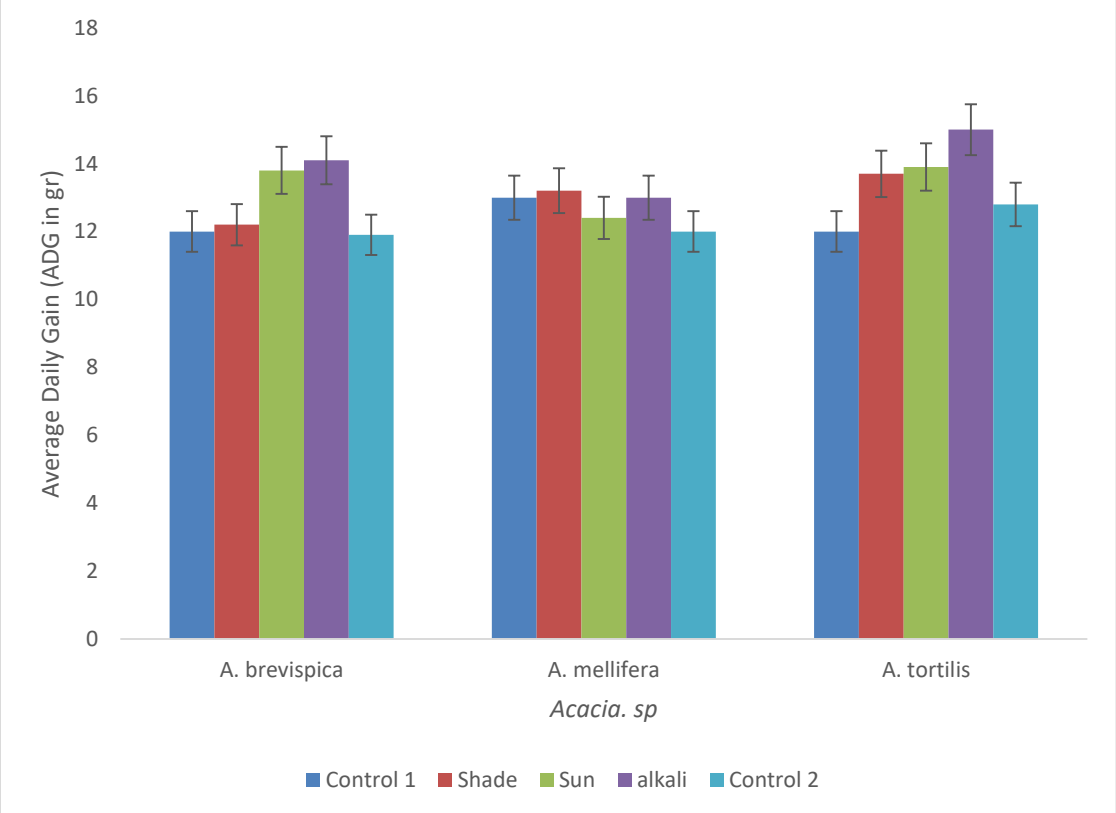


Figure 4.6: Average Daily Gain (ADG) in g of the goats fed with differently processed mature green pods of *Acacia sp.*

4.3.5 Feed Conversion Efficiency (FCE)

There was a significant difference in food conversion ration within the *Acacia sp*ecies pods treated differently. High feed conversion efficiency was recorded in *Acacia tortilis* treated in alkali as compared to other treatments as illustrated in Figure 4.7.

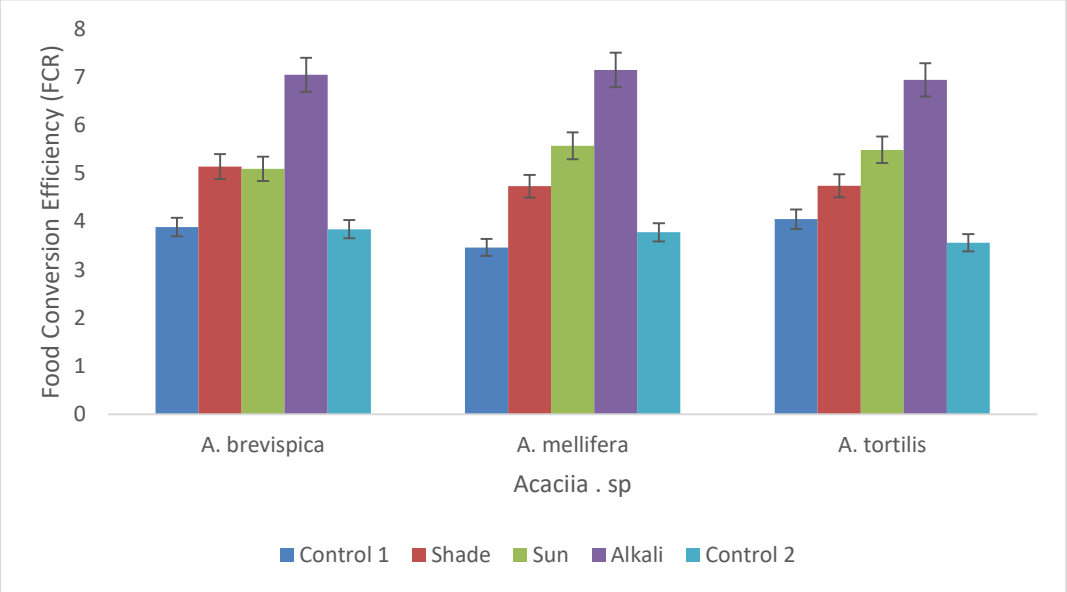


Figure 4.7: Feed conversion efficiency of the goats fed with differently processed mature green pods of *Acacia sp.*

4.4 Tannin effects on carcass yields and organs weights of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

4.4.1 Pre -slaughter weight in kg of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

Pre-slaughter weight (kg) for goats fed with *A. mellifera* under control experiment was (13.07± 2.07) with no significant difference with other species ($F_{0.05\ (4,\ 10)} = 1.18$, p= 0.3764). SEAG fed on mature green pods of *A. mellifera*, and *A. tortilis* dried under the shade dried resulted to a higher pre-slaughter weight of 13.23± 0.57 and 13.37± 0.51 respectively with no significant difference ($F_{0.05\ (4,\ 10)} = 1.16$, p= 0.2346). Feeding the SEAG Green pods of *A. mellifera* processed by drying under the sun dried resulted to a lower insignificant pre slaughter weight (p>0.05 with others as portrayed in Figure 4.8. In alkali and control 2 processed mature green pods of *A. tortilis*, fed to SEAG resulted to pre- slaughter weight insignificantly high than in *Acacia. Brevispica* and *A. mellifera* species.

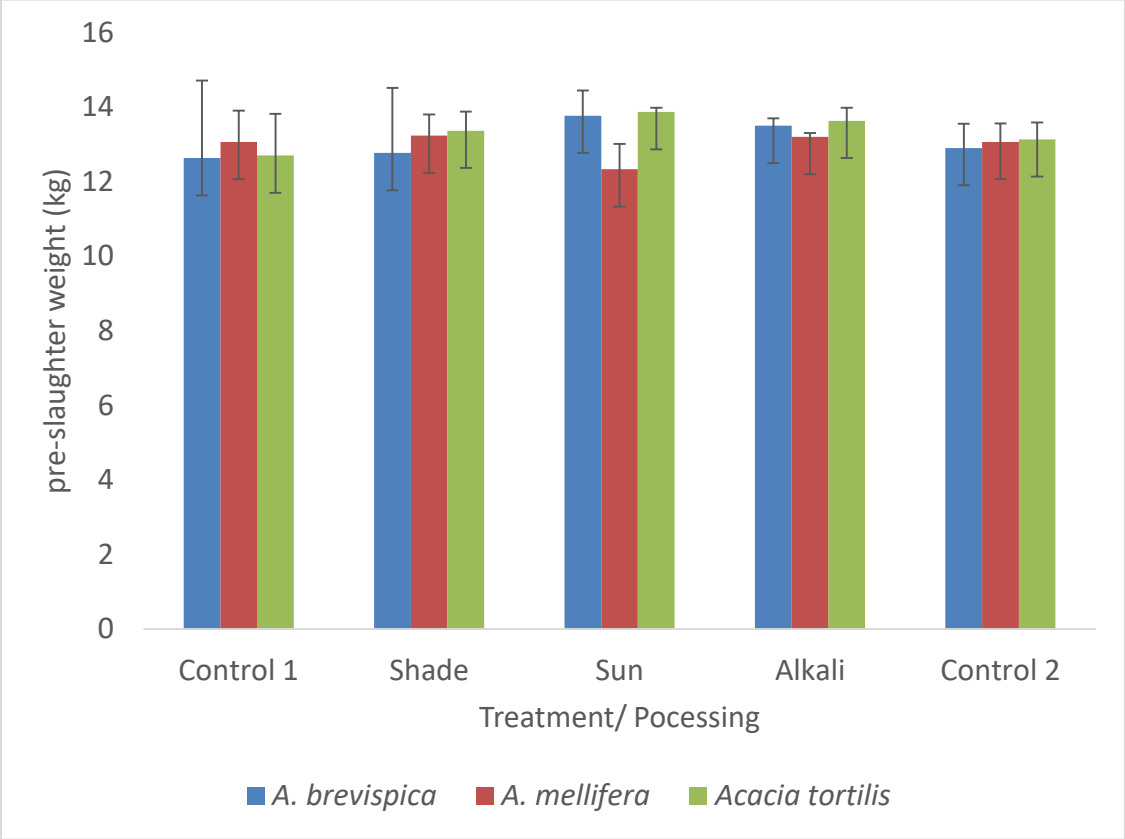


Figure 4.8: Pre slaughter weight in kg of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

4.4.2 Post- slaughter weight in kg of (SEAG) fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

Post -slaughter weight of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements did not differ significantly ($p>0.05$). Post -slaughter weight for goats fed with *A. tortilis* processed in control 1 (5.07±0.32) and shade dried (5.30±0.52) were insignificantly low ($p>0.05$) while in alkali was insignificantly higher (6.30±0.61) when compared with other treatments as portrayed in Figure 4.9. Forage feed of mature green pods of *Acacia brevispica,* resulted to a high

insignificant (p>0.05) post- slaughter weight processed in Control 1 (6.27±0.46), Shade (6.00±1.00), sun dried (5.83±0.76) and Control 2 (5.50±0.44) portrayed in Figure 4.9.

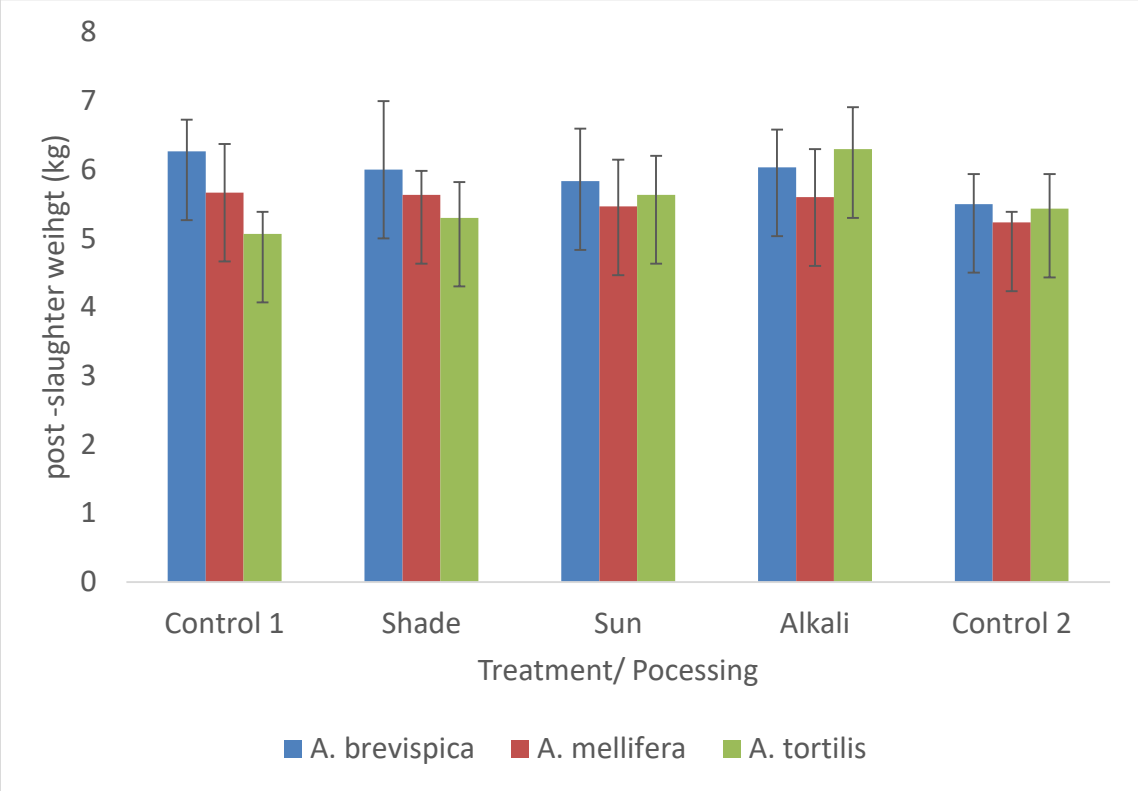


Figure 4.9: Post -slaughter weight in kg of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements.

4.4.3 Killing- out percentage of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements

Killing-out percentage of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements did not differ significantly (p>0.05). Killing-out percentage for goats fed with *A. tortilis* processed in control 1 (60.01±2.50), shade dried (60.34±3.77) and sun dried (59.39±3.87) were insignificantly

higher (p>0.05) while in alkali was insignificantly lower (53.84± 3.43), when compared with other treatments as portrayed in Figure 4.10. Killing-out percentage for goats fed with mature green pods of *Acacia. brevispica,* was insignificantly low in Control 1 (49.75±6.46), Shade dried (53.13±1.42) and Control 2 (57.40±1.47) processed (p>0.05) as portrayed in Figure 4.10.

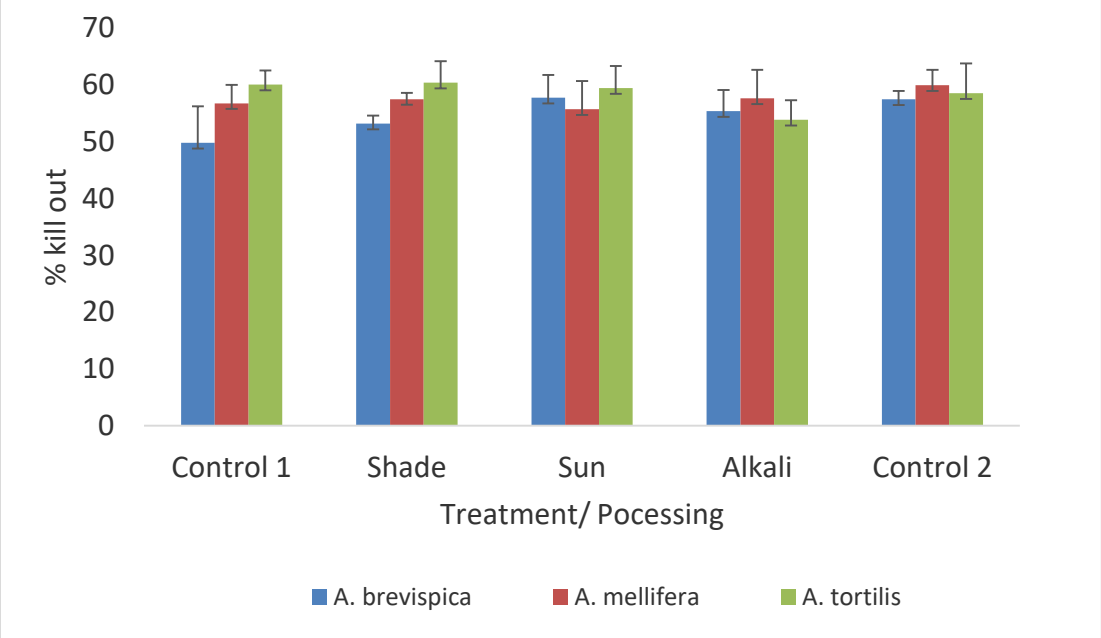


Figure 4.10: Killing- out percentage of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis processed* differently as supplements

4.4.4 Organs of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements

Effects of mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently and fed as supplements on organs of SEAG was assessed. Feed supplement from *A. mellifera* led to a higher average liver weight in control 1 (213.33±15.28)

significantly different with others ($F_{0.05\,(4,\,10)} = 16.00$, p= 0.0039). In shade processed, *A. brevispica* and *A. mellifera* resulted to in significantly similar liver weight but significantly different to that of *A. tortilis* ($F_{0.05\,(4,\,10)} = 57.00$, p= 0.0001). Similar trend was observed for supplements processed under the sun dried, alkali and control 2 as illustrated in Table 4.6. Liver weights resulting from feed supplements of *A. brevispica, A. mellifera* and *A. tortilis* processed differently did not differ (p>0.05). Processing method of supplements did not influence the average kidney weight (p>0.05). *A. brevispica* and *A. mellifera* supplements resulted to the highest average lungs weight when compared with *A. tortilis* supplements. Processing method of supplements did not influence the average kidney weight (p>0.05). *A. mellifera* supplements resulted to the highest average spleen weight (*spleenomegaly*) when compared with *A. brevispica* and *A. tortilis* supplements and can be attributed to the high tannins level in *A. mellifera.*

Table 4.6: Organs of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* processed differently as supplements

Organ	Treatment	*A. brevispica*	*A. mellifera*	*A. tortilis*	F-Ratio	p-value
Liver	Control 1	173.33± 5.77a*	213.33± 15.28b	173.33± 5.77a	16	0.0039
	Shade dried	236.67± 15.28a**	246.67± 5.77a	166.67± 5.77b	57	0.0001
	Sun dried	240.00± 10.00a**	253.33± 5.77a	166.67± 5.77b	117.6	0.00001
	Alkali	246.67± 5.77a**	260.00± 10.00a	166.67± 5.77	137.6	0.00001
	Control 2	33.33 5.77	26.67 15.28	106.67 5.77	59.11	0.0001
Kidney	Control 1	43.33± 11.55a*	110.00± 10.00b	30.00± 10.00a	49.6	0.0002
	Shade dried	43.33± 5.77a*	106.67± 5.77b	43.33± 5.77a	120.33	0.00001
	Sun dried	36.67± 5.77a*	103.33± 5.77b	36.67± 5.77a	133.33	0.00001
	Alkali	33.33± 5.77a**	103.33± 5.77b	43.33± 5.77a	129	0.00001

	Control 2	33.33 5.77a**	106.67 5.77c	26.67 15.28b	59.11	0.0001
Lungs	Control 1	166.67± 5.77a*	166.67± 5.77a	103.33± 5.77b	120.33	0.00001
	Shade dried	166.67± 5.77a*	176.67± 5.77a	103.33± 5.77b	142.33	0.00001
	Sun dried	146.67± 5.77a**	166.67± 15.28b	103.33± 5.77c	31.44	0.0007
	Alkali	153.33± 5.77a**	173.33± 5.77b	103.33± 5.77c	117	0.00001
	Control 2	156.67 5.77	153.33 5.77	103.33 5.77	80.33	0.00001
Spleen	Control 1	103.33± 5.77a*	426.67± 25.17b	103.33± 5.77a	448.05	0.00001
	Shade dried	103.33± 5.77a*	436.67± 11.55b	103.33± 5.77a	1666.67	0.00001
	Sun dried	103.33± 5.77a	450.00± 20.00b	103.33± 5.77a	772.57	0.00001
	Alkali	100.00± 0.00a	330.00± 26.46b	103.33± 5.77a	213.32	0.00001
	Control 2	103.33 5.77	286.67 32.15	103.33 5.77	91.67	0.00001

Means followed with the same letter in the same row are insignificantly different (0.05)

4.5 The effects of *Acacia brevispica, A. mellifera,* and *A. tortilis* tannins processed differently on rumen cellulolytic bacteria- quantification and characterization, on growing SEAG

4.5.1 Rumen cellulolytic bacteria quantification on growing (SEAG)

Average microbial counts were assessed to determine effects of *A. brevispica*, *A. mellifera*, and *A. tortilis* tannins processed differently on rumen cellulolytic bacteria-quantification on growing SEAG. Processing method of *A. brevispica* influenced average microbial counts (Cfus/ml) with sun dried (431.50± 1.41), shade dried (417.00± 26.16), and alkaline treated (417.00± 40.31) in decreasing order while Control 1 (181.00± 39.60) ($F_{0.05\ (4,\ 5)} = 31.71$, p= 0.0010) as portrayed in Figure 4.11. Processing method of *A. mellifera* influenced average microbial counts (Cfus/ml) with alkaline treated (463.00± 28.28), sun dried (428.00± 11.41) and shade (418.00± 62.23) having the largest microbial

counts while Control 1 (209.50± 53.03) and Control 2 (245.00± 55.15) had the lowest bacterial count with a significant difference ($F_{0.05\,(4,\,5)} = 12.87$, p= 0.0076) as portrayed in Figure 4.11. Processing method of *A. tortilis* influenced average microbial counts (Cfus/ml) with alkaline treated (462.00± 11.31), sun dried (452.50± 10.61) and shade dried (390.00± 69.30) having the largest microbial counts while Control 1 (166.50± 65.76) and Control 2 (241.00± 28.28) had the lowest bacterial count with a significant difference ($F_{0.05\,(4,\,5)} = 17.19$, p= 0.0040) as portrayed in Figure 4.11. Average microbial counts (Cfus/ml) did not differ among the feed supplements species (p>0.05).

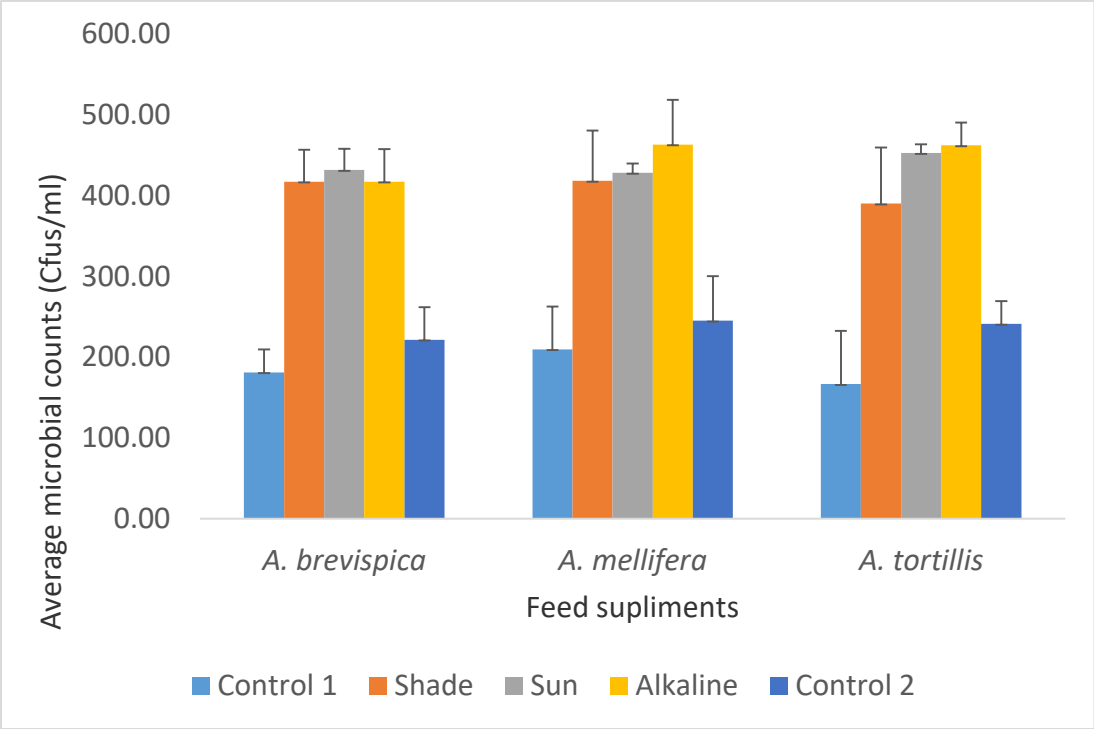


Figure 4.11: The effects of *Acacia brevispica, A. mellifera* and *A. tortilis* tannins processed differently on rumen cellulolytic bacteria- quantification and characterization, on growing SEAG

4.5.2 Microbiological assessment

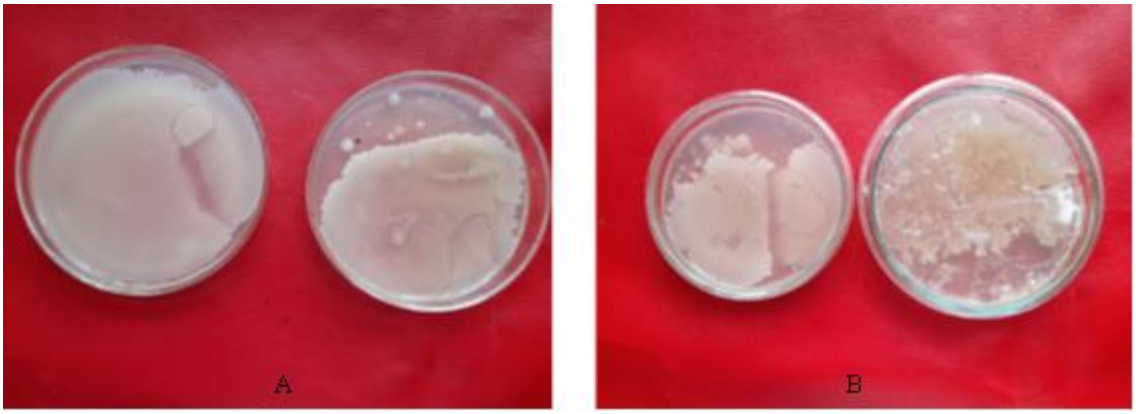


Plate 4.1: Some bacterial cells spread very fast outgrowing the rest of the cells. The other cells can however be seen within the larger colony while in B: Some fast-growing colonies engulf smaller colonies.

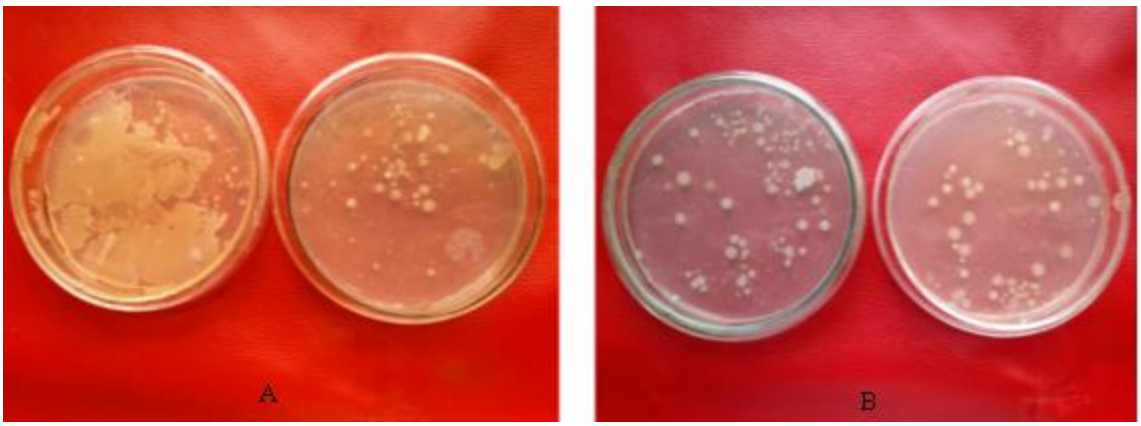


Plate 4.2: Distinct colonies can be seen form the petri dishes.

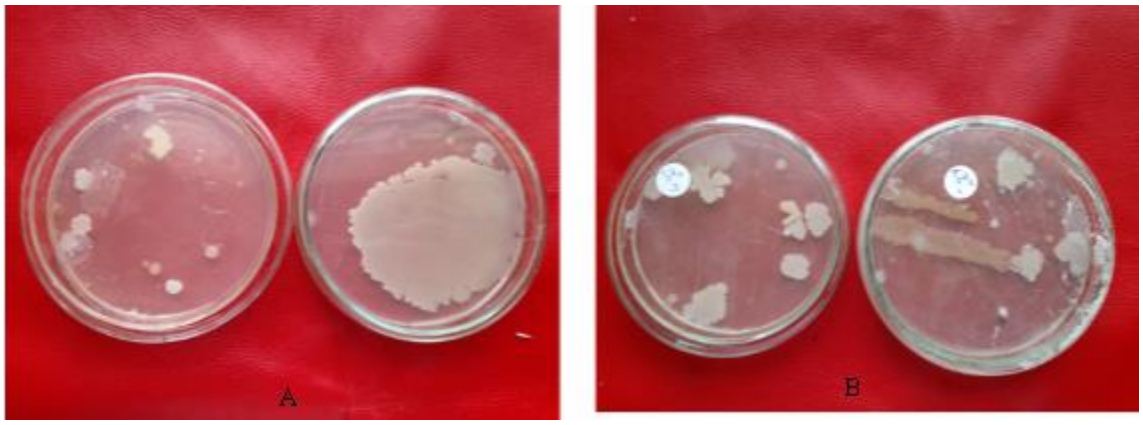


Plate 4.3: Colonies with different growth forms and colors can be seen

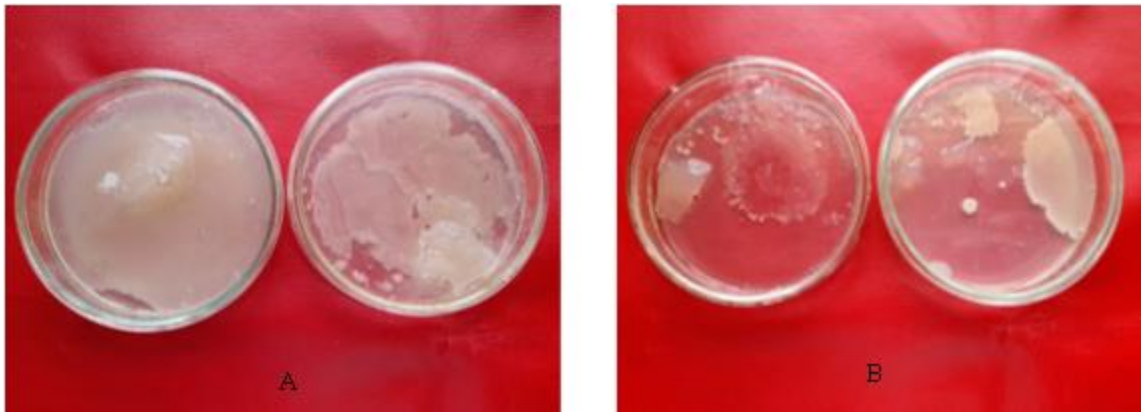


Plate 4.4: Another petri dish illustrating cells with different growth rates with the fast growing surrounding the slow growing cells. Many colonies can however be seen growing within the large colonies. T4 had the highest number of colonies Colonies displaying different forms of growth.

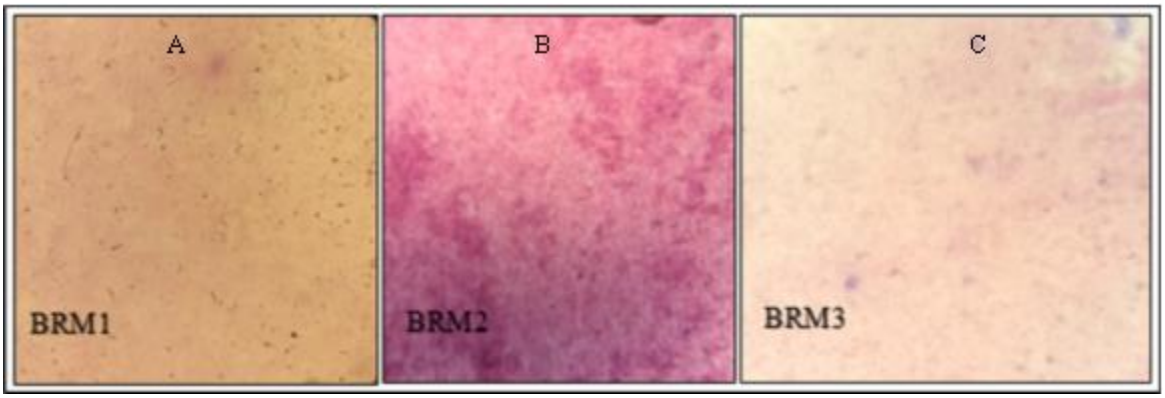


Plate 4.5: Micrographs illustrating the gram reaction of the three dominant bacterial colonies. Though the micrographs did not fully capture the cell morphology the general pink appearance hints to gram negative cells.

CHAPTER FIVE

DISCUSSION

5.1 Nutrient composition and tannin bioassay of mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* pods processed differently

5.1.1 Nutrient composition of mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently

Results established that the nutrient composition of mature green pods of *A. brevispica, A. mellifera*, and *A. tortilis* pods processed differently differed among and within the species. From the results, *A. mellifera* had the highest percentage moisture content, average crude fiber, percentage of ash, crude protein, ADL and NDF when compared with the other *Acacia sp*ecies. This may be due to their adaptability differences in their habitats. The findings are in line with those of Hlatini & Chimonyo (2016) that the crude protein content of *Acacia mellifera* and *A. tortilis* have a range of between 10-15%. Other studies have demonstrated that most *Acacia sp*. have crude protein ranging from 12-18% on DM basis. Similarly, Ndamitso *et al*. (2017) added that *Acacia mellifera* pulps (pods without seeds), have more proteins in comparison with other *Acacia sp*ecies. They also have more fiber (ADF) of 21% at the 17-21% DM basis, but the seeds have more protein at 19%, and crude fiber at 29% on DM basis respectively. According to Melesse *et al*. (2019) there are recognized variations in nutritional composition and anti-nutritional factors of the leaves and the pods of *Acacia mellifera* and the comparative tannin levels in pods, leaves, bark, and twigs respectively.

The findings concur with those of Dambe *et al*. (2015) who established that *Acacia mellifera* pods processed differently had nutritive value of between 96.13% and 98.12% dry matter and between 10.13% and 13.25% of crude protein when processed differently and fed to Red Sokoto goat.

5.1.2 Tannin bioassay of mature green pods of *Acacia brevispica, A. mellifera,* and *A. tortilis* pods processed differently

The condensed tannins have their pre-dilection sites in many plant species for example they are found only in the white clovers' flowers and in Lucerne legume seed coat (Hemingway & Karchesy, 2012). Research established no significant difference in absorbance and transmission strength of tannin from mature green pods of *A. brevispica, A. mellifera,* and *A. tortilis* pods processed differently. The findings are in line with those of Bayssa (2016) who highlighted in his report that crude protein levels in forages treated with alkali did not show much difference but the condensed tannins in leaves treated with Ca $(OH)_2$ at 3% (W/W) depicted significant reduction of total phenolics (TP), total tannins (TT), total condensed tannins (CT). The findings are not in agreement with Hemingway & Karchesy (2012). who indicated that different treatments especially heat treatment could result in the condensed tannins to complex covalently with forage compounds and lower the levels of free CT which could bind ruminal proteins.

5.2 Effects of tannins in mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently on in-vitro organic matter digestibility (IOMD) and growth performance when used as supplements on growing SEAG

The rumen cellulolytic bacteria in the rumen fluid used in the fermentation of the differently processed *Acacia sp*ecies pods utilizes their bacterial enzymes to degrade the organic matter to yield the end products of fermentation such as the Short Chain Fatty Acids (SCFAs) and carbon dioxide (CO_2). However, high tannin concentration leads to tannin-protein complexation, hence inhibiting protein degradation by bacterial enzymes. The results in this current study indicate a negative correlation between the amount of HTs and the volume of gas produced.

In this study, the aforesaid assertion is corroborated by the apparent relationship between the amount of Hydrolysable tannins (HTs) and the volume of gas produced during the digestibility test. *A. tortilis* produced a high gas production as compared with *A. mellifera* and *A. brevispica*. The low gas production in *A. mellifera* may be associated with the *Delphinident / Cyanidine* ratio (D/C), as different tannin monomers which differ in ratios and concentrations with the different *Acacia sp*ecies (Bayssa *et al*., 2021). These findings lend credence to the significance and the negative effects of tannins present in unprocessed mature green pods of *Acacia sp*ecies.

The alkali processed pods for the three *Acacia sp*ecies gave the best results in the digestibility experiment. Alkali method works through a pH. mediated chemical (oxidation) reaction that leads to the neutralization of the acidic tannins by the basic alkali, resulting in the release of the bound proteins to undergo enzymatic fermentation

by the rumen cellulolytic bacteria to yield gases. The resultant increase in the production of gas can be taken as an indicator of the biological anti nutritive action of the tannins. Organic matter in-vitro digestibility (OMD) of *Acacia mellifera* treated with PEG and Sodium hydroxide (NaOH) and incubated for 24 hr. period showed significant increase in digestibility. The use of wood ash to reduce tannins in *Acacia spp.* resulted in better digestibility of the forage in sheep concurring with Tshabalala, Sikosana, & Chivandi (2013). Some communities have used wood ash to alleviate excess tannins in sorghum and millet used by man and the use of wood ash being a less expensive option has the potential in the reduction of tannins in forages, but requires more validation by animal experiments (Mlambo & Mapiye, 2015). This study has tried to validate this point through the use of field feeding trials on live animals given acacia pods processed differently amongst which was alkali treatment which gave the best digestibility results.

Sun drying was very close to alkali processing among the processing methods tested but shade -drying and the control (un- processed pods) gave lower gas production results suggesting that the efficacy of these methods is less than the alkali and the sun drying methods. The composition of mainly crude fiber, crude protein and tannins chemical composition in terms of condensed and hydrolysable tannins and their monomers, could be attributed to the differences in fermentation and the rates of gas production (digestibility). This is in agreement with the findings of Smeriglio *et al*. (2017), who reported the use of wood ash to reduce tannins in *Acacia cyanophylla* resulted in better digestibility of the forage in sheep.

Mlambo and Mapiye (2015) indicated that condensed tannins (CT), have the tendency to lower the palatability and nutritive value of the *Acacia mellifera.* The in-vitro digestibility results in the current study depicting significant difference in tanniniferous *Acacia sp*ecies in East Africa regions which agrees with those recorded by Kandie *et al* (2020). According to Bayssa (2016), condensed tannins in leaves treated with the Alkali-Ca $(OH)_2$ at 3% (W/W) showed significant reduction of total tannins, total *phenolics* and total condensed tannins.

Sun drying of pods to reduce tannins is based on the principle that the ultraviolet sun dried rays transfixes the tannins onto the plant cell walls making them unavailable to form complexes with plant proteins hence freeing proteins to undergo enzymatic digestion. In this study, sun drying performed almost at same level with the use of alkali. The major challenge in the use of sun drying method is that in practical nutrition in Kenya's pastoralist ASAL, Acacia pods are used when they have over-dried. This results in a higher buildup of Acid Detergent Fiber (ADF) and lignin Detergent Fiber (LDF) which has low digestibility. On the contrary, treating acacia pods with alkali, whether at early green stage as was the case in this study, or at late dry stage as is the practice by the pastoralists, will have best results in tannin reduction regardless of the levels of ADF and LDF already present in the pods.

In this study, all the processing methods except shade drying and the control (unprocessed pods) had significant increase in gas yield. It has been said that the occurrence of some condensed tannins in forages resulted in reduced protein digestibility hence lowered free amino acids and reduced conversion of this acids into the branched –

chain volatile fatty acids (VFAs) preferred by bacteria as growth-promotors and microbial protein synthesis (Costa *et al.*, 2018). It has also been reported that there is negative digestion co-efficient for neutral detergent insoluble nitrogen (NDIN) and also acid detergent lignin (ADL), due to the complexation of soluble tannins with the plant cell wall components. This results in the complexes being rendered indigestible or inaccessible by bacterial digestive enzymes according Gemede & Ratta (2014). It has been proposed that in-vitro carbohydrate and dry Matter degradability has a negative correlation with hydrolysable and the condensed tannins level by Chingala (2018).

The presence of tannins in feeds reduces the nutritional value by binding with proteins rendering proteins unavailable for microbial degradation. The higher the cellulose, hemicellulose and lignin in a plant, the lower the digestibility. Most plants with anti-nutritional factors (ANF) like tannins binds with dietary proteins hence rendering the proteins unavailable for digestion by the ruminal micro-flora (Katunga *et al.*, 2020). They also affect palatability, voluntary feed intake and digestibility and N retention in ruminants (Chingala, 2018). It has been demonstrated that tannins lower organic matter and crude fiber digestibility and in-vivo studies have shown that protein degradability is significantly reduced when tannin rich forages are part of the diet. It has been proposed that in-vitro carbohydrate and Dry Matter degradability has a negative correlation with hydrolysable and the condensed tannins levels (Chingala 2018; Katunga *et al.*, 2020). This is in agreement with the findings of this study.

5.3 Feed intake and feed conversion efficiency (FCE) of the SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements

Tannin production and protein precipitation differs with plant species, parts of the plant, different time on the same species and different environments. Studies have demonstrated that the availability of protein in *Acacia sp*. to ruminal microbes is interfered with by tannins hence affecting dry matter intake (DM). The carbon-nutrient hypothesis posits that the formation of tannins is due to the hunting of the primary metabolites like carbon –skeletons, during unfavorable periods like when there is poor nutrient levels and growth is hampered. It has also been observed that some tannins depict characteristics of both hydrolysable tannin (HT) (Patra & Saxena, 2011). Condensed tannins can be either cis or Trans based on the orientation of the functional group located on the C-3 and C-4 positions relative to Benzene ring, and this affects their capacity to form complexes with proteins, carbohydrates, lipids and minerals depending on the pH of the rumen environment (Piluzza, Sulas & Bullitta, 2014).

Average mean daily feed (supplements) intake for the entire period was assessed. Between the *Acacia sp*ecies, all supplements processed in alkaline were ingested in large amounts with a significant difference with others treatments. *A. tortilis* pods processed differently as supplements were taken in large amounts when processed in alkaline compared with the others. This is attributed to low levels of tannin as compared to *A. mellifera* and *Acacia. brevispica* pods. Condensed tannins are often present in leguminous tropical forages more so those found in arid zones and acidic soils. The levels of the polyphenolic compounds in the pods (pulp and seeds) of *A. mellifera* and *Acacia.*

brevispica have lower palatability and nutritive value. *Acacia mellifera* leaves have more total extractable tannins at 25% and 5% condensed tannins (CT), (>60g/kg digestible matter) and these phenolics have the tendency to lower the species palatability and nutritive value (Mlambo and Mapiye 2015). According to Katanga (2015), the raised contents of condensed tannins cause reduced forage degradability, low growth rate and palatability.

The findings are in line with those of Mlambo and Mapiye (2015) but are not in line with those of Katanga (2015) that pods of *Acacia mellifera* have the least levels of tannins. The findings are in line with those of Kremp *et al*. (2012) who posited that phenolic compound levels in leguminous plants differently affects animals fed on such plants as protein supplements and there is need to pinpoint the particular compounds that causes lowered palatability intake and digestibility. It has been reported that the section of diet, protective responses of animals and the interactions of fed diet components and the rumen microbes' digestive enzymes, significantly influence the degree of nutritional effects of tannins.

Treatment or processing method also influence palatability of the species pods. This is attributed to the fact that environmental effects like temperature and nutrient stress affects tannin levels and certain plants reacts to browsing by ruminants through quick rise in condensed tannin levels (Tadele, 2015). This is in agreement with the findings in this current study where feed intake and fed conversion efficiency varied significantly ($p \leq 0.05$). Low feed conversion ratio was recorded in *Acacia tortilis* treated in alkali resulting in the highest feed conversion efficiency (FCE). Research has established that

treatment of tanniniferous forages with alkalis reduces their total extractable tannins and phenols and /or condensed tannins levels significantly. The reduction is achieved through conversion of tannins to quinones which have no capacity to form complexes with proteins and increases crude protein content and NDF digestibility (MacAdam & Villalba, 2015).

The presence of tannins in feeds reduces the nutritional value by binding with proteins rendering proteins unavailable for microbial degradation (Mlambo and Mapiye, 2015). However, this largely depends on the type of tannins and it is perceived that hydrolysable tannins (HT) are more hazardous than condensed tannins (CT) and it is said that (CT) have less harm when less than 5% of the dry matter in the feed, and that there is a negative correlation between (CT) levels in forages (>50GCT/kg DM) and their palatability, voluntary feed intake, digestibility and N retention in ruminants. Also, goats and sheep fed on diets of *Calliandra callothyrsus* and *Desmodium intortum* and legumes possessing 9.5g/kg and 22.5g/kg of condensed tannins respectively resulted in over 21% more Nitrogen reaching the abomasum when compared to the ones fed on tannin-free diet which directly affects food conversion efficiency (Herremans *et al*., 2020).

Duvaux-Ponter (2017) reported that goats fed high levels of tannins resulted in clinical manifestation of lethal poisoning like abortion, ruminal atony, and hyper glycaemia. Condensed dietary tannin concentrations of less than about 100g/kg DM in the diet may improve ruminant performance, and that dietary tannins concentration of 20-45 g/kg DM in the diet increased Nitrogen efficiency resulting in increased daily weight gain in lambs feed on temperate fresh forages e.g.; *Lotus cornicalatus*, (Min 2013). Other studies have

demonstrated that the optimal balance between positive and negative effects of condensed tannins was observed in sheep when the dietary concentration was 3-4%, and it has been proposed that tannin –rich legumes should be used to enhance by-pass protein and boost ruminant performance (Crawford *et al*., 2020). Many alternative hypotheses regarding the "bypass protein" which elucidates the impact of *proanthocyanidines* on protein degradation and utilization by the ruminants are in agreement with other studies on legume forages (Addisu, 2016). Livestock which eats diets having (>5% w/v tannin) level normally develop negative nitrogen balance, reduced feed digestibility and animal performance.

The findings concur with those of Lawa *et al*. (2017) who found a conversion efficiency of 6.37kg of feed to 1 kg of meat in Awassi male ramp lambs subjected to a feed containing 40% Acacia leaves resulted in a feed conversion efficiency (FCE) of 6 body weight gain. However, these results were comparable to the ones recorded in another finding where a concentrate ration was used resulting in a FCE of 6.55. This finding agrees with those of this study where the food conversion efficiency (FCE) for the best performing tannins reducing method (alkali) was 7.00.

5.4 Tannin effects on carcass yields and organs weights of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements

Effects of mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently and fed as supplements on organs of SEAG was assessed. Feed supplement from *A. mellifera* led to a higher average liver weight significantly different with others

This can be attributed to the negative effects of high tannins levels as is the case in A. mellifera. This caused Hepatomegaly (enlarged liver). Pre-slaughter weight (kg) for goats fed with *A. mellifera* processed in control experiment did no significant difference with those fed with other *Acacia sp*ecies. SEAG fed on mature green pods of *A. mellifera,* and *A. tortilis* dried under the shade resulted to a higher pre-slaughter weight with no significant difference. Similarly, post slaughter weight and killing-out percentage of SEAG fed on mature green pods of *Acacia brevispica, A. mellifera* and *A. tortilis* processed differently as supplements did not differ significantly. Killing-out percentage for goats fed with *A. tortilis* processed in control 1, shade and sun dried were insignificantly higher while in alkali was insignificantly lower when compared with other treatments. The findings are in line with those of Mousa *et al*. (2011) on fat-tailed Awassi ram lambs in North Sinai (Egypt) fed on a ration with 40% *Acacia sp*ecies pods which reported higher averages of fasting weights, hot carcass and cold-dressed weights as compared to other rations but with no significant difference among them.

5.5 The effects of Acacia *brevispica, A. mellifera,* and *A. tortilis* tannins processed differently on rumen cellulolytic bacteria- quantification and characterization, on growing SEAG

From the findings, processing method of *A. brevispica* influenced average microbial counts in terms of colony forming units (Cfus/ml) with shade, sun dried and alkali in increasing order. Alkali processing method resulted to the largest microbial counts while Control 1 and Control 2 (negative control on basal diet with no tannins diet supplements) had the lowest bacterial count. Processing method directly correlate with microbial counts as it reduced the tannin levels with example in alkali treatment. This is in line with

findings of Wallace (2014) that levels of condensed tannins could affect the performance of the growth of microbes. In his findings, protozoa multiplied more in the rumen of sheep fed on *L. corniculatus* forage mixed with PEG to cancel tannins effects, while condensed tannins in the forage - *Quebracho* lowered the quantity of protozoa in rumen simulation experiments.

Similarly, Solís-Dominguez *et al*. (2012) indicated that processing method of Acacia species influenced average microbial counts (Cfus/ml) similar with the findings of this study where alkaline treated, sun dried and shade in decreasing order, processing resulted to the largest microbial counts. The findings are in line with those of Mlambo and Mapiye (2015), that processing methods of *Acacia sp*ecies used as supplements influence the rumen cellulolytic bacteria- quantification and characterization on growing small ruminal animals such as goats and sheep. In addition, Ribeiro *et al*. (2016) indicated that ruminant animals cannot fully digest fibrous forages owing to cell walls of polysaccharides due to both physical and chemical agents found in the ingested forage, and also by the retention time limits on the intake material within the rumen, notwithstanding other feed compounds e.g. *phenolics, saponins*, *silica* and *lignin* which possess inhibitory effects on cellulolytic action. Selzer, (2017) observed that plant cuticle resistance to microbial digestive enzymes could be improved by forage mastication and pre-treatment of forages so as to reduce their negative effects on digestion.

A study by Jones *et al*. (2004), found that the bacteria *Prevotella ruminocola* had the least effects even with raising amounts of cell-associated condensed tannins in the culture, and that the bacteria *Streptococcus bovis* was very sensitive to condensed tannins

and showed lowered cell multiplication and changed cellular morphology in the presence of 500ugmL-1. Tannins concentration. This may explain why the presence of gram positive bacteria as can be seen in plate 5, was not evident, unlike the gram negative bacilli which picked the Gram –stain.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Acacia tortilis had the lowest tannins concentration among the three species hence least effect on nutrient bio- availability. In-vitro Organic Matter Digestibility and growth performance of goats was highest in the goats supplemented with alkali-processed mature green pods of *acacia tortilis.*

Feed intake and Feed Conversion Efficiency was highest in goats supplemented with alkali-processed mature green pods of *Acacia tortilis*, with a food conversion efficiency of 7:1, comparable to that of conventional feed supplements. Bucks fed on *Acacia tortilis* alkali-treated-pods resulted in best average final weight of 15.03±2.01.

Pre-slaughter weights did not vary with different processing methods but it varied with post –slaughter weights with the alkali processed *Acacia tortilis* pods supplements giving the best post slaughter weight (kg) and killing out percentage.

As far as average microbial counts were concerned, processing method influenced average microbial counts (Cfus/ml) with shade, sun dried and alkaline treated in ascending order resulting to the largest microbial counts. Alkali processing method gave the best results in almost all the parameters under study.

As far as average microbial counts were concerned, processing method influenced average microbial counts (Cfus/ml) with shade, sun dried and alkaline treated in ascending order resulting to the largest microbial counts. Alkali processing method gave the best results in almost all the parameters under study.

6.2 Recommendations

Acacia tortilis should be planted under commercial establishment by the pastoralists in the Arid and Semi-Arid (ASAL) zones of Kenya.

The alkali method of reducing tannins in Acacia species pods should be adopted in order to reduce the negative effects of tannins in tanniniferous forages.

A further study to develop the rumen bacterial isolates to be undertaken to establish novel rumen cellulolytic bacteria that can be incorporated in ruminant supplementation value-chain and can improve on ruminant productivity.

REFERENCE

Abdel-Shafy, H. I., & Mansour, M. S. (2018). Solid waste issue: Sources, composition, disposal, recycling, and valorization. *Egyptian journal of petroleum*, *27*(4), 1275-1290.

Abd'Quadri-Abojukoro, A. N. (2021). *Effects of selected medicinal plant extracts on bacterial activity, cytotoxicity, rumen fermentation, nutrient utilization, and methane mitigation* (Doctoral dissertation).

Addisu, S. (2016). Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. *Online J. Anim. Feed Res*, *6*(2), 45-56.

Agassiz, D. J. L., & Bidzilya, O. V. (2016). Gelechiidae (Lepidoptera) bred from acacia in Kenya with description of eight new species. *Annals of the Ditsong National Museum of Natural History*, *6*(07), 116-145.

Alders, R. G., Campbell, A., Costa, R., Guèye, E. F., Ahasanul Hoque, M., Perezgrovas-Garza, R., ... & Wingett, K. (2021). Livestock across the world: diverse animal species with complex roles in human societies and ecosystem services. *Animal Frontiers*, *11*(5), 20-29.

Amadi, J. A., Olago, D. O., Ong'amo, G. O., Oriaso, S. O., Nanyingi, M., Nyamongo, I. K., & Estambale, B. B. (2018). Sensitivity of vegetation to climate variability and its implications for malaria risk in Baringo, Kenya. *PLoS One*, *13*(7), e0199357.

Asmare, B. (2014). Biotechnological advances for animal nutrition and feed improvement. *World Journal of Agricultural Research*, *2*(3), 115-118.

Assogbadjo, A. E., Kakaï, R. G., Vodouhê, F. G., Djagoun, C. A. M. S., Codjia, J. T. C., & Sinsin, B. (2012). Biodiversity and socioeconomic factors supporting farmers' choice of wild edible trees in the agroforestry systems of Benin (West Africa). *Forest Policy and Economics*, *14*(1), 41-49.

Avornyo, F. K., Partey, S. T., Zougmoré, R. B., Asare, S., Agbolosu, A. A., Akufo, N. M., ... & Konlan, S. P. (2020). In vivo digestibility of six selected fodder species by goats in northern Ghana. *Tropical Animal Health and Production*, *52*(2), 473-480.

Bayssa, M., Negesse, T., & Tolera, A. (2016). Leaf biomass yield, chemical composition, in vitro gas and methane production and rumen degradation characteristics of some woody plant species in afar rangeland of north eastern Ethiopia. *Middle-East J. Scientific Res*, *24*(4), 1252-1265.

Bayssa, M., Yigrem, S., Betsha, S., & Tolera, A. (2021). Production, reproduction and some adaptation characteristics of Boran cattle breed under changing climate: A systematic review and meta-analysis. *PloS one*, *16*(5), e0244836.

Behnke, R. H., & Nakirya, M. (2012). The contribution of livestock to the Ugandan economy. *IGAD LPI Working Paper 02-12*.

Belay, D., Getachew, E., Azage, T., & Hegde, B. H. (2013). Farmersâ€™ perceived livestock production constraints in Ginchi watershed area: Result of participatory rural appraisal. *International Journal of livestock production*, *4*(8), 128-134.

Bhat, T. K., Kannan, A., Singh, B., & Sharma, O. P. (2013). Value addition of feed and fodder by alleviating the antinutritional effects of tannins. *Agricultural Research*, *2*(3), 189-206.

Bhat, T. K., Kannan, A., Singh, B., & Sharma, O. P. (2013). Value addition of feed and fodder by alleviating the antinutritional effects of tannins. *Agricultural Research*, *2*(3), 189-206.

Bompadre, T. F. V., Neto, O. B., Mendonca, A. N., Souza, S. F., Oliveira, D., Fernandes, M. H. M. R., ... & Teixeira, I. A. M. A. (2014). Energy requirements in early life are similar for male and female goat kids. *Asian-Australasian Journal of Animal Sciences*, *27*(12), 1712.

Boudet, A. M. (2012). Polyphenols: From plant adaptation to useful chemical resources. *Recent advances in polyphenol research*, *3*, 41-70.

Brewbaker, J. L., & Hutton, E. M. (2019). Leucaena: versatile tropical tree legume. In *New agricultural crops* (pp. 207-259). CRC Press.

Castillo-Martínez, J. C., Martínez-Castañón, G. A., Martínez-Gutierrez, F., Zavala-Alonso, N. V., Patiño-Marín, N., Niño-Martinez, N., ... & Cabral-Romero, C. (2015). Antibacterial and antibiofilm activities of the photothermal therapy using gold nanorods against seven different bacterial strains. *Journal of Nanomaterials*, *2015*.

Chen, H. (2014). Chemical composition and structure of natural lignocellulose. In *Biotechnology of lignocellulose* (pp. 25-71). Springer, Dordrecht.

Chibinga, O. C., Nyangito, M. M., Musimba, N. R., Simbaya, J., & Daura, M. T. (2016). Effect of coppicing levels on the re-growth of Julbernardia globiflora. *Livestock Research for Rural Development*, *28*(3).

Chingala, G. (2018). Beef production and quality of Malawi Zebu steers fed diets containing rangeland-based protein sources under feedlot conditions. *PhD (Animal Science) thesis, University of Stellenbosch, South Africa*.

Chingala, G., Raffrenato, E., Dzama, K., Hoffman, L. C., & Mapiye, C. (2019). Intake, digestibility, rumen protein synthesis, and growth performance of Malawi Zebu steers fed diets containing rangeland-based protein sources. *Tropical animal health and production*, *51*(1), 199-204.

Cirkovic Velickovic, T. D., & Stanic-Vucinic, D. J. (2018). The role of dietary phenolic compounds in protein digestion and processing technologies to improve their antinutritive properties. *Comprehensive Reviews in Food Science and Food Safety*, *17*(1), 82-103.

Coburn, J. W., & Bronner, F. (Eds.). (2014). *Disorders of mineral metabolism: Calcium Physiology* (Vol. 2). Elsevier.

Crawford, C. D., Mata-Padrino, D. J., Belesky, D. P., & Bowdridge, S. A. (2020). Effects of supplementation containing rumen by-pass protein on parasitism in grazing lambs. *Small Ruminant Research*, *190*, 106161.

Dambe, L. M., Mogotsi, K., Odubeng, M., & Kgosikoma, O. E. (2015). Nutritive value of some important indigenous livestock browse species in semi-arid mixed Mopane bushveld, Botswana. *Livestock Research for rural development*, *27*(10), 1-10.

Daoub, R. M., Elmubarak, A. H., Misran, M., Hassan, E. A., & Osman, M. E. (2018). Characterization and functional properties of some natural Acacia gums. *Journal of the Saudi Society of Agricultural Sciences*, *17*(3), 241-249.

Derero, A., & Kitaw, G. (2018). Nutritive values of seven high priority indigenous fodder tree species in pastoral and agro-pastoral areas in Eastern Ethiopia. *Agriculture & Food Security*, *7*(1), 1-9.

Dinnage, R., Simonsen, A. K., Barrett, L. G., Cardillo, M., Raisbeck-Brown, N., Thrall, P. H., & Prober, S. M. (2019). Larger plants promote a greater diversity of symbiotic nitrogen-fixing soil bacteria associated with an Australian endemic legume. *Journal of Ecology*, *107*(2), 977-991.

Dinnage, R., Simonsen, A. K., Barrett, L. G., Cardillo, M., Raisbeck-Brown, N., Thrall, P. H., & Prober, S. M. (2019). Larger plants promote a greater diversity of symbiotic nitrogen-fixing soil bacteria associated with an Australian endemic legume. *Journal of Ecology*, *107*(2), 977-991.

Duvaux-Ponter, B. A. J. A. (2017). *Improving the success of the critical peri-partum transition period in small ruminants: access to physiological and metabolic disturbances and prospection of effects of natural products to prevent them* (Doctoral dissertation, Paris, Institut agronomique, vétérinaire et forestier de France).

Emire, S. A., Jha, Y. K., & Mekam, F. (2013). Role of anti-nutritional factors in food industry. *Beverage & Food World*, *2*, 23-28.

Engida, E., Guthiga, P., Nyota, H., & Karugia, J. (2015). The role of livestock in the Kenyan economy: Policy analysis using a dynamic Computable General Equilibrium model for Kenya.

Fanzo, J. (2014). Strengthening the engagement of food and health systems to improve nutrition security: Synthesis and overview of approaches to address malnutrition. *Global food security*, *3*(3-4), 183-192.

Fanzo, J. (2019). Healthy and sustainable diets and food systems: the key to achieving Sustainable Development Goal 2?. *Food ethics*, *4*(2), 159-174.

Feichtner, K. S., & Gessner, V. H. (2018). Cooperative bond activation reactions with carbene complexes. *Chemical Communications*, *54*(50), 6540-6553.

Fterich, A., Mahdhi, M., & Mars, M. (2012). Impact of grazing on soil microbial communities along a chronosequence of Acacia tortilis subsp. raddiana in arid soils in Tunisia. *European Journal of Soil Biology*, *50*, 56-63.

Gardarin, A., Garnier, É., Carrère, P., Cruz, P., Andueza, D., Bonis, A., ... & Kazakou, E. (2014). Plant trait–digestibility relationships across management and climate gradients in permanent grasslands. *Journal of Applied Ecology*, *51*(5), 1207-1217.

Gemede, H. F., & Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International journal of nutrition and food sciences*, *3*(4), 284-289.

Githae, E. W., & Mutiga, I. M. (2021). Ecological restoration of pastoral landscapes in the drylands of East Africa. *Journal of Dryland Agriculture*, *7*(3), 34-41.

Gruninger, R. J., Ribeiro, G. O., Cameron, A., & McAllister, T. A. (2019). Invited review: Application of meta-omics to understand the dynamic nature of the rumen microbiome and how it responds to diet in ruminants. *Animal*, *13*(9), 1843-1854.

Habchi, J., Tompa, P., Longhi, S., & Uversky, V. N. (2014). Introducing protein intrinsic disorder. *Chemical reviews*, *114*(13), 6561-6588.

Helal, H. G. (2018). Productive and reproductive performance of Barki ewes fed on sprouted barley grains on desert by-products during lactating period. *Res. J. Anim. Vet. Sci*, *10*, 37-48.

Hermes, T. R., Frachetti, M. D., Voyakin, D., Yerlomaeva, A. S., Beisenov, A. Z., Doumani Dupuy, P. N., ... & Makarewicz, C. A. (2020*).* High mitochondrial diversity of domesticated goats persisted among Bronze and Iron Age pastoralists in the Inner Asian Mountain Corridor. *PloS one*, *15*(5), e0233333.

Herremans, S., Vanwindekens, F., Decruyenaere, V., Beckers, Y., & Froidmont, E. (2020). Effect of dietary tannins on milk yield and composition, nitrogen partitioning and nitrogen use efficiency of lactating dairy cows: A meta-analysis. *Journal of animal physiology and animal nutrition*, *104*(5), 1209-1218.

Heuzé, V., Tran, G., Sauvant, D., Bastianelli, D., & Lebas, F. (2015). Lentil (Lens culinaris).

Hlatini, V. A., & Chimonyo, M. (2016). Nutritionally-related blood metabolites and liver enzymes in growing pigs fed on Acacia tortilis treated with polyethylene glycol. *Livestock Science*, *187*, 158-161.

Hlatini, V. A., Ncobela, C. N., Zindove, T. J., & Chimonyo, M. (2018). Use of polyethylene glycol to improve the utilisation of leguminous leaf meals in pigs: a review. *South African Journal of Animal Science*, *48*(4), 609-620.

Howieson, J., Conradie, P., Ballard, N., & Jordaan, G. (2014). Pasture development for community livestock production in the Eastern Cape province of South Africa.

Kamo, T., Takemura, T., Wasano, N., Fujii, Y., & Hiradate, S. (2012). Quantification of cyanamide in young seedlings of Vicia species, Lens culinaris, and Robinia pseudo-acacia by gas chromatography-mass spectrometry. *Bioscience, biotechnology, and biochemistry*, *76*(7), 1416-1418.

Kandie, h. C. (2022). *Identification, preference and nutritional evaluation of Acacia species as browse feed for goats in asal region, baringo county-kenya* (Doctoral dissertation, University of Eldoret).

Katunga, M. M. D., Mushagalusa, P. B., & Kambale, B. (2020). Productivity and Participatory Evaluation of Forage Legumes and Grasses in Pour Soils of Ngweshe Kingdom, DRC. *Open Access Library Journal*, *7*(9), 1-9.

Katz, S. E. (2016). *Wild fermentation: The flavor, nutrition, and craft of live-culture foods*. Chelsea Green Publishing.

Koerner, T. B., Abbott, M., Godefroy, S. B., Popping, B., Yeung, J. M., Diaz-Amigo, C., ... & Koehler, P. (2013). Validation procedures for quantitative gluten ELISA methods: AOAC allergen community guidance and best practices. *Journal of AOAC International*, *96*(5), 1033-1040.

Kremp, A., Godhe, A., Egardt, J., Dupont, S., Suikkanen, S., Casabianca, S., & Penna, A. (2012). Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. *Ecology and Evolution*, *2*(6), 1195-1207.

Krishna, H., Saroj, P. L., Maheshwari, S. K., Singh, R. S., Meena, R. K., Chandra, R., & Parashar, A. (2019). Underutilized fruits of arid & semi-arid regions for nutritional and livelihood security. *Int. J. Minor Fruits Med. Aromat. Plants*, *5*, 1-14.

Lamidi, A. A., & Ologbose, F. I. (2014). Dry season feeds and feeding: a threat to sustainable ruminant animal production in Nigeria. *Journal of Agriculture and Social Research (JASR)*, *14*(1), 17-30.

Lawa, E. D. W., Marjuki, M., Hartutik, H., & Chuzaemi, S. (2017). Effect of white kabesak (Acacia leucophloea Roxb) leaves level in the diet on feed intake and body weight gain of kacang goat. *Journal of Indonesian Tropical Animal Agriculture*, *42*(4), 255-262.

Lelenguyah, G. L. (2013). Effects of climate variability on pastoral livelihoods in Marigat District, Baringo County, Kenya, M. *Env. Studies thesis, Kenyatta University*.

Lengarite, M. I., Mbugua, P. N., Gachuiri, C. K., & Kabuage, L. W. (2012). Mineral status of sheep and goats grazing in the arid rangelands of northern Kenya. *Pakistan Journal of Nutrition*, *11*(4), 383.

Li, Y., Zhang, W., Ma, L., Wu, L., Shen, J., Davies, W. J., ... & Dou, Z. (2014). An analysis of C hina's grain production: looking back and looking forward. *Food and Energy Security*, *3*(1), 19-32.

MacAdam, J. W., & Villalba, J. J. (2015). Beneficial effects of temperate forage legumes that contain condensed tannins. *Agriculture*, *5*(3), 475-491.

Makkar, H. P. (2003). *Quantification of tannins in tree and shrub foliage: a laboratory manual*. Springer Science & Business Media.

Makkar, H. P. S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small ruminant research*, *49*(3), 241-256.

Marchiosi, R., dos Santos, W. D., Constantin, R. P., de Lima, R. B., Soares, A. R., Finger-Teixeira, A., ... & Ferrarese-Filho, O. (2020). Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochemistry Reviews*, *19*(4), 865-906.

McGregor, B. A. (2016). The effects of nutrition and parity on the development and productivity of Angora goats: 1. Manipulation of mid pregnancy nutrition on energy intake and maintenance requirement, kid birth weight, kid survival, doe live weight and mohair production. *Small Ruminant Research*, *145*, 65-75.

McSweeney, C., & Mackie, R. (2012). Commission on genetic resources for food and agriculture. *Micro-organisms and ruminant digestion: State of knowledge, trends and future prospects. Background Study Paper (FAO)*, *61*, 1-62.

Melesse, A., Steingass, H., Schollenberger, M., Holstein, J., & Rodehutscord, M. (2019). Nutrient compositions and in vitro methane production profiles of leaves and whole pods of twelve tropical multipurpose tree species cultivated in Ethiopia. *Agroforestry systems*, *93*(1), 135-147.

Mendieta-Araica, B., Spörndly, E., Reyes-Sánchez, N., Salmerón-Miranda, F., & Halling, M. (2013). Biomass production and chemical composition of Moringa oleifera under different planting densities and levels of nitrogen fertilization. *Agroforestry Systems*, *87*(1), 81-92.

Mertens, D. R., & Grant, R. J. (2020). Digestibility and intake. *Forages: the science of grassland agriculture*, *2*, 609-631.

Mlambo, V., & Mapiye, C. (2015). Towards household food and nutrition security in semi-arid areas: What role for condensed tannin-rich ruminant feedstuffs?. *Food Research International*, *76*, 953-961.

Mlambo, V., Marume, U., & Gajana, C. S. (2015). Utility of the browser's behavioural and physiological strategies in coping with dietary tannins: Are exogenous tannin-inactivating treatments necessary?. *South African Journal of Animal Science*, *45*(5), 141-451.

Muriithi, G. M. (2018). *Impacts of climate variability and weather forecasts on livestock production and marketing in Baringo County, Kenya* (Doctoral dissertation, university of nairobi).

Mutai, P. A. (2022). Nutritive Value, Tannin Bioassay and Processing Effects of *Acacia brevispica, A. mellifera* and *A. tortilis* Pods as Potential Supplements for Growing Small East African Goats (SEAG) in Baringo County-Kenya.

Mutai, P. A. (2017). *Evaluation of Nutritional Value of Field Legumes Using Rabbit (Oryctalogus Cunniculus) As the Animal for Bioassay* (Msc Thesis, University of Eldoret).

Naumann, H. D., Muir, J. P., Lambert, B. D., Tedeschi, L. O., & Kothmann, M. M. (2013). Condensed tannins in the ruminant environment: a perspective on biological activity. *Journal of Agricultural Sciences*, *1*(1), 8-20.

Naumann, H. D., Tedeschi, L. O., Zeller, W. E., & Huntley, N. F. (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Revista Brasileira de Zootecnia*, *46*, 929-949.

Ndamitso, M. M., Mustapha, S., Etsuyankpa, M. B., Ajai, A. I., & Mathew, J. T. (2017). Evaluation of chemical composition of Acacia nilotica seeds.

Needham, B. D., Funabashi, M., Adame, M. D., Wang, Z., Boktor, J. C., Haney, J., & Mazmanian, S. K. (2022). A gut-derived metabolite alters brain activity and anxiety behaviour in mice. *Nature*, *602*(7898), 647-653.

Oba, G. (2012). Harnessing pastoralists' indigenous knowledge for rangeland management: three African case studies. *Pastoralism: Research, Policy and Practice*, *2*(1), 1-25.

Okello, A. O., Nzuma, J. M., Otieno, D. J., Kidoido, M., & Tanga, C. M. (2021). Farmers' perceptions of commercial insect-based feed for sustainable livestock production in Kenya. *Sustainability*, *13*(10), 5359.

Omoyo, N. N., Wakhungu, J., & Oteng'i, S. (2015). Effects of climate variability on maize yield in the arid and semi-arid lands of lower eastern Kenya. *Agriculture & Food Security*, *4*(1), 1-13.

Owen, E., Smith, T., & Makkar, H. (2012). Successes and failures with animal nutrition practices and technologies in developing countries: A synthesis of an FAO e-conference. *Animal Feed Science and Technology*, *174*(3-4), 211-226.

Patra, A. K., & Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, *91*(1), 24-37.

Paul, A. A., Kumar, S., Kumar, V., & Sharma, R. (2020). Milk Analog: Plant based alternatives to conventional milk, production, potential and health concerns. *Critical reviews in food science and nutrition*, *60*(18), 3005-3023.

Piluzza, G., Sulas, L., & Bullitta, S. (2014). Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. *Grass and Forage Science*, *69*(1), 32-48.

Quintero-Anzueta, S., Molina-Botero, I. C., Ramirez-Navas, J. S., Rao, I., Chirinda, N., Barahona-Rosales, R., ... & Arango, J. (2021). Nutritional evaluation of tropical forage grass alone and grass-legume diets to reduce in vitro methane production. *Frontiers in Sustainable Food Systems*, *5*, 663003.

Randall, D. G., Krähenbühl, M., Köpping, I., Larsen, T. A., & Udert, K. M. (2016). A novel approach for stabilizing fresh urine by calcium hydroxide addition. *Water Research*, *95*, 361-369.

Ratemo, C. M., Ogendi, G. M., Huang, G., & Ondieki, R. N. (2020). Application of traditional ecological knowledge in food and water security in the Semi-Arid Turkana County, Kenya. *Open Journal of Ecology*, *10*(6), 321-340.

Ribeiro, G. O., Gruninger, R. J., Badhan, A., & McAllister, T. A. (2016). Mining the rumen for fibrolytic feed enzymes. *Animal Frontiers*, *6*(2), 20-26.

Robinson, T. P., Wint, W., Conchedda, G., Cinardi, G., Boeckel, T. P. V., Macleod, M., ... & Gilbert, M. (2015). The global livestock sector: Trends, drivers and implications for society, health and the environment.

Ronaldo, S. G., Cleverson, F. D. A., Jose, R. D. S. C., Ronaldo, M. J., Fabio, T. D., Francisco, C. D. S. S., & Derly, J. H. D. S. (2016). Genetic diversity in sweet cassava from the Brazilian Middle North Region and selection of genotypes based on morpho-agronomical descriptors. *African Journal of Agricultural Research*, *11*(38), 3710-3719.

Rosa Perez-Gregorio, M., & Simal-Gandara, J. (2017). A critical review of the characterization of polyphenol-protein interactions and of their potential use for improving food quality. *Current Pharmaceutical Design*, *23*(19), 2742-2753.

Rosales, R. B. (2014). The use of leguminous shrubs and trees as forages in tropical ruminant production systems. *Seminario Taller Internacional: Manejo de la proteína en la Producción de Ganado Bovino*.

Saminathan, M., Tan, H. Y., Sieo, C. C., Abdullah, N., Wong, C. M. V. L., Abdulmalek, E., & Ho, Y. W. (2014). Polymerization degrees, molecular weights and protein-binding affinities of condensed tannin fractions from a Leucaena leucocephala hybrid. *Molecules*, *19*(6), 7990-8010.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, *2*(1), 1-14.

Schmitt, M. H., Ward, D., & Shrader, A. M. (2020). Salivary tannin-binding proteins: a foraging advantage for goats. *Livestock Science*, *234*, 103974.

Schwab, N., Bürzle, B., Böhner, J., Chaudhary, R. P., Scholten, T., & Schickhoff, U. (2022). Environmental drivers of species composition and tree species density of a near-natural central Himalayan treeline ecotone: Consequences for the response to climate change. In *Mountain Landscapes in Transition* (pp. 349-370). Springer, Cham.

Selzer, K. (2017). *Effects of supplementing different proportions of cellulase and xylanase enzyme mixtures on intake and digestibility of high forage total mixed ration by sheep* (Doctoral dissertation, University of Pretoria).

Serhan, M., & Mattar, J. (2017). The goat dairy sector in Lebanon. *Goat science*.

Shelton, M. H. (2021). Advances in forage legumes: shrub legumes.

Shorrocks, B., & Bates, W. (2015). *The biology of African savannahs*. Oxford University Press, USA.

Sinha, N. K., Sidhu, J., Barta, J., Wu, J., & Cano, M. P. (Eds.). (2012). *Handbook of fruits and fruit processing*. John Wiley & Sons.

Skenjana, A. (2012). *The potential nutritive value of waste products from the sub-tropical fruit processing industry as livestock feed* (Doctoral dissertation, University of Pretoria).

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British journal of pharmacology*, *174*(11), 1244-1262.

Soares, S., Mateus, N., & de Freitas, V. (2012). Carbohydrates inhibit salivary proteins precipitation by condensed tannins. *Journal of agricultural and food chemistry*, *60*(15), 3966-3972.

Solís-Dominguez, F. A., White, S. A., Hutter, T. B., Amistadi, M. K., Root, R. A., Chorover, J., & Maier, R. M. (2012). Response of key soil parameters during compost-assisted phytostabilization in extremely acidic tailings: effect of plant species. *Environmental science & technology*, *46*(2), 1019-1027.

Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: environmental issues and options*. Food & Agriculture Org..

Su, B., & Chen, X. (2020). Current status and potential of Moringa oleifera leaf as an alternative protein source for animal feeds. *Frontiers in veterinary science*, *7*, 53.

Tadele, Y. (2015). Important anti-nutritional substances and inherent toxicants of feeds. *Food science and quality management*, *36*, 40-47.

Thakur, A., Sharma, V., & Thakur, A. (2019). An overview of anti-nutritional factors in food. *Int. J. Chem. Stud*, *7*(1), 2472-2479.

Trikilidou, E., Samiotis, G., Tsikritzis, L., & Amanatidou, E. (2020). Performance of Semi-Micro-Kjeldahl Nitrogen Method–uncertainty and nitrate interference. *International Journal of Environmental Analytical Chemistry*, 1-11.

Tshabalala, T., Sikosana, J. L. N., & Chivandi, E. (2013). Nutrient intake, digestibility and nitrogen retention in indigenous goats fed on Acacia nilotica fruits treated for condensed tannins. *South African Journal of Animal Science*, *43*(4), 457-463.

Tufarelli, V., & Laudadio, V. (2011). Dietary supplementation with selenium and vitamin E improves milk yield, composition and rheological properties of dairy Jonica goats. *Journal of dairy research*, *78*(2), 144-148.

Villot, C., Meunier, B., Bodin, J., Martin, C., & Silberberg, M. (2018). Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. *Animal*, *12*(3), 481-showing490.

Wangai, P., Muriithi, J. K., & Koenig, A. (2013). Drought related impacts on local people's socio-economic life and biodiversity conservation at Kuku Group Ranch, southern Kenya. *International Journal of Ecosystem*, *3*(1), 1-6.

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany*, *89*, 164-175.

Wu, C., Han, T., Lu, H., & Zhao, B. (2016). The toxicology mechanism of endophytic fungus and swainsonine in locoweed. *Environmental Toxicology and Pharmacology*, *47*, 38-46.

Wu, G., Fanzo, J., Miller, D. D., Pingali, P., Post, M., Steiner, J. L., & Thalacker-Mercer, A. E. (2014). Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. *Annals of the New York Academy of Sciences*, *1321*(1), 1-19.

Xue, D., Chen, H., Luo, X., Guan, J., He, Y., & Zhao, X. (2018). Microbial diversity in the rumen, reticulum, omasum, and abomasum of yak on a rapid fattening regime in an agro-pastoral transition zone. *Journal of Microbiology*, *56*(10), 734-743.

Zhou, J., Lin, Z., Ju, Y., Rahim, M. A., Richardson, J. J., & Caruso, F. (2020). Polyphenol-mediated assembly for particle engineering. *Accounts of Chemical Research*, *53*(7), 1269-1278.

APPENDICES

Appendix I: Experiment two (a): experimental layout for quantification of gm^+ and gm^- bacteria using gram-staining procedures:

*ACACIA SP*ECIES	T1 Number /type of cellulolytic bacteria:	T2 Number/type of cellulolytic bacteria:	T3 Number /type of cellulolytic bacteria	T4 Number /type of cellulolytic bacteria	T5 Number /type of cellulolytic bacteria
A. Brevispica	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =
A. Melliferra	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =
Acacia tortilis	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =	GM+= GM^- =

Appendix II: NACOSTI license

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **134683**

Date of Issue: **12/September/2019**

RESEARCH LICENSE

This is to Certify that Mr.. MUTAI PAUL of University of Eldoret, has been licensed to conduct research in Baringo on the topic: NUTRITIVE VALUE, TANNIN BIOASSAY AND PROCESSING EFFECTS OF ACACIA SPECIES PODS AS SUPPLEMENTS FOR GOATS IN BARINGO. for the period ending : 12/September/2020.

License No: **NACOSTI/P/19/1193**

134683

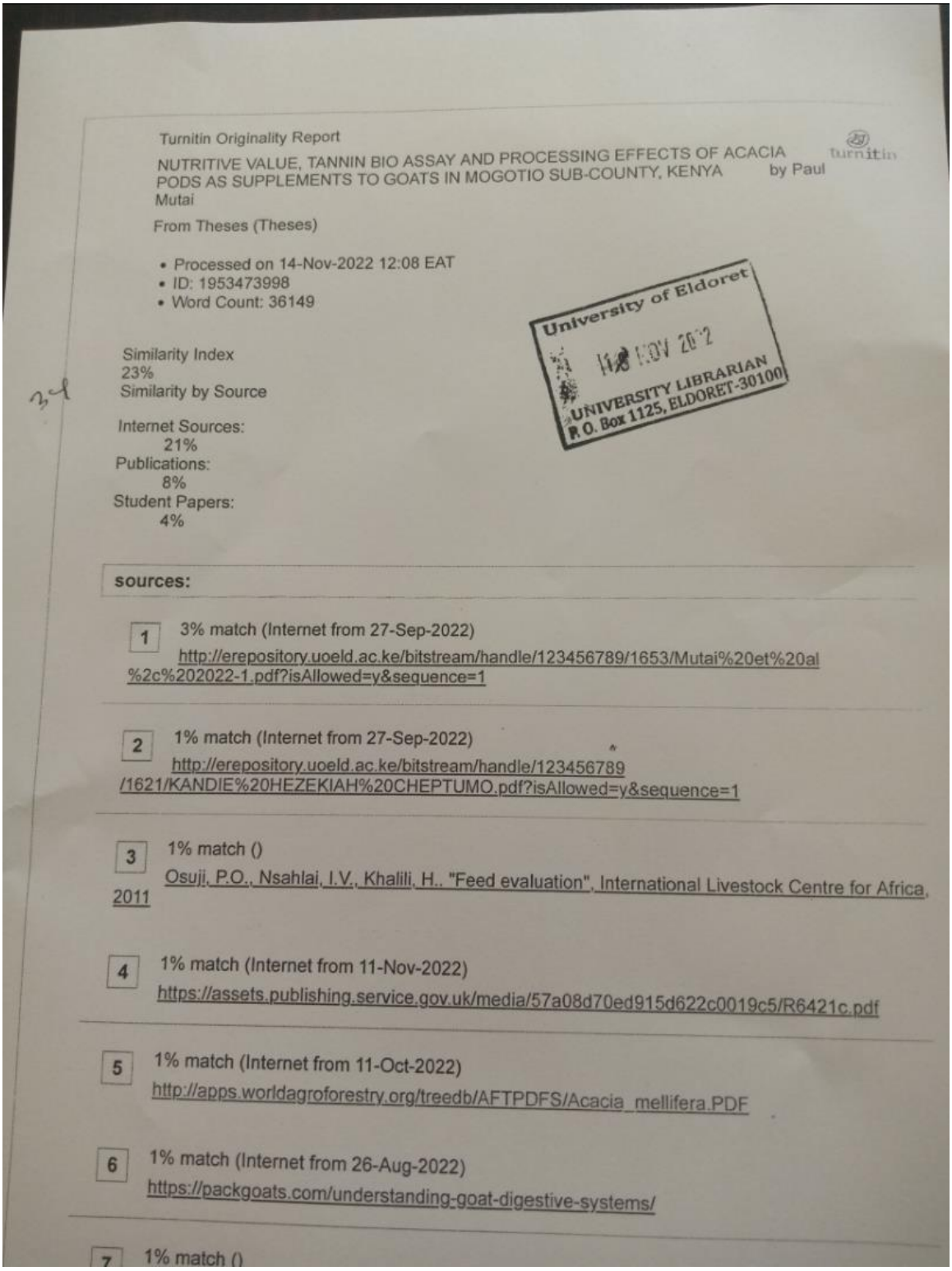
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix III: Similarity Report



Turnitin Originality Report

NUTRITIVE VALUE, TANNIN BIO ASSAY AND PROCESSING EFFECTS OF ACACIA PODS AS SUPPLEMENTS TO GOATS IN MOGOTIO SUB-COUNTY, KENYA by Paul Mutai

From Theses (Theses)

- Processed on 14-Nov-2022 12:08 EAT
- ID: 1953473998
- Word Count: 36149

University of Eldoret
14 NOV 2022
UNIVERSITY LIBRARIAN
P.O. Box 1125, ELDORET-30100

Similarity Index
23%
Similarity by Source

Internet Sources:
21%
Publications:
8%
Student Papers:
4%

sources:

1 3% match (Internet from 27-Sep-2022)
http://erepository.uoeld.ac.ke/bitstream/handle/123456789/1653/Mutai%20et%20al%2c%202022-1.pdf?isAllowed=y&sequence=1

2 1% match (Internet from 27-Sep-2022)
http://erepository.uoeld.ac.ke/bitstream/handle/123456789/1621/KANDIE%20HEZEKIAH%20CHEPTUMO.pdf?isAllowed=y&sequence=1

3 1% match ()
Osuji, P.O., Nsahlai, I.V., Khalili, H.. "Feed evaluation", International Livestock Centre for Africa, 2011

4 1% match (Internet from 11-Nov-2022)
https://assets.publishing.service.gov.uk/media/57a08d70ed915d622c0019c5/R6421c.pdf

5 1% match (Internet from 11-Oct-2022)
http://apps.worldagroforestry.org/treedb/AFTPDFS/Acacia_mellifera.PDF

6 1% match (Internet from 26-Aug-2022)
https://packgoats.com/understanding-goat-digestive-systems/

7 1% match ()