

**AN *IN VIVO* AND *IN VITRO* EVALUATION OF FEED
POTENTIALS OF LEAVES OF CONDENSED TANNIN
RICH TREE (*Albizia gummifera*, (J.F. Gmel.) C.A.SM.)**

MSc. THESIS

BIRUK KEBEDE

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JIMMA UNIVERSITY

**AN *IN VIVO* AND *IN VITRO* EVALUATION OF FEED
POTENTIALS OF LEAVES OF CONDENSED TANNIN RICH
TREE (*Albizia gummifera*, (J.F. Gmel.) C.A. SM.)**

**An MSc Thesis Submitted to the Department of Animal Sciences, School of
Graduate Studies, Collage of Agriculture and Veterinary Medicine, Jimma
University**

**In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Animal Production**

By

Biruk Kebed

**February, 2011
Jimma University**

APPROVAL SHEET

Jimma University College of Agriculture and Veterinary Medicine Graduate Studies

As thesis research advisor, we hereby certify that we have read and evaluated this thesis prepared, under our guidance by Biruk Kebede, entitled ‘**An In vivo and In vitro Evaluation of Feed Potentials of Leaves of Condensed Tannin Rich Tree (*Albizia gummifera* (J.F. Gmel.) C.A. Sm)**’ and recommend that it be submitted as fulfilling the thesis requirement.

Yisehak Kechero (Ass.Proff.)

_____	_____	_____
Major advisor	Signature	Date

Abegaz Beyene (PhD)

_____	_____	_____
Co-advisor	Signature	Date

As a member of the Board of Examiners of the MSc. Thesis Open Defense Examination, We certify that we have read, evaluated the Thesis prepared by Biruk Kebede and examined the candidate. We recommended that the Thesis be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Animal Production.

_____	_____	_____
Chairman person	Signature	Date

_____	_____	_____
Internal Examiner	Signature	Date

_____	_____	_____
External Examiner	Signature	Date

DEDICATION

I dedicate this thesis to my father Kebede Biru and to all people that gave me a positive inspiration to continue my studies.

STATEMENT OF THE AUTHORS

I, the undersigned, declare that this thesis is my work and is not submitted to any institution elsewhere for the award of any academic degree, diploma or certificate and all sources of materials used for this thesis have been accordingly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers under the rules of the library.

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Name: Biruk Kebede

Date of submission: January, 2011

Signature: _____

Place: Jimma University

BIOGRAPHICAL SKETCH

The author, Biruk Kebede Biru, was born on May 23, 1983 G.C at Gera *Wereda* in Jimma zone, south western Ethiopia. He attended his elementary school in the same district and later attended his junior school education and high school education at Jiren compressive secondary school. After successful completion his high school education in 2004, he joined Mekelle University and graduated with a Bachelor of Science (B.Sc) degree in Animal, Range, and Wildlife science on July 2007.

After completion of the B.Sc degree, he was employed by MoARD and served as animal production expert for two years. He joined the School of Graduate Studies of Jimma University in 2009/10 academic year to pursue his post graduate study for the Master of Science (M.Sc) degree in Animal production.

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LIST OF ABBREVIATIONS

AA	Amino Acid
ADF	Acid detergent fiber
ADG	Average daily gain
AG	<i>Albizia gummifera</i>
AGL	<i>Albizia Gummifera</i> Leaf
ARC	Agricultural research center
BDL	Below determination limit
CA	Crude Ash
CHO	Carbohydrate
CP	Crude protein
CPI	Crude Protein Intake
CRD	Completely randomized design
CT	Condensed tannin
DCP	Digestible protein
DM	Dry matter
DMI	Dry Matter Intake
DOMD	Digestible organic matter
EED	Ether extract digestibility
FAO	Food and Agriculture Organization
FCE	Feed conversion efficiency
FI	Feed Intake
FI	Feed Intake
GLM	General Linear Model
JUCAVM	Jimma University College Of Agriculture And Veterinary Medicine
Km	Kilometer
mg	Milligram
OMD	Organic matter digestibility
PSCs	Polyphenolics secondary compounds
TDN	Total digestible nutrient
WS	Weight At Slaughter

LIST OF ABBREVIATIONS (*Continued*)

GC	Gas Chromatograph
ADL	Acid Detergent Lignin
BW	Body Weight
CPD	Crude Protein Digestibility
DMD	Dry Matter Digestibility
HC	Hemicellulose
HGT	Hohenheim Gas Test
ILRI	International Livestock Research Institute
LWG	Live Body Weight Gain
ME	Metabolizable Energy
MEI	Metabolizable Energy Intake
ml	Milliliter
MOA	Ministry of Agriculture
MoARD	Ministry of Agriculture and Rural Development
MPTS	Multi - Purpose Tree Species
NDFD	Neutral Detergent Fiber Digestibility
NDFI	Neutral Detergent Fiber Intake
NFE	Nitrogen Free Extract
NFE	Nitrogen Free Extracts
°C	Degree Celsius
OMD	Organic Matter Digestibility
OMD	Organic Matter Digestibility
PEG	Polyethylene Glycol
PER	Protein Efficiency Ratio
RDP	Degradable Protein
SAS	Statistical Analysis System
SE	Standard Error of Means
USAID	United States Agency for International Development

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An *in vivo* and *in vitro* evaluation of feed potentials of leaves of condensed tannin rich tree (*Albizia gummifera*, (J.F. Gmel.) C.A. Sm.)

By: Biruk Kebede (BSc. in Animal science)

**Advisors: Ato Yisehak Kechero (Ass.Proff.) JUCAVM P. O. Box 307, Jimma
Abegaz Beyene (PhD) JUCAVM P. O. Box 307, Jimma**

Abstract

*The objectives were to evaluate the effect of dietary inclusion of leaves of the condensed tannin-rich plant, *Albizia gummifera* (AG), in grass-based hay with or without polyethylene glycol (PEG) on nutrient utilization, growth performance in ram lambs and assess the correlation between CP and some selected *in vivo* and *in vitro* nutritive value parameters. A 7-days digestibility and 90-day feed intake and weight gain trials were conducted using lambs with an initial body weight (BW) of 24.50 ± 0.02 kg (mean \pm SE). The experiment was arranged with three treatments and eight animals per treatment in a completely randomized design. The dietary treatments consisted of grass based-hay (T1, control), daily supplementation of lambs with the AG at 30% of control diet + control diet (T2), and T2 + PEG (T3; 40g PEG: 1kg AG on DM basis per head). The lambs were individually fed at 3% of their live body weight and had free access to clean drinking water and mineralized salt licks. Apparent digestibility coefficients were determined for dry matter (DM), crude protein (CP), crude fat (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF). A gas production test was performed after 2, 4, 6, 8, 12, 24, 32, 48, 58, 72 and 96 h of incubation. Nutrient intake was higher in lambs supplemented with PEG followed by T2 as compared to the T1 ($p < 0.001$). A loss in body weight was observed in the control group. The average daily body weight gain and apparent digestibility of all the chemical nutrients were significantly higher for T3 and T2 as compared to T1 ($p < 0.001$). The highest and lowest weights of carcass parameters were recorded for PEG supplemented and control groups, respectively ($p < 0.05$). The PEG supplementation had also a significant effect on the cumulative gas production (GP), organic matter digestibility (OMD), short chain fatty acid (SCFA) and ME content of diets ($p < 0.001$). The highest (12.00 MJ/kg) and lowest ME contents (8.20 MJ/kg) of feedstuffs were recorded for T3 and T1, respectively ($p < 0.001$). CP content was positively correlated with IVGP ($r = 0.71$), IVOMD ($r = 0.72$) and ME ($r = 0.87$) contents of feedstuffs ($p < 0.05$). The improvement in GP, OMD, SCFA and ME with PEG supplementation emphasizes the negative effect of CTs on digestibility. Feeding local grass hay would be inadequate as the sole source of nutrients for sheep. Therefore, 30% AG or 30% AG + PEG supplementation could maintain the optimum utilization of the grass based hays and resulted in better feed intake, digestibility and carcass parameters.*

Keywords: *Albizia gummifera*; carcass; digestibility; feed intake; performance; polyethylene glycol; sheep

1. INTRODUCTION

In Sub-Saharan Africa, the dry season is always a critical period when most feed resources especially grasses and herbaceous forages dry out (Devendra, 1992; Kanani *et al.*, 2006; Cheema *et al.*, 2011). Foliages of multi-purpose tree species (MPTS) have been recognized as reliable substitute either as sole feed or as protein supplements which can be utilized to prevent a decline in productivity of animals during this period (Arigbede *et al.*, 2011). The foliage of MPTS has been used to supplement hays and straws to alleviate CP deficiency in fibrous feeds, reduce rumen acidosis and other health-related problems, and improve intake and productivity of animals (Owens *et al.*, 1998; Salem *et al.*, 2006). The increasing demand for protein by the increasing population in developing countries urges for cheap feedstuffs. Currently small-holder farmers in many Sub-Saharan African countries in general are increasingly relying on various potential browse plants, underutilized potential feed resources, to supplement their herbivore livestock especially in dry seasons (Kanani *et al.*, 2006; Aremu and Onadeko, 2008). During feed scarcity in the dry season MPTS such as *Albizia gummifera* can provide a green feed throughout the year which may be particularly useful as a feed supplement to the typical low-quality diets (Yisehak and Belay, 2011). The presence, however, of secondary plant compounds could present major constraints to their use (Makkar, 2003). The primary antinutritional agent in *A. gummifera* species and many other browse species are condensed tannins (CTs, proanthocyanidins) (Yisehak *et al.*, 2010).

Tannin effects on ruminant nutrition have been studied for several years and are often seen only in terms of their negative impacts on intake and production: decreased nutrient utilization, particularly protein (Waghorn *et al.*, 1994a); decreased palatability and consequently the amount of feed ingested; decreased digestibility (Silanikove *et al.*, 1993; Perevolotsky *et al.*, 1993); volatile fatty acids production reduction and decreased digestibility of organic matter and fiber (Ben Salem *et al.*, 1997). As well as binding to protein, tannins can also bind to carbohydrates (Tiemann *et al.* 2008a) leading also to a reduction in ruminal gas production (Silanikove *et al.*, 1993; Makkar *et al.*, 1995a). Inhibition of digestibility can be attributed mostly to soluble CT binding proteins and digestive enzymes and antimicrobial characteristics. The formation of indigestible fiber-

bound CT, protein macrostructures and increased lignifications within the fodder prior to consumption also contribute to losses in feeding value (Krebs *et al.*, 2007).

The determination of the *in vitro* gas production is of value to nutritionist because it provides information on fermentation kinetics of forage consumed by ruminants, which is dependent on the rate of passage and the degradation rate (Mould *et al.*, 2005). The *in vitro* gas production technique has proved to be a potentially useful technique for feed evaluation (Menke and Steingass, 1988; Blummel and Ørskov, 1993; Herrero *et al.*, 1996; Getachew *et al.*, 2004), as it is capable of measuring rate and extent of nutrient degradation (Groot *et al.*, 1996; Cone *et al.*, 1997). In addition, *in vitro* gas production technique provide less expensive (Getachew *et al.*, 2004), easily to determine tannin effects (Khazaal *et al.*, 1993) and suitable for use in developing countries (Blummel *et al.*, 1997). With a better understanding of tannin properties and proper management, MPTS like *Albizia gummifera* could become an invaluable source of protein for strategic supplementation. As the demand for feed rises, tanniniferous plants must become an increasingly important part in the diet of animals, in particular for ruminants in smallholder subsistence farming in developing countries. It is therefore critical that techniques be developed to measure and manage the anti-nutritional components that they contain (Makkar, 1993 and Waghorn *et al.*, 1997).

Actually, a growing number of methods are available for analysis of tannins; although these assays, due to the complex and diverse nature of tannins, do not provide satisfactory results. For a quick assessment and initial screening of tannins in browse species, it would be cheaper if tannins could be predicted from the use of relatively simple methods such as a tannin bioassay. It is evident from a number of studies that where high tannin feed is the main source of fodder, detannification through complexation of soluble CT is beneficial in retention of digestive capacity and optimization of nutrient availability to the ruminant. This assay is based on an *in vitro* rumen fermentation system coupled with the use of a tannin-complexing agent, polyethylene glycol (PEG). PEG is a well-recognized biologically detannification agent, with PEG appearing to be the major form used in trials involving high CT feed despite the fact that PEG has been shown to be more effective, *in vitro* (Makkar *et al.*, 1995b).

Sheep are the predominant small ruminant species kept in the Gilgel Gibe catchments of Jimma zone, southwestern Ethiopia, where almost all of them are kept under traditional extensive management system and depend almost exclusively on natural grazing. During the dry season, the nutritive value of natural pastures deteriorates and becomes deficient in many nutrients, especially proteins. As a result, animals that depend on those pastures progressively lose weight until the wet season comes. Alternatively, animals can be supplemented to minimize or to prevent weight losses (Yisehak *et al.*, 2011).

A. gummifera is a tropical multipurpose tree indigenous to southwest Ethiopia (Bekele *et al.*, 1993) and widely abundant in the Gilgel Gibe catchments of Jimma zone, containing high amount of CTs which is well known to affect the efficiency of nutrient digestion in ruminants (Yisehak *et al.*, 2010). However, to our knowledge, the response in sheep's nutrient utilization, performance and carcass quality to *A. gummifera* leaf feeding have not been investigated. Therefore, the present study was undertaken to investigate the effects of dietary inclusion of *A.gummifera* leaves with or without PEG on feed intake, apparent digestibility, growth performance and carcass characteristics of local ram lambs, to determine the effect of PEG on the *in vitro* fermentation parameters(gas production, organic matter digestibility, short chain fatty acids) and metabolizable energy and to determine the relationship between crude protein content of dietary feedstuffs with feed intake, weight gain, apparent digestibility and *in vitro* gas production.

2. LITRATURE RIVIEW

2.1 Browsers utilization and the constraint to their utilization

More than 200 species of leguminous trees are reported to be used for forage, with most species being tropical or subtropical in origin. The most commonly used species come from the genera *Acacia*, *Albizia*, *Calliandra*, *Desmanthus*, *Desmodium*, *Gliricidia*, *Leucaena*, *Prosopis* and *Sesbania* (Brewbaker, 1986) and effects of tannins on palatability, nutritive value and production of tropical fodder tree species suited to tropical environments are well documented (Shelton *et al.*, 1995; Evans 1996; Stewart, 1996; Shelton *et al.*, 1998). Tree leaves and pods form a natural part of the diet of many ruminant species and have been used traditionally as sources of forage for domesticated livestock in Asia, Africa and the Pacific (Le Houerou, 1980).

Devendra (1995) has noted some broad estimates of the importance of tree browses (shoots, twigs, leaves, fruits and pods of trees and shrubs), which are generally high in tannins and important in arid and semi-arid regions. Gutteridge and Shelton (1994) have reviewed the role of forage tree legumes, noting that at least 75% of the shrubs and trees of Africa serve as browse plants. In northern Africa browse forms 60 - 70% of rangeland production and 40% of the total available feed, other estimates put such feeds as making up 40 - 50% of the total available feed (FAO, 1992). About 45% of smallholder farmers in the sub-humid coastal region of Kenya used browse from fodder trees. In Eastern Kenya dairy cows have been successfully fed on a diet containing 25 to 30% of daily intake as the fodder tree *Calliandra calothyrsus*, and growing heifers about 15% of intake (Wood, 1996). Tree browses form about 1- 2% of the feed of cattle in the Gambia during the dry season, but for goats such feed may constitute 10% of the diet Reynolds *et al.* (1993). Tree fodders contribute an estimated 8.2 million tones/year of fresh feed in South-East Asia, about 4% of the available feedstuffs, excluding grasses (Devendra, 1993). In India 60 - 70% of the forage requirements for goats are tree browses, tree legumes being particularly important (Devendra, 1995). Foran (1984) noted that in central Australia, *Acacia aneura* and *Acacia kempeana* contributed up to 40% of the diet for cattle during the hardest times of the year, but when grass was abundant, the level normally fell to less than 20%. About

40% of the fodder available annually in Nepal comes from forest trees and contributed over 15% of the dry matter and over 20% of the crude protein to the diet, but actual usage on individual farms varied depending on the ethnic and social group of the farmers, possible as a result of different livestock holdings and opportunities for planting trees (Panday, 1982). Livestock in the Sub-Saharan Africa are dependent primarily on native grasslands and crop residues (Ibrahim, 1999). In Ethiopia, there are extensive areas where the raising of livestock on the natural vegetation is the possible types of land use (Coppock, 1994). The rangelands of Ethiopia are presently being extensively deteriorated both in quantity and quality (Tamene, 1990; Belaynesh, 2006). In seasonally dry environments, the main limitations to animal production are lack of green feed for at least half of the year. The other is the low nutritive quality of forages during most of the period of active pasture growth (Jones and Wilson, 1987). Alemayehu, (1998) estimated that 80-85 % of the livestock feed in Ethiopia comes from natural pasture. Alemayehu (2003), reported Ethiopia's Livestock feed resources are mainly natural grazing and browse, crop residues, improved pasture, and agro-industrial byproducts.

Therefore multipurpose trees and shrubs (MPTs) play a vital role in agro forestry systems and have a good potential in raising the ruminants' productivity by lessening the gap between demand and supply of fodders, the deficit of feeds being the main constraint in most parts of the world (Datt *et al.*, 2008). Browse species play a major role in providing feed for ruminants in arid and semi-arid regions, particularly during the dry season when poor quality roughage and crop residues prevail (Kibon and Orskov, 1993; Ahn *et al.*, 1989; Kamalak *et al.*, 2004), because fodder trees are an important source of supplementary protein, vitamins and minerals in developing countries (Singh *et al.*, 1989; Baumer, 1992), and nutritious like leguminous fodder crop (Akram *et al.*, 1990) for small ruminant to supplement grass and other low quality feeds to improve their nutritive value for higher animal performance (Reed *et al.*, 1990). During dry period forage trees remain green and maintain a relatively high crude protein (CP) content (D'Mello, 1992). It is rarely realized that probably more animals fed on shrub and tree fodder than on grasses for productive and persistent forage sources for the tropics (Cheema *et al.*, 2011). Thus, the importance of forage trees and legumes to agriculture and the environment in reducing soil erosion and fixing nitrogen, together with uses of trees for timber etc. must also be noted in maintaining and increasing the productivity of many tropical regions.

2.1.1 Nutritive value of browses

Nutritive value is a function of the feed intake (FI) and the efficiency of extraction of nutrients from the feed during digestion (digestibility). Feeds of high nutritive value promote high levels of production (live weight gain); and feed intake in ruminants consuming fibrous forages is primarily determined by the level of rumen fill, which in turn, is directly related to the rate of digestion and passage of fibrous particles from the rumen (Norton, 1994, McDonald *et al.*, 2002). MPTS foliage has been recognized as reliable substitute either as sole or protein supplements which can be utilized to prevent decline in the productivity of animals during dry period (Kanani *et al.*, 2006).

Tree fodders are rich protein and mineral contents as compared to grasses and thus the feeding value of low quality roughages and grasses can be greatly improved by foliage from trees, which can be grown integrated directly to pastures and in fence (Aganga *et al.*, 2003). These nutrient contents are subject to less variation than with grasses and this particularly enhances their value as dry season feeds for livestock. Le Houerou (1980), Shelton (1994), and Dzewela *et al.* (1997) recognized the role of tree in supplying dietary nitrogen, energy, mineral, and vitamins in arid and semi arid region. Leaves and fruits of ligninous species have a much higher level of digestible protein (DCP) than other fodder sources; in Senegal, has reported to have 180 to 200 g DCP/kg DM in browse compared to 100 to 130 for groundnut leaves and 50 to 70 for leaves and fruits of various herbaceous species (Guerin *et al.*, 1986).

Although many investigations have been carried out on the nutritive potentials of MPTS in several African countries (Gasmi-Boubaker *et al.*, 2005; Salem *et al.*, 2006; Tefera *et al.*, 2008), there are still several species yet to be studied in these countries. Despite these benefits, adoption of MPTS into farming systems by resource poor farmers was generally poor. This is due to several reasons among which were slow establishment and high labor requirements to lop the tree foliage especially in cut and carry systems as well as poor adaptation to the prevailing soil and climatic conditions (Afneta, 1996). Some plants evolved PA production as a defense strategy, first against invasion by pathogenic bacteria and fungi, then against being eaten by insects and herbivores, with the mechanism being protein precipitation (Barry, 1989).

Generally the potential roles of MPTS' foilages in ruminant nutrition are high quality and high digestibility biomass resource, available in and around the farm, supplement to provide nutrients deficient in the diet and reduction in the requirements for purchased concentrates and as a result decrease in cost of feeding (Singh, 1982). Thus, to encourage the adoption of MPTS by farmers, evaluation of different species peculiar to different geographical areas is necessary. Also, the levels of various secondary metabolites present in each species as well as their effects on availability of other nutrients and nutritional quality of the plants should be well documented. Therefore this will help to expand the range of tree species with forage value available for use as protein supplements by farmers during periods of feed scarcity.

2.1.2 Limitations to nutritive value of browses

MPTS contain a wider range of anti-nutritional factors than more conventional fodder species (D'Mello, 1992). These compounds exert both beneficial and harmful effects on ruminant digestive physiology (rate of passage, rumen metabolism and microbe's activity) and dietary nutrients use efficiency, intake and digestibility, and hence the level of supplementation of browse species in roughage based diets of small ruminants vary with their availability, nutritional quality, animal species and concentration of toxic constituents (Singh and Kundu, 2010). Although they may contain adequate concentrations of nutrients, the presence of secondary plant compounds could present major constraints to their use (Dzowela *et al.*, 1987). For this reason, depending on the species, tree legume foliage may be of lower nutritive value as a sole feed than as a supplement to other feeds. The significance of secondary plant compounds becomes more evident when tree foliage is the only feed consumed (Norton, 1994). Hence, when tannin-rich leaves are offered as a sole feed to sheep and goats they may not provide the maintenance requirement despite their relatively high-protein and low-fiber contents (Silanikove *et al.*, 1996a). Where fresh *Acacia saligna* was the sole feed for rams, and not supplemented with PEG, DMI were lower, but the DMI of *A. saligna* with PEG exceeded 800 g/d. Their higher DMI corresponded to a higher DMD of 54.2% (Krebs *et al.*, 2007).

Plants can be rendered unattractive as forage by their effects on any of the senses of the animal (reference). *Acacia lysiphloia* has sticky foliage, and *Acacia coriacea* gives off an offensive odour, both of which may be repellent to livestock (Fox, 1987). Species of many genera, for example, *Acacia*, *Erythrina*, *Mimosa* and *Prosopis*, have large thorns and/or spines. Some *Acacia* spp. form symbiotic associations with ants of the genus *Pseudomyrmex*, which swarm to protect the tree when it is disturbed (Allen and Allen, 1981). Most trees will rapidly grow out of the reach of livestock unless they are carefully managed and use of fodder trees is labor-intensive and more suited to small-scale intensive farming than to larger, more extensive operations (Paterson *et al.*, 1998).

2.2 Chemical composition of browses

2.2.1 Protein

Compared with tropical grasses, browse appears to be richer in protein and minerals (except for protein for young grasses) as already shown by Rose-Innes some 30 years ago (1964, 1966, 1967) and later by (Boudet, 1975; Le Houérou (1965, 1966, 1972, 1978) replace by other reference and others. Protein is necessary for the production of milk, muscles, wool and hair and to replace the protein unavoidably lost during maintenance (Minson, 1990). The foliage of MPTS has been used to supplement straws to alleviate crude protein (CP) deficiency in fibrous feeds, reduce rumen acidosis and other health-related problems, and improve intake and productivity of animals (Owens *et al.*, 1998; Salem *et al.*, 2006).

When the quality of grasses fall below the maintenance requirement during a long dry season tree fodders can normally provide sufficient CP in order to complement poorer quality pastures and crop residues (Paterson *et al.*, 1998). The mean CP value of leguminous browse is 15% against 13% for non leguminous browse (Jones and Wilson, 1987; Valli *et al.*, 1998; Datt *et al.*, 2007). The CP content of forage tree legume leaves (12-30%) is usually high compared with that of mature grasses (3-10%); Feeds containing less than 1.3% N (8% CP) are considered deficient as they cannot provide the minimum ammonia levels required. All forage tree legumes have N contents higher than this value,

and may be judged adequate in CP (Norton, 1994). Most of the MPTs possessed more than 10% of CP and below this level; rumen fermentation is adversely affected (Alam *et al.*, 1994; Mandal, 1997; Datt *et al.*, 2006).

2.2.2 Mineral content of browses

Mineral are required for both plants and animals in critical amounts and balance, the access and deficiency both reduces the efficiency of vegetation and dependant livestock production (Cheema *et al.*, 2011). Most of the MPTs were adequate to rich sources of Ca, Fe and Mn; however, about 75% were deficient in P, 67% in Ca, 58 % in Zn and 25% in Co, (Datt *et al.*, 2004) when compared to critical levels (Prabowo *et al.* 1990), also the proportion of silica is two to three times lower in browse. Therefore, these minerals should be supplemented in order to avoid production losses considering the essentiality of minerals (Chew, 2000) for growth, health, reproduction, production as well as normal biological functions of animals (Datt *et al.*, 2008).

The optimum level of Ca in plants is 0.4-0.6 and levels above 1.0% are considered high (Georgievskii, 1982). The minimum requirement of ruminants for phosphorus (P) varies from 1.2 to 2.4 g/kg feed dry matter depending on physiological function. Forage tree leaves generally have high P concentrations. Calcium (Ca) is closely associated with P metabolism in the formation of bone, and a Ca: P ratio of 2:1 is usually recommended for ruminant diets. Ca is rarely limiting in forage diets and the same is true for forage trees. Magnesium (Mg) and potassium (K) are found in excess of requirements in tree leaves and are seldom a limiting dietary factor in ruminants (reference). Shah and Gupta (1987) reported that 40% of fodder tree leaves evaluated contain TDN 26.9 to 70.22% and were rich in calcium and poor in phosphorus which can result in phosphorus deficiency in cattle if not given supplementary phosphorus.

The recommended requirement for Na in ruminant diets is 0.7 g/kg dry matter. Some tree species appear to be marginal in Na, but deficiencies are probably not common as forage tree leaves usually form only part of a ruminant's diet (Norton, 1994). Sodium levels of tree fodders are often low, but in many areas of the tropics, animals on grass alone also

need a salt supplement to prevent deficiency problems (Paterson *et al.*, 1998). Sulphur (S) in plant material is mainly found in the form of sulphur amino acids and is required together with N for microbial protein synthesis in the rumen. Concentrations greater than 1.5 g/kg dry matter or N: S ratios less than 15:1 are considered adequate. However, where protein digestion in the rumen is limited by complexing with tannins, S rather than N may become the limiting factor in microbial protein synthesis (Norton, 1994). However, abnormally high concentrations of some minerals such as sodium chloride and selenium may be found in browse (Olsson and Welin-Berger, 1989).

2.3 Voluntary intake of browses species

In general, there is an inverse relationship between condensed tannin (CT) concentrations in browse sources and voluntary feed intake by herbivores (Silanikove *et al.*, 2001). An animal may consume a nutritious food containing toxins but limit the intake to stay below a toxicity threshold (Marsh *et al.*, 2006). Several studies have reported that tannins do not influence feed intake (McNabb *et al.*, 1993; Wang *et al.*, 1994). On the other hand, in other studies the presence of tannins has been associated with reduced feed intake (Wiegand *et al.*, 1995; Barahona *et al.*, 1997; Vasta *et al.*, 2009).

CT produces adverse postingestive effects that cannot be accounted for by digestion inhibition alone, because they cause such rapid (within few to 60 min) and dramatic decreases in food intake (Provenza *et al.*, 1994). Silanikove *et al.*, (1997) in goats and Landau *et al.*, (2000) in heifers have shown that feeding ruminants with diet rich in CT was associated with lowered feed intake and shorter duration of eating bouts, mainly of the first eating bout, immediately after distribution of the diet. Astringency due to the interaction of tannins with salivary and mucosa proteins in the mouth signals the ingestion of CT rich diets (Prinz and Lucas, 2000). The complex formed between salivary CT binding proteins and tannin is independent of the pH range observed in the digestive tract and soluble in nature (Austin *et al.*, 1989), and to a certain extent this characteristic is very important for protecting feed protein and animals against dietary tannins; so the complex formed between tree leaf tannin and salivary tannin binding protein during the mastication and rumination process enters the rumen and remains as such without any dissociation and the effect is avoided within minutes by ingesting PEG (Landau *et al.*, 2000).

2.4 Digestibility of browse nutrients

A one percent increase in digestibility results in 9% higher feed efficiency and 10% increase in milk yields, which represent important economic gains (Dzowela *et al.*, 1997). Chewing during eating ruptures about 60% of plant cells and the CT are able to bind with salivary and plant proteins and the extent to which CT interfere with digestion is a function of astringency, concentration and potential sites for binding (Waghorn, 2008). CT are not digested by the micro flora (McSweeney *et al.*, 2001) and the carbon skeleton is not absorbed (Terrill *et al.*, 1994), so high concentrations of CT reduce the DM potentially available for digestion (Patra *et al.*, 2006). This was due to tannins particularly condensed tannins, bind to dietary constituents, (with proteins, carbohydrates and minerals), intestinal enzymes and bacteria (Makkar, 2003); thereby decrease the availability of dietary constituents, particularly proteins and dramatically inhibit digestive and absorptive processes in the gastro-intestinal tract of animals.

Higher concentrations of CT in diets, which remain free after binding with proteins, may depress fiber digestion by complexing with lignocelluloses thus preventing microbial digestion (Patraa and Saxena, 2010) or by directly inhibiting cellulolytic microorganisms and activities of fibrolytic enzymes (Bae *et al.*, 1993) or both (Jones *et al.* 1994; McAllister *et al.* 1994); and reduce cell-wall digestibility by binding bacterial enzymes and forming indigestible complexes with cell wall carbohydrates (Leinmüller *et al.*, 1991). The reduced rate of carbohydrate digestion, especially fiber, may decrease total volatile fatty acid concentrations in the rumen (Beauchemin *et al.*, 2007); (Patra *et al.*, 2006), because rumen pool sizes tend to increase as a consequence of a slower rate of digestion (Waghorn and McNabb, 2003).

Excessive intakes of CT in shrub species reduced the whole tract apparent digestibility and increased faecal nitrogen loss by sheep (McNeill *et al.*, 2000). Similarly greater faecal N excretion has been shown in many studies with tannin-containing diets (Komolong *et al.*, 2001; Animut *et al.*, 2008; Grainger *et al.*, 2009), and resulting in apparent negative digestibility of lignin in sheep fed carob (Silanikove *et al.*, 1994) or negative NDF digestibility in goats fed Pistacia (Silanikove *et al.*, 1996a), probably due to tannin–protein complexes were not completely dissociated in the abomasums and lower digestive tract.

2.5 Effect of tannins on animal production

The mechanisms and effects of tannin-ruminant interactions are so varied that accurate predictions of animal performance (i.e. milk, meat or wool production) as influenced by tannin consumption cannot be easily made (Mueller-Harvey, 2006). Forages containing moderate concentrations of CT (20–40 g kg⁻¹ of DM) may exert beneficial effects on protein metabolism; however, high dietary CT concentrations (60–120 g kg⁻¹ of DM) may depress nutrient availability, voluntary feed intakes, digestive efficiency and animal productivity (Aerts *et al.*, 1999) due to the complexes formed between tannins and several types of macromolecules (Frutos *et al.*, 2004a). The effects of tannins on ruminant productivity depend upon, quality (e.g. degradability and composition of essential amino acids) and quantity of dietary protein, requirements of amino acids and status of other nutrients and quantity of tannins consumed, since in several types of forages moderate amount of tannin have beneficial effects (Barry and McNabb, 1999; Min *et al.*, 2003; Waghorn and McNabb, 2003).

The quantity of protein flowing from the rumen is a major factor in determining the productivity of ruminant livestock (Patraa and Saxena, 2010), therefore greater animal performance observed when diets contain moderate levels of tannins, due to the protection of feed protein from ruminal hydrolysis, hence improving their nutritional value by altering rumen fermentation patterns to increase efficiency of microbial protein synthesis (Barry *et al.*, 1986; Terril *et al.*, 1992; Bhatta *et al.*, 2000), which intern increase the flux of essential amino acids to the small intestine and an increase in the absorption of essential amino acids to blood (Waghorn and Shelton, 1997; Makkar, 2003). More over the decreased rate and extent of protein degradation in the rumen due to feeding of tannin-rich feeds is advantageous environmentally because this could lower ammonia concentrations in the rumen West *et al.*, (1993) and hence urea N excretion in urine, (Aufrere *et al.*, 2008; Tiemann *et al.*, 2008b; Grainger *et al.* 2009).

2.5.1 Body weight gain

The effect of CT on lamb growth depends on the degree of tannin activity (Priolo and Ben Salem, 2004). Inclusion of quebracho tannins at a dosage of 20 g kg⁻¹ (equivalent to 15 g CT kg⁻¹) to *lucerne* hay increased body weight gain and feed conversion efficiency in lamb compared with control, while dosages of 10 and 30 g kg⁻¹ had intermediate values (Al-Dobaib, 2009). Concentrations of CT beyond 6% in the four browse species tended to relate negatively to body weight gain which supports the observations of reduced body weight gain in lambs fed feed with CT concentrations of 76-90 g/kg DM (Frutos *et al.* 2004a). Similarly diets containing as low as 26 g tannins kg⁻¹ from carob pulp (*Ceratonia siliqua*) resulted in a decrease of growth rate of lambs from 140 to less than 50 g per day Priolo *et al.*, (2000), furthermore, CT concentrations exceeding 60 mg/g DM have been observed to depress voluntary feed intake, fiber and protein digestibility and subsequently reduce the growth rate of ruminants (Huang *et al.* 2010), whereas the CT in *H. coronarium* (72 g kg⁻¹ of DM) did not affect daily gain of lambs (Douglas *et al.*, 1999). Additionally Mbatha *et al.* (2002) observed a decrease of liver mass with increasing CT concentrations over 6 weeks, which signifies the negative effect of CT on goat body weight. However, after a long time the negative effects of CT can diminish as goats become adaptable to CT.

Therefore the growth is the function of interaction of CT in the diet with food proteins, digestive enzymes and microbes. It was expected that goats given low CT concentration diets would lose less body weight than those fed high CT concentration diets (Silanikove *et al.*, 2006). It has been observed that the low body weight gain of kids after feeding CT rich diets is because of the low protein (Mole *et al.*, 1993), dry matter and organic matter digestibility (Silanikove *et al.*, 2006). This also suggests that CT reduce the body weight through the reduction of feed intake. Thus, this suggested that pre- and post-ingestive effects affect the body weight gain of ruminants (Dludla, 2010).

However, there was no difference in growth for goats fed cassava without or with PEG (Frutos *et al.*, 2004a). Thus the higher the difference between with PEG and without PEG incubations, indicate the higher the tannin content and activity in the browse material (Ammar *et al.*, 2004). Barry (1985) observed that daily administration of polyethylene

glycol to sheep grazing *Lotus pedunculatus* (which has a high CT content; 76-90 g kg⁻¹ DM) diets increased live weight gain by 41-61 g/d. Conversely, the lower level of PEG may have been insufficient to deactivate all the anti-nutritive effects of CT, thus weight loss by sheep and goats as response to the higher PEG level may have been related to a protein–energy imbalance resulting from PEG effects on protein release together with low energy content in the diet (Narvaez *et al.*, 2011). Therefore, it should not be assumed that the effects of PEG in tanniniferous diets on body weight gain can only be affected by levels of tannins (Waghorn, 2008). Also the nature of tannins in different fodder species and the species' stage of growth can have different effects on body weight gain (Dludla, 2010). CT from carob (*Ceratonia siliqua*) reduced lamb growth rate whilst Sulla (*Hedysarum coronarium*) with double CT concentration did not reduce daily gain of lambs (Waghorn, 2008). Thus studying the nutritional effects of tannins in plants is complicated because different plants contain a great diversity of tannins and other PSCs that have contrasting effects which changes with the plant age and season (Krueger *et al.*, 2010).

2.5.2 Milk production

With respect to the effect of ct on reproductive efficiency Min *et al.* (1999) observed that sheep grazing *L. Corniculatus* (17 g ct kg⁻¹ dm) increased their production of lambs by 25% due to increased rates of ovulation and a subsequently increased lambing percentage, possibly related to protein utilization. Condensed tannin from *L. Corniculatus* increased milk yield, protein and lactose percentage, reducing fat percentage in ewes' lambs; the higher protein percentage could be due to an increase of AA and especially essential AA flows from the small intestine (Wang *et al.*, 1996a). This increased concentration of protein might be explained by the greater availability of intestinal amino acids, especially of methionine and lysine, which are thought to limit milk production. The greater concentration of lactose can be explained by greater glucose supply; most lactose synthesis in the mammary gland relies directly on blood glucose, and in ruminants gluconeogenesis mainly involves propionic acid and amino acids. Thus, a greater availability of amino acids would contribute to greater synthesis of glucose. The reduction in the concentration of fat was attributed to a simple dilution effect as the concentrations of lactose and protein increased (Frutos *et al.*, 2004b). Similarly inclusion of 75 g tamarind seed husk, a tannin rich by-product (140 g ct kg⁻¹) per kg diet of cross-bred dairy cows

also resulted in increased body weight gain and milk protein content in mid lactation (Bhatta *et al.*, 2000).

Recently, Grainger *et al.* (2009) reported that milk yield, fat and protein percentage of milk reduced when dairy cows were dosed daily with 163–326 g CT from *Acacia mearnsii*. In this experiment, feed intake and digestibility were also decreased by *A. mearnsii* tannins. These results indicate that CT from different plant species has different effects, depending on different levels and activity (Priolo and Ben Salem, 2004).

However the benefits of PEG have been evaluated in Mediterranean regions and in another place in Africa, with clear benefits for live weight gain and lactation (Waghorn, 2008). In Anglo-Nubian goats, a breed selected for milk production, which is able to utilise body depots for lactation to a great extent (Landau *et al.*, 1993). PEG greatly enhanced milk production throughout a long lactation, independently of kid survival and growth (Silanikove, 2000). When PEG was given to Mamber goats, PEG was associated with more rapid growth of the litter (Gilboa *et al.*, 2000), most likely resulting from increased milk yield at the onset of lactation. Therefore, it is important to note that PEG alters osmotic pressure in the rumen fluid by increasing the rate of fermentation and that water intake is the mechanism by which ruminant adjusts the rumen osmolality (Moujahed *et al.*, 2000).

2.5.3 Wool production

CTs' seem to have different effect on wool growth depending on the concentration. At low concentrations of ct seem to increase wool growth (Terrill *et al.*, 1992). Montossi *et al.* (1996) observed that grazing on *expand h. lanatus*, with its much lower ct concentration (4.2 g ct kg⁻¹ dm), increased wool production by 10%; the reduced protein degradation in the rumen and the better AA absorption from the small intestine noted by (Barry *et al.*, 1986 and Waghorn *et al.*, 1987), which could be responsible for the increased wool growth when *Hedysarum coronarium* (Terrill *et al.*, 1992) and *Lotus corniculatus* were fed to sheep; and the reverse situation may be observed when CT level in the diet is high. Barry, (1985) suggested that the reduction of wool growth by tannins could be due to 1)

decreased protein absorption, limiting the amount of sulphur amino acids available for wool production, and 2) an elevation of plasma growth hormone concentration which would divert amino acid away from wool synthesis. Latter Barry (1987), again observed a decreased rates of body weight gain and wool growth when, *Lotus pedunculatus* containing 76–90 g ct kg⁻¹ of dm, which has been explained due to the presence of high concentrations and different types of CTs in forages (Mueller-Harvey, 2006; Waghorn and Shelton, 1997).

Several authors (Wang *et al.*, 1994; Min *et al.*, 1999 and 2003), using PEG for comparisons, indicate that the grazing of *L. corniculatus* (30-35 g CT kg⁻¹ DM) increases wool production by 10-14%, which they attribute to a greater absorption of essential amino acids (especially sulphur amino acids) in the intestine. Prasad *et al.* (1997) reported that sheep fed khejri (*Prosopis cineraria*) and supplemented with polyethylene glycol had higher wool yields (587 vs. 444 g) than unsupplemented sheep. Therefore wool growth has a direct correlation with protein utilization (Priolo and Ben Salem, 2004).

However, later work by McNabb *et al.* (1993) and Wang *et al.* (1994) appeared to suggest that tannins do increase the availability of sulphur for body synthetic reactions. Likewise, other workers have failed to record changes in the levels of growth hormone (Wang *et al.*, 1996a). In other reports, consumption of *Lotus pedunculatus* with and without polyethylene glycol supplementation had no effect in the growth rates or wool production of sheep (Waghorn *et al.*, 1994a; Waghorn and Shelton, 1995).

2.6 Anti-nutritional factors in browses

Anti-nutritive factors are those physical and chemical features of plants potentially available for grazing or browsing, may be generated in natural feedstuffs by the normal metabolism of species and by different mechanisms such as inactivation of some nutrients, reduction of the digestive process or metabolic utilization of feed, which result in lower levels of animal productivity than would be expected from proximate and mineral analyses of the foliage (Akande *et al.*, 2010). There are about 8,000 polyphenols, 270 non-protein amino acids, 32 cyanogens, 10,000 alkaloids and several saponins which have been

reported to occur in various plant species (Kumar, 2003). Many features of plants are capable of reducing their acceptability to animals and they range from the purely physical, to symbiotic relations and chemical reactions (Paterson, 1993). Being an ANF is not an intrinsic characteristic of compounds, but depends up on the digestive process of the ingesting animals can have beneficial or deleterious effect on organisms consuming them (Aganga and Tshwenyane, 2003). They diminish animal productivity but may also cause toxicity during period of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities.

2.6.1 Physical future

Physical features of plants generally affect their acceptability to livestock and consequently the level of voluntary feed intake (Paterson, 1993). Growth form is the most obvious physical deterrent to consumption by herbivores. Most trees will rapidly grow out of the reach of domestic grazing animals unless they are suitably managed. Species such as *Leucaena leucocephala* will readily regrow after severe cutting or defoliation, even to ground level, while others such as *Sesbania* species are much less tolerant of complete defoliation (Skerman *et al.*, 1988). Within any given genus there may be both armed and unarmed species, but *Acacia*, *Caesalpinin*, *Erythrina*, *Mimosa* and *Prosopis* are all recognized for their dangerous thorns and spines (Allen and Allen, 1981). In Chile, goats are, to some extent, able to avoid the spines while browsing the green foliage (Habit *et al.*, 1981). In Australia, *Acacia polyacantha* has spines that protect it from most grazing animals (Skerman *et al.*, 1988), while in Africa, species such as *A. detinens*, *A. karoo* and *A. tortilis* form impenetrable thickets which can impale competition (Allen and Allen, 1981). Thus thorns restrict leaf accessibility and retard rate of nutrient ingestion by restricting bite size of browsers (Cooper and Owen-Smith, 1986; Teague, 1989; Belovsky *et al.*, 1991). Removal of thorns is however, not a feasible alternative, especially when feeding medium to large herds. It is worthwhile to develop a more practical and low cost strategy of collecting leaves for feeding such herds (Mapiye *et al.*, 2011).

2.6.2 Chemical features

Despite the relatively high crude protein contents found in many forage trees/shrubs, anti-nutritive factors (ANF) profoundly limit their nutritive value (Carolyn and Peter, 2008). The major anti-nutrients mostly found in plant protein sources are toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors (Akande et al 2010).

2.6.3 Phenolics

Phenolic compounds represent a group of bioactive compounds that are derivatives of the pentose phosphate and phenylpropanoid pathways in plants (Balasundram *et al.*, 2006), and commonly found in the plant kingdom, include several groups of different substances, among them tannins, flavonoids and phenolic acids, are one of the most important classes of compounds for their biological activities, like antioxidant properties (Skerget *et al.*, 2005; Conforti *et al.*, 2008; Liu *et al.*, 2008); and their implications in animal nutrition (Tedesco *et al.*, 2001).

Among them, tannins can be defined as an extremely complex group of polyphenolics compound and represent an important class of plant secondary metabolites that are produced by the plants in their intermediary metabolism (Jayanegara *et al.*, 2011). Chemically, they are polyphenolics compounds with varying molecular weights, and they have the ability to bind natural polymers such as proteins and carbohydrates (Mueller-Harvey, 2006). Based on their molecular structure, tannins are classified as hydrolysable tannins (HT; polyesters of Gallic acid and various individual sugars) being more widely distributed in nature (Hagerman *et al.*, 1992) and condensed tannins (CT; polymers of flavonoids), also named proanthocyanidins (PA) because this term is more correlated with their chemical structure, present several implications on the nutritive value of temperate forages fed to ruminants and have been studied by various authors (Wang *et al.*, 1996b; Douglas *et al.*, 1999; Min *et al.*, 2003; Haring, *et al.*, 2008; Wolfe *et al.*, 2008).

With respect to ruminant nutrition, tannins are considered to have both beneficial and detrimental nutritional effects and their relationship with animal nutrition involves a number of different research areas such as the interactions between condensed tannin and bacterial cells (Min BR *et al.*, 2003) and the interactions between condensed tannin and forage proteins (Haslam *et al.*, 1989). The beneficial effects may include better utilization of dietary protein, faster body weight gain or wool growth, higher milk yield, increased fertility, and improved animal welfare and health through the prevention of bloat and reduced worm burdens (Mueller-Harvey, 2006). However, effects of condensed tannins on digestibility and utilization of feeds depend on the source and level of tannins applied, structure and chemical nature, the nature of tannin activity, structure–activity relationships, and biological activity (Schofield *et al.*, 2001; Mueller-Harvey, 2006; McSweeney *et al.*, 2008) and the environmental factors influencing their content in plants (Piluzza and Bullitta, 2010). Hence, parameters such as molecular weight and solubility of tannins in water versus organic solvents have become more important than simply tannin concentration (Mueller-Harvey *et al.*, 2007). Also many authors underline that the content of tannins varies with the growth stage of species (Wolfe *et al.*, 2008; Marais *et al.*, 2000; Haring *et al.*, 2007) and stress the fact that care must be taken to define the growth stage of plant materials extracted for analysis (Koupai-Abyazani *et al.*, 1993).

On the other hand tannins have been considered to represent a promising group of compounds for decreasing enteric CH₄ emissions from ruminants by dietary means (Animut *et al.*, 2008a, b; Bhatta *et al.*, 2009). It was estimated that, globally, ruminants produce 80 million tons of CH₄ annually, which accounts for 28% of anthropogenic emissions (Beauchemin *et al.*, 2008). However, it seems that findings on whether tannins are able to genuinely suppress ruminal CH₄ formation per unit of digestible nutrient, and the extent to which this effect occurs, appear to be inconsistent. For example, Beauchemin *et al.* (2007) found that feeding up to 20 g quebracho (*Schinopsis* species) per kg of dietary dry matter (DM) failed to reduce enteric CH₄ emissions from growing cattle, although the protein binding effect of the CT was evident. Further publications reported a lack of effect on CH₄ emissions of chestnut (*Castanea sativa*) wood tannins (Śliwiński *et al.*, 2002a, b) and sorghum (*Sorghum bicolor*) tannins (De Oliveira *et al.*, 2007). It remains unclear whether these sources of CT were simply inefficient or whether the level of supplementation was too low (Jayanegara *et al.*, 2011). Negative effects of tannins have

been associated with their potential toxicity to some rumen micro-organisms and in the metabolism of the ruminant (Goel *et al.*, 2005); low palatability and impaired diet digestibility, resulting in a reduced performance, in particular when HT are involved (Tiemann *et al.*, 2008a)

2.7 Methods used to alleviate deleterious effects of tannins in browse trees

Various methods have been used to alleviate deleterious effects of tannins in a wide range of browse species, grain seeds and agro-industrial by-products (Makkar, 2000). These methods have included mechanical or physical techniques (e.g. wilting, processing, ensiling, etc.), inoculation with tannin resistant bacteria (Miller *et al.*, 1995) and chemical techniques (treatment with alkalis, (e.g. urea, ammonia, calcium hydroxide, sodium hydroxide, potassium hydroxide) Makkar and Singh, 1993; Vitti *et al.*, 2005), organic solvents, precipitants (Ben Salem *et al.*, 2007); chelating metal ions (Price *et al.*, 1979), and oxidising agents (e.g. potassium dichromate, potassium permanganate) (Makkar and Singh, 1993).

Although being effective for overcoming toxic effects of tannins (Mueller-Harvey, 2006), alkalis, metal ions and oxidising agents require expertise, result in large losses of soluble nutrients and are corrosive (Vitti *et al.*, 2005). Moreover, if mismanaged, they can be poisonous to people and animals and are not environmentally friendly (Vitti *et al.*, 2005).

Other de-tanninification approaches involve use of microbial enzymes (McSweeney *et al.*, 2001) and tannin binding compounds such as polyethylene glycol and polyvinylpyrrolidone (Priolo *et al.*, 2005; Mlambo *et al.*, 2007). The use of polyethylene glycol (PEG; MW 4 000 or 6 000), for which tannins have higher affinity than for proteins, is by far the most used reagent to neutralize these secondary compounds (Provenza, 2001). Consequently, it would be possible to increase the nutritive value of tanninrich browse by adding compounds such as PEG, which preferentially binds the tannins, making plant proteins more available for digestion. This strategy is very useful in situations where foodstuffs contain high concentrations of tannins. However, the limited availability and high cost of microbial enzymes and tannin binding compounds makes their application

impractical and unprofitable under low input cattle production systems (Ben Salem *et al.*, 2005a). Oven, freeze, and sun air drying techniques have also been used to lessen the adverse effects of phenolics in browse legumes (Dzowela *et al.*, 1995; Stewart *et al.*, 2000).

2.7.1 Oven, freeze, and sun air drying techniques

The form in which the leaves are fed (fresh, wilted or dry) recognized to affect both intake and digestibility of browse species (Palmer and Schlink, 1992). Ahn *et al.* (1989) have shown that the drying of tree legume leaf decreases tannin content and, in the case of *gliricidia* and *Tipuana tipu*, removes all tannin. Hagerman (1988); Mole and Waterman (1987) reported variable effects of drying at 40°C and freeze-drying on extractable tannin contents. Oven and freeze drying methods require expertise, sophisticated equipment and electricity (Ahn *et al.*, 1997; Stewart *et al.*, 2000), which may not be available in rural communities. When Bolivian tree leaves were oven dried at 50°C for 16-24 hours losses of extractable tannins ranged from 19 to 70% compared to fresh leaves, depending on species (AminiPour, 2011). Compared to other methods, sun air drying is a simple, inexpensive and sustainable technique that makes use of readily and abundantly available resources (Dzowela *et al.*, 1995). Sun air drying could, therefore, be a more acceptable and feasible alternative for resource poor producers (Mapiye *et al.*, 2011).

The drying of tropical forages also decreases the apparent content and activity of CT tannins (Ahn *et al.*, 1989), and increases the digestibility of organic matter, fiber and N and performance of sheep fed diets supplemented with *Calliandra calothyrsus* leaf (Norton and Ahn, 1997). Improvement on dried tree legume leaf meal diets may be due to increased nutrient concentration, improved utilization of endogenous N in the rumen, increased rumen escape CP due to the change in the solubility of the CP, and amount and quality of post-ruminal amino acid absorption of leaf meal (Rubanza *et al.*, 2007). Air drying in the sun has been reported to reduce condensed tannins by 15–30% relative to fresh foliage (Ahn *et al.*, 1997). Sun air drying has been proven to improve palatability of some browse species (Nitis, 1986). In practice, it has been recommended to reduce astringency of *Acacia* species, thus increasing intake by ruminants (Ben Salem *et al.*,

1997), thus air drying improves ruminal degradability and digestibility of leguminous tree leaves (Lowry *et al.*, 1996; Hove *et al.*, 2001) and, consequently, animal performance (Rubanza *et al.*, 2005) compared to fresh leaves. However, depending on prevailing conditions, drying for a long period at temperatures above 40 °C can cause losses in water soluble carbohydrates, proteins and vitamins due to respiration, decomposition and Maillard reactions, which in turn reduce digestibility (Ahn *et al.*, 1997).

In contrast Kaitho *et al.* (1997) and Maasdorp *et al.* (1999) found that the palatability of *Acacia* species remains relatively low even after drying. Drying of mature oak leaves under different conditions (90 °C for 24 h, 60 °C for 48 h, shade drying for 24, 48 and 72 h and sun drying for 24 and 48 h) had no effect on the levels of total phenols, condensed tannins, protein precipitation capacity, degree of polymerisation, specific activity of tannins and bound condensed tannins (Mole and Waterman, 1987; Kumar and D'Mello 1995). One of the reasons for this difference was found to be different level of moisture in these leaves (Makkar, 2003). Cassava and leucaena leaves had about 65% moisture whereas oak leaves had 40%. Increase of moisture of oak leaves followed by the heat treatment decreased tannin levels (Makkar and Singh, 1991b). Similarly, removal of water from leucaena leaves by lyophilisation also decreased the extent of tannin inactivation by the heat treatment. The steaming or autoclaving (1.05 kg/cm²) for 10 or 20 min of fresh oak leaves did not decrease the level of total phenols, condensed tannins, ellagitannin, and protein precipitation capacity. Increase of treatment time did not increase the reduction in tannin. The above drying conditions do not appear to hold promise for inactivation of tannins in oak leaves, but could be effective for feedstuffs having higher moisture content (Makkar, 2003).

2.7.2 Chopping and storage techniques

The chopping of leaves and then storage' has found practical application at the farmer's door and is being adopted without any problem, as it requires only a slight change in normal management practices (Makkar, 2003). A decrease of phenolic compounds occurs when the plant material is chopped due to the facilitated contact between tannins and plant enzymes which resulted in decrease of the level of extractable tannins. Makkar and Singh

(1993) concluded that storing leaves of *Q. incana* at 37°C in an aerobic medium decreased the tannin level to a lower extent than in leaves stored under anaerobic conditions. Similarly chopping, anaerobic conditions and extending of the storage duration resulted in an increase in DM and CP content and degradation of acacia leaves and increased the volume of gas produced as a consequence of greater fermentation (Ben Salem *et al.*, 2005b). The inverse relationship between tannin levels and in situ degradability (Khazaal *et al.*, 1994) and gas production from woody species has been demonstrated (Getachew *et al.*, 2002a). Instead of feeding the leaves on the same day as they are lopped, they need only be chopped and stored for about 5–10 days before feeding. The higher extent of inactivation of tannins by chopping of leaves could be due to oxidation of tannins by phenol oxidases present in leaves, as chopping is expected to increase the availability of tannins to the enzyme (Makkar, 2003). Inactivation of tannins during storage was due to their polymerisation to higher ‘inert’ polymers (Makkar and Singh, 1991a, 1993). Tannin concentrations decreased with storage duration, leading to improved degradation of acacia in the rumen. Water treatment in association with anaerobic storage would cause further deactivation of phenolic compounds. The for example to soak acacia leaves in water and to store under air tight conditions would be an ideal alternative (Ben Salem *et al.*, 2005b).

2.7.3 Browse mixtures techniques

Farmers usually minimize antinutritional problems by feeding leaf mixtures, which dilute or reduce toxic effects (Lowry, 1990). Recent research shown that mixtures produced less deleterious effects than tanniniferous browses fed as sole feeds (Pamo *et al.*, 2006; Melaku *et al.*, 2005; Melaku, 2004). This supports the hypothesis derived from ecological studies that the best strategy for herbivores would be to mix diets in order to minimize the energy costs of detoxification (Foley *et al.*, 1999). Free-ranging animals can select their diets so as to avoid the worst effects of tannins. It is said that incidence of fatalities in which tannins have been implicated occur when animals are very hungry and are unable to select alternative feeds. Using tree fodders as supplements to roughages, a common feeding practice, may coincidentally help limit the intake of tannins, however, this is an area which has been little investigated (AminiPour, 2011).

The complementary role between spineless cactus and saltbushes, mainly *Atriplex nummularia* and benefit from the association of these drought tolerant species was highlighted. Cactus pads high in soluble carbohydrates make better use of the high amount of soluble nitrogen in atriplex foliage. Abundant water in cactus pads facilitates excretion of the excessive salt in atriplex foliage. On the other hand, atriplex may overcome nitrogen and fiber deficiency in cactus pads (Ben Salem *et al.*, 2002).

2.7.4 Alkaline treatment techniques

It is possible that tannin may undergo oxidation under alkaline conditions to be converted to inert forms (Swain, 1965; Reichert *et al.*, 1980). Several investigations have demonstrated beneficial effects of soaking of high tannin ingredients in alkaline solutions (Chavan *et al.*, 1979; Laurena *et al.*, 1986; Mohammed and Ali, 1988). Salunkhe *et al.*, (1990) concluded that alkali solutions were generally more efficient in deactivating tannins than acidic or ash treatments. Alkaline treatments such as Ca (OH) ₂, NaOH or wood ash can be very effective in preventing the toxic or antinutritional effects of tannins and/or associated phenolics in the leaves of *Quercus stellata* Wangenh, *Quercus incana* or in sorghum grain (Murdiati *et al.*, 1990), Reichert *et al.*, 1980, Price *et al.*, 1979). The fact that ammonia is just as effective as these metal hydroxides demonstrated that high OH⁻ concentrations, rather than chelating metal ions, were responsible for improving the nutritive value (Makkar and Singh, 1992b).

In the (Mahmood *et al.* (2007) study alkali treatment reduced the total tannin contents of salseed meal by 83% when incubated for 72 h. These results are comparable with the findings of Wah *et al.* (1977) who observed a similar reduction in tannin content of salseed meal when treated with alkaline solution (NaOH) but 37% dry matter was lost when the treated material was subjected to extensive washing with water. Rao and Rao (1986) have also reported 20-71% and 60% losses in dry matter of salseed meal as a result of washing after soaking in sodium hydroxide and sodium carbonate, respectively. These losses in dry matter are undesirable because they lower nutritional quality of feed ingredients due to loss in appreciable amount of protein being extracted with tannin because tannins have greater affinity for protein due to strong hydrogen bonding with

carboxyl oxygen of peptide group (Evers *et al.*, 1999; Kumar *et al.*, 2005; Sathe and Salunkhe, 1981) reported the treatment solution was merely added to the material and was oven-dried rather than washing with water, therefore chances of dry-matter loss by leaching were almost negligible. In addition to that soaking of the material requires a large volume of solution which may increase the cost of chemical and subsequent drying (Mahmood *et al.*, 2007). Wood ash, a cheap source of alkali and easily (potentially) available to farmers, was effective in detanninification of tannin-rich feedstuffs, and also mediated by high pH-mediated oxidation of tannins (Makkar, 2003). Wood ash solutions have also been used traditionally for treatment of high-tannin-containing sorghum and millet for human consumption (Makkar and Singh, 1992a). Magadi soda (sodium sesquicarbonate, sodium carbonate and sodium bicarbonate), another unrefined material containing alkalis has also been shown to reduce tannins in sorghum by 40–50%. A 10% solution of oak wood ash decreased the content of total phenols, condensed tannins and protein precipitation capacity by 66, 80 and 75% in oak leaves, whereas these values for pine wood ash were 69, 85 and 80%, respectively (Makkar and Singh, 1992a). Higher effectiveness of pine wood ash was attributed to a higher level of alkalis in the pine wood ash (pH values of 10% ash solution of pine and oak woods were 11.3 and 10.5, respectively)

Interestingly, NaOH treatment was more effective with whole than with ground sorghum grain, (Mukuru *et al.*, 1992) which could be due to the fact that most of the sorghum phenolics are located in the outer pericarp. The nutritive value of *L. leucocephala*, however, was not improved by urea or wood ash treatment which is not surprising, as these tannins appear to be particularly suited for generating ruminal escape protein (McNeill, 1998). It may be concluded that alkaline treatments are probably most useful for overcoming the acute toxic effects of certain tannin or phenolic compounds. It is notable that deactivation by acid was less than that achieved by alkali treatment. It is possible that some of this difference may be due to different affinities of phenol groups in the acid treated material and phenate groups in the alkali treated material (Mahmood *et al.*, 2007).

2.7.5 Urea treatment techniques

It has been proposed that urea deactivates tannins (Russell and Lolley, 1989) and used in many countries to improve the nutritive value of poor quality roughages like cereal straws, as an aqueous solution of urea under anaerobic conditions releases ammonia, which is alkaline and efficient in disrupting lignin-carbohydrates complexes, thus improving fiber digestion; and it also increases the N content of the feeds (Ben Salem *et al.*, 2005c). This characteristic of urea was utilized to improve the nutritive value of some tannin-rich feedstuffs (Makkar, 2003). The tannin deactivation effect of urea was noted on sorghum grains (Russel and Lolley, 1989), treating sorghum grains with urea solutions containing 2, 3 and 4 g urea/100 ml water with moisture content of 0.26, 0.34 and 0.34 decreased the level of tannins by 0.66–0.70 compared to untreated material. Another factor responsible for enhanced effects observed on urea addition could be the higher pH caused (Makkar and Becker, 1996b) by evolution of ammonia from urea.

2.7.6 Other chemicals

Extraction with organic solvents (acetone, methanol, and ethanol) and treatment with oxidizing agents (potassium dichromate, potassium permanganate and alkaline hydrogen peroxide) were very effective and removed/inactivated up to 90% of the tannins in oak leaves (Makkar and Singh, 1992c) and up to 99% in agro-industrial and forestry by-products (Makkar and Becker, 1996b). The oxidizing agents convert tannins to quinone, which are not capable of forming tannin–protein complexes under normal physiological conditions. The reduction in tannins in oak leaves using alkalis ranged between 70 and 90%, which is due to oxidation of phenolics by oxygen (present in air) at higher pH values and due to alkalinity in the extraction medium. Extraction with aqueous organic solvents (30% acetone, 50% methanol, 40% ethanol) removed about 70% tannins from oak leaves (Makkar, 2003).

Some agro-industrial by-products (seeds of *A. nilotica*, *Mangifera indica* and *Tamarindus indica*) and oak leaves were detanninified by using hydrogen peroxide (a strong oxidizing agent) in the presence of sodium hydroxide. The decrease in tannin content was as high as

99% (Makkar and Becker, 1996b). Ferrous sulphate (0.015 M), a tannin-complexing agent reduced tannins by 85%. Ferrous sulphate is known to form complex with tannins (Deshpande *et al.*, 1986) and increase in the degree of polymerisation in the treated material could be due to binding of phenolics through ferrous ions. These treatments affected the extractable condensed tannins to a larger extent than the bound condensed tannins. The oxidizing agents, potassium permanganate (0.03 M) and potassium dichromate (0.02 M) also decreased tannin level by about 95% (Makkar and Singh, 1992c). Potassium permanganate is easily available in villages in developing countries (generally used for cleaning water in wells), is non-corrosive and hence farmers can use this chemical at home for detanninification of tannin-rich feedstuffs. The use of oxidizing agents holds promise for a large-scale detoxification of tannin-rich feedstuffs because of their low cost. These approaches are very simple, do not require complex equipment and are likely to be adopted by the feed industry in the future both in developing and developed countries; however the use of organic solvents is expected to be more expensive, provided the cost of tannins recovered is higher than the cost of organic solvents used in the treatment (Makkar, 2003).

2.7.12 Addition of tannin-binding polymers

For about 3 decades, it has been known that tannins bind to water-soluble polyvinyl pyrrolidone (PVP), water-insoluble polyvinyl polypyrrolidone (PVPP), and water-soluble polyethylene glycol PEG, which contain a large number of oxygen atoms capable of forming hydrogen bonds with the phenolic groups in tannins, to bind strongly with tannins and precipitate them from solutions and can reduce their antinutritional or toxic effects *in vitro* and *in vivo*, (Villalba and Provenza, 2002; Gunjan *et al.*, 2005; AminiPour, 2011). This property of tannin-binding agents has been exploited for separation of plant metabolites in tannin rich environments and for neutralizing their negative effects in animals. Jones and Mangan, (1977) first used PEG (molecular weight, MW, 4000) to prevent the formation of sainfoin tannin and protein complexes over a wide range of pH, and its presence release protein from the complexes. Also Silanikove *et al.* (2001) reported the use of PEG as a tannin-neutralizing agent to improve the nutritive value of tanniniferous feeds.

2.7.12.1 Polyethylene glycol (PEG)

Polyethylene glycol is a non-ionic polyhydroxyl compound and polyether-diols with two terminal hydroxyl groups and many alternating ether linkages which binds to the hydroxyl groups of tannins, and reduces the negative effects of secondary compounds (Ellen, 2009). PEG is an inert and unabsorbed molecule, which binds with tannins to form a stable, insoluble complex that prevents tannins from binding to protein in the rumen (Decandia, 2000). Polyethylene glycol has been used to counteract the adverse effects of tannins and can be added to the feed to improve digestibility, palatability and intake of tannins in ruminants (Titus *et al.*, 2001). Supplemental PEG can cause increased intake of tannin containing plants by animals as diverse as rats (Horigome *et al.*, 1988); hoggets (Kumar and Vaithiyanathan, 1990); sheep and goats (Pritchard *et al.*, 1988; Titus *et al.*, 2000a, b; and cattle (Landau *et al.*, 2000). Different molecular weights have different solubility, surface tension, viscosity, freezing point, and melting points, thus it has been used to alleviate negative effects of CT in sheep and goats kept at maintenance level (Motubatse, 2008).

2.7.12.2 Poly ethylene glycol role as a detannifying agent

Herbivores provided with a supplement containing peg consumed more tannin-rich forage in feeding (Moujahed *et al.*, 2000) and grazing trials (Bhatta *et al.*, 2004). Prevent tannin-protein complex formation, increase efficient utilization of diets, increased dry matter intake, digestibility, wool growth and live weight gain or reduced live weight loss (Motubatse, 2008). Though it is not clear whether peg can neutralize all tannins present in feeds (Gunjan *et al.*, 2005). Peg supplementation had a significant effect on gas production and volatile fatty acid production, OMD and ME content of different leaves; this improvement in gas production, OMD and me depended on the level of peg and could be due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen (Ahmed, 2008). Also peg supplementation had increased microbial activity and mass, and *in vitro* dry matter disappearance (IVDMD) when rumen microbes are exposed to tannin-rich forages (Cheeseman, 2006).

Besides concentration of tannins, their nature is another factor that would also influence response of animals to PEG incorporation. So there were instances where PEG effects were species-dependent and removed the beneficial tannin effects (Mlambo *et al.*, 2004, Frutos *et al.*, 2004a). The effect of PEG also depends on the level of proteins in the diet, thus higher the level of proteins, lesser is the effect of PEG (Makkar and Becker, 1996a). Furthermore incorporation of PEG has been shown to have beneficial effects in monogastrics (Horigome *et al.*, 1984; Yu *et al.*, 1996a, b), and both beneficial and adverse effects in ruminants. It is therefore not necessary to promote the widespread use of a relatively expensive feed additive like PEG, as has happened during a drought in the 1990s in southern Africa (Mueller, 2006) without prior feeding trials to determine whether or not it is economical for large scale implementation.

2.7.12.4 Means of administering PEG and effectiveness

Different means of administering PEG have been used in the literature to assess the fodder potential of tanniniferous plant species. PEG was included either in concentrate supplement (Decandia *et al.*, 2000), dissolved in drinking water (Ben Salem *et al.*, 1999), infused orally (Wang *et al.*, 1996b; Gilboa *et al.*, 2000) or sprayed in solution on browse foliage given to ruminant animals. Industry can incorporate PEG in a pelleted diet composed of ingredients including tannin-rich by-products (Ahmed, 2008). Ben Salem *et al.* (1999) reported a comparison of the three methods of PEG provision for sheep on *Acacia cyanophylla* foliage; thus on the basis of improvement in rates of acacia intake, apparent diet digestibility, rumen fermentation and microbial synthesis, PEG in concentrate ranked first, followed by PEG in drinking water and then PEG sprayed on foliage. However, they are either impractical under field conditions (drenching) or uneconomical (spraying, harvesting and mixing) (Silanikove, 2000).

Polyethylene glycol is more effective in *in vitro* when delivered in a supplement block or in several small doses than when added all at once in drinking water, sprayed on forage, or provided in concentrate (Getachew *et al.*, 2001). When the animals lick these blocks the PEG will be released slowly, and this is expected to supply higher microbial protein post-ruminally as a result of higher efficiency of microbial protein synthesis mediated probably

via better synchronization of ATP production and release of nutrients; inclusion of energy sources with the aim of synchronizing nitrogen degradability and availability of energy increased the efficiency of microbial protein synthesis in the presence of PEG (IAEA, 2002). Therefore incorporating PEG in the 'lick blocks' is likely to decrease the cost of PEG treatment through lower PEG requirement (Getachew *et al.*, 2001) and possible self-regulation of PEG intake (Villalba and Provenza, 2001).

However, administration of PEG to sheep and goats once daily by mixing it with small amount of concentrates (Decandia *et al.*, 1998) or by mixing with the drinking water has been proposed as practical for field application. Similarly Motubatse (2008) reported mixing with water is by far the most simple and practical method of applying PEG. In other study Narvaez *et al.* (2011) supplied PEG in drinking water at 0.15% BW to sheep resulted in considerable increases in the intake of DM (24.5% in goats and 25.9% in sheep), ME (22.2% in goats and 28% in sheep) and CP (10.8% in goats and 8.3% in sheep), of *Arctostaphylos* per unit of metabolic weight. Thus, despite the rapid washout of PEG from the rumen as a water-soluble molecule, the typical mean retention time of fluid in the entire gastrointestinal tract approximately 40 h, (Silanikove *et al.*, 1993), and allow effective neutralization of ruminal and postruminal effects of tannins by PEG.

2.7.11.5 Level of PEG used to attenuate the tannin effects

There are variations on level of peg used to attenuate the tannin effects on *in vitro* assays. The amount of peg incorporated into the diets varied from 3 to 120 g per day, with varying responses (Getachew *et al.*, 1998). Min *et al.* (1998) reported that polyethylene glycol 4000 ho (c2h4o)_n h was mixed in water at the rate of 0.5 g peg/ml; then the solution was drenched to sheep at a rate of 48 ml respectively with 6.3 % condensed tannin on dry matter (dm) basis. Many studies use peg based on weight of samples (Jones *et al.*, 2000; baba *et al.*, 2002; Muetzel and Becker, 2006). For example Jones *et al.* (1998) tested 10 levels of peg 4000 ranging from 0 to 1000 mg/g sample and they recommend the highest level (1 g/g) to be used in further gas production studies using peg to bind tannins. Similarly Vitti *et al.* (2005), used 1 g/g sample, also Jones *et al.* (2000) used 160 mg peg/g sample; Tolera *et al.* (1997) and Rubanza *et al.* (2005) used 2 g/g sample. Even in these

experiments with peg dose based on sample weight, the peg: substrate ratio varies considerably and standardization is still necessary (Bueno *et al.*, 2008).

And few others based on amount of tannins (Salawu *et al.*, 1997), this outhor used the syringe method of (Menke and Steingass, 1988) to determine the effect of three concentrations of PEG: flavanol ratios 2:1, 1.5:1 and 1:1 (on weight basis) and mentioned that addition of PEG slightly improved the gas production for leaves, however, it increased gas production for stems and suggested that it is not tannins alone that limit gas production in leaves. Getachew *et al.* (2001) also evaluated three plants with the syringe gas method, using two PEG levels to attenuate the tannin effects: 38.5 and 77 mg PEG per 385 mg sample; the addition of PEG to tannin-containing browses increased *in vitro* gas, SCFA and ammonium N production, and improved microbial growth and the efficiency of microbial protein synthesis without affecting the SFCA production.

Studies to know the *in vivo* optimum tannin: PEG ratio for ‘near-complete’ inactivation of tannin-rich feedstuffs, by taking into account the tannin level and activity and intake of tannins are needed. The treatment increased apparent digestibility of nitrogen by 0.26 and also led to modest increases in cellulose and hemicellulose digestibility. Oral administration of PEG (MW 3350) at the rates of 40 and 60 g per day to lambs grazing on Sulla and pasture was used by (Terrill *et al.*, 1994) and no consistent effect showed on the sheep grazing tanniferous Sulla or (tannin free) pasture. The amount and frequency of dosing PEG depends on the tannin content of the diet, which varies with environment and season. One solution to such problems is to let animals’ self-regulate intake of PEG (Silanikove, 2000).

2.7.14 *Albizia* species its potential and limitations as a ruminant feed

The genus *Albizia* is distributed in Asia, Africa, Australia, and tropical and subtropical America. Most species are deciduous woody trees and shrubs. They are easily identified by their bipinnately compound leaves. *Albizia* is a pan tropical genus that includes at least 470 names. Lewis and Rico Arce (2005) gave a figure between 120-140 species; in Africa there are 36 endemic species and in the Neotropics 22. Twelve species of *Albizia* are

native or naturalized in Mexico and Central America, this figure does not include the 'rain tree' *Albizia saman* currently recognized as *Samanea saman* (Polhill, 1994). *Albizia* are adapted to a variety of soils and environment (Allen and Allen, 1981) is among the most widely planted trees in the world (Evans, 1982). Many of these trees grow fast, compete well with other vegetation, and provide multiple services and products. They are commonly planted for soil reclamation, as ornamentals in home garden systems, and as a shade for tea and other plantation crops. *Albizia* species are also a valuable source of fuel wood, fodder, green manure and timber (Parrotta, 1987a; Parrotta, 1987b). *Albizia* species are highly valued in Central America as understory shade trees for crop plantations, soil erosion stabilizers, and soil improvers and as nitrogen fixing species, providers of livestock fodder with high crude-protein content, as timber trees and also as providers of water-soluble gum (Allen and Allen, 1981 and Sprent, 2001).

Albizia gummifera belongs to the family Fabaceae, subfamily Mimosoideae. Whereas many activities have relied on well known species, *A. gummifera* is a lesser known leguminous tree species with potential for multifunctional benefits. It is a source of timber and medicine and also has potential as an agro forestry tree species. It forms mycorrhizal associations and has the ability to associate with many crops (Katende *et al.*, 1995). The ability of the species to form mycorrhizal and rhizobial symbiotic associations coupled with fast growth in gaps makes it suitable for use in ecological restoration of degraded forests (Judith Ssali Nantongo, 2008). *Albizia* species are nitrogen fixing trees that provide high protein (around 20%) fodder useful for raising small livestock and large. Moreover, *A. gummifera*, is indigenous to Ethiopia, which grows in 1500-2300m above sea level becoming a sustainable fodder source for herbivore livestock in south west Ethiopia. It is the most widely spread species and plays a number of very important roles for people and their livestock in Ethiopia (Yisehak and Belay, 2011).

2.7.15 Nutritive and feeding values of *albizia* species

Within *Albizia*, a truly pan tropical genus comprising 100–150 species (Nielsen, 1981). Several Asian species are known as useful providers of leaf fodder, most notably *Albizia lebeck* (Lowry *et al.*, 1994), as well as *Albizia procera*, *Albizia julibrissin* and *Albizia*

chinensis (Stewart and Dunsdon, 2000). In East Africa, *Albizia amara* leaf, though not highly preferred by livestock, is eaten when alternatives are not available (Gohl, 1981). In contrast a summary of the uses of *Albizia* species by (Roshetko, 1997), showed only one Neotropical species, *Albizia carbonaria* used as fodder. This could be a reflection of the generally low level of use of tree fodder. For a species to be useful as fodder, it must be productive in terms of leaf biomass yield and also respond well to lopping (Mejia, 1997). It should also be noted, that relative palatability will also vary with animal species for instance Kaitho *et al.* (1997) found that relative palatability index values for goats were more than double those for sheep.

A variety of secondary compounds have been isolated from *Albizia* species, some having biological activity. A range of sterols (taxerol, cycloartemol, lupeol, campesterol and sitosterol) have been found in the flowers of *A. lebbbeck* (Asif *et al.*, 1986) and a saponin (echinocystic acid) was reported in root extracts (Shrivastava and Saxena, 1988). Saponins are glycosides of steroid or triterpenoid compounds (e.g. ursane, oleanane and lupane) and, by their detergent action, have been implicated in the formation of bloat in cattle grazing white clover pastures. Triterpenic substances and glycosides of echinocystic acid (saponin) have been isolated from the bark of *A. chinensis*, and these bark extracts have been found to have molluscicidal (Ayoub and Yankov, 1986), spermacidal (Rawat *et al.*, 1989) and insecticidal (Tripathi and Rizvi, 1985) properties.

Rahman *et al.* (1986) also reported that alkaloids from the seeds of *A. lebbbeck* are fungicidal and cytotoxic to selected lines of cancer cells growing *in vitro*. As the name suggests, the neutral non-protein amino acid albizzine was first isolated from *Albizia lebbbeck*, but no toxic activity has been reported. Whilst these compounds may provide some protection against plant predators, they do not appear to affect the palatability and intake of forage trees by ruminants. It has been observed that whilst goats will eat the bark of some browse trees, little bark damage is found when goats browse *A. chinensis*. The high content of saponins in bark may be deterring consumption. There appear to be no reports of saponins in *Albizia* leaf and the dried leaf is non-toxic when fed to rats (Ahn, 1990). Although there is a paucity of information on the effects of *A. chinensis* fed to ruminants, it appears that *Albizia* species may prove to be a valuable new source of forage

for ruminants. A major difference between the species is in tannin content. *Albizia chinensis* contains significant levels of condensed tannins and proanthocyanidins while *A. lebbbeck* contains no extractable tannins (Ahn *et al.*, 1989). The pods and bark of several *Albizia* species are known to contain toxic saponins; for instance fallen pods of the African species *Albizia versicolor* caused a serious outbreak of poisoning in sheep and goats in Malawi (Soldan *et al.*, 1996), several other African species contain compounds used in traditional medicine. Green leaf, fallen leaf and flowers of *A. lebbbeck* have all been shown to be highly palatable and of high nutritive value for sheep (Lowry, 1989). Less is known about *A. chinensis* although it is readily accepted (either fresh or dried) by young goats as a supplement to low quality straws (Robertson, 1988; Ash, 1990) and is eagerly browsed by does and their kids. Comparison by Stewart and Dunsdon (2000) between the treatments on the basis of the index derived from the CP and DOMD data suggested that *Albizia adinocephala* are the most promising of the species tested. *Albizia tomentosa* (syn. *Albizia purpusii*) appeared by far the least promising, with a combination of very low digestibility (DOMD = 16.5%) and relatively low CP (196 mg g⁻¹). The other *Albizia tomentosa* attribute had much higher CP (234 mg g⁻¹) and almost double the digestibility (DOMD = 31.8%), as well as much lower fiber values. *Albizia* species from other parts of the tropics have been showed relatively a high crude protein content (190 mg g⁻¹). Gohl (1981) reported similar CP values, of 151 mg g⁻¹ and 181–220 mg g⁻¹, for fresh leaves of *Albizia chinensis* and *A. lebbbeck*, respectively. *Albizia lebbbeck* is the most well-known of the *Albizia* species used as fodder, and has been the subject of several reviews (Prinsen, 1986; Lowry *et al.*, 1994). Moreover, *A. gummifera* is known for its fast growth rate, high biomass productivity and as an important component of diet for goat, sheep and cattle and play an important role in the nutrition of herbivore animals where there are available fed alternatives are few. On DM basis *Albizia gummifera* leaf has 108-308g CP kg⁻¹, 367-396g NDF kg⁻¹, 283-313g ADF kg⁻¹, 68- 72g CT kg⁻¹ DM kg⁻¹, 379.8-430g IVDMD kg⁻¹, 492.5-683.5g CHO kg⁻¹, 6.1-6.9g MJ ME kg⁻¹, 9.4-28.3g DCP kg⁻¹, 7.02-8.2g DE kg⁻¹, and 400.4-460.3g TDN kg⁻¹. Thus, *A.gummifera* has a potential to be used as a protein supplementation and generally with proper feeding management can be a source of fodder to herbivour livestock fed low quality roughages (Yisehak and Belay, 2011).

3. MATERIALS AND METHODS

3.1 The study area

The experiment was conducted at Jimma University Small Ruminant Research farm, south western Ethiopia located at 7°40'N and 36°50'E and at an altitude of 1780 m above sea level (http://en.wikipedia.org/wiki/Jimma_Zone). The climate of the area, where *A. gummifera* grown and sheep bred, (GOR, 2006) is characterized as humid tropical with bimodal heavy rainfall which is uniform in amount and distribution, ranging from 1200 to 2800 mm per year. The ten years mean annual minimum and maximum temperature of the area was 11.3°C and 26.2°C, respectively. Farmers in the area carry out mixed crop-livestock agriculture. Sheep production is characterized by traditional smallholders that are kept mainly in severely overgrazed private and communal rangelands throughout the year. Foliages of fodder trees and shrubs (Yisehak *et al.*, 2010; Yisehak and Belay, 2011) are becoming potential supplements of ruminants especially in the dry season.

3.2 Animals, feeding management and apparent digestibility of nutrients

Twenty four ram lambs/yearlings with comparable body weight 24.5 ± 0.02 (mean \pm SE) kg and similar body condition score (Campbell *et al.*, 2006) were purchased from local livestock market. The sheep were then transported to Jimma University College of Agriculture and Veterinary Medicine small ruminant research farm for 45 min. immediately, after arrival they were administered ivermectin, a broad spectrum anthelmintic, and vaccinated against pasteurellosis. Later, each treatment group was separately penned, fed and watered in tie stall pens in a well-ventilated barn with a concrete floor. The sheep were fed on hay (the hay was composed of plant mixtures about 82% *Poaceae*, 10% *Astraceae*, 7.5% *Fabaceae*, 0.5% *Cyperaceae* and *Juncaceae* as a control or basal diet) and *A.gummifera* leaves with and without PEG as a supplementary/experimental/diet. The 24 sheep were randomly categorized in to three groups. Each group of sheep was randomly assigned to one of the treatments. The design of the experiment was a completely randomized design with three treatments and eight animals per treatment. The treatments included hay alone (3% of their body weight BW on

DM basis) (T1), inclusion of *A.gummifera* leaf (AG) leaf to the basal diet at a rate 30 % (this rate was based on Devendra, 1988) + basal feed (T2) and AG + PEG (T3). PEG, MW4000, HO (C₂H₄O) nH was purchased from Micron International Trading House Private Limited Company, Addis Ababa.

The live weight basis of feeding was based on NRC (1985) and Osuji *et al.* (1993) in addition to one month pre-experimental trial in the farm; this was done for adaptation in the research station, health checks, and to measure dry matter intake of basal and test diets. In the 1st week of experimental period, second month, initial weights of all animals were re-taken for actual experiment. The treatments are then randomly assigned to the experimental units in each group - one treatment to a unit in each group of sheep. Thereafter, animals were weighed weekly and the amounts of feed offered were adjusted according to the changes in the body weight. Feed offered and refused were measured on daily basis until 13th week. The diets were formulated weekly to make sure that sheep were consuming *A.gummifera* at a rate of 30% of total daily DM requirement. The leaves of *A.gummifera* were collected from farm grown trees between 3 to 4 years. *A.gummifera* leaves were fed (8:00AM) prior to the provision of basal diet up to 10:00 AM in a separate trough. The sheep had free access to water. PEG was mixed in water at a rate of 0.5 g PEG/ml (Getachew *et al.*, 2001). The solutions were given (drenched) to sheep every sheep at rate of 40g PEG to 1 kg of AG.

After the end of the 12th week of feeding, total collection of faeces was carried out for seven consecutive days. The faeces collection bags were harnessed to the animals for daily faeces collection during the collection period. All animals from each treatment were used for faecal collection at a time. Feed offered and refused were weighed and recorded for each animal. Representative samples were then taken for the feed offered every morning before feeding, placed in deep freezer to minimize loss of ammonia until a sub-sample was taken for analysis. Feed refusals were pooled over the experimental period and sub-sampled for analysis. Supplement and basal feed offers and refusals were weighed for each animal daily and their differences were recorded as a daily feed intake per animal. The total faeces voided during the day and nights were weighed, thoroughly mixed for each animal. About 10% of the total weights were sampled every morning, placed in plastic bags and stored at -20°C until analysis. At the end of the experiment sub-samples

from frozen samples were taken, mixed /agitated and oven-dried at 60°C for 48 hr. The dried faeces were grounded in a Wiley mil to 1mm screen and stored at an airtight container until analysis (AOAC, 2005; Osuji *et al.*, 1993). DM intake and apparent DM digestibility of the feed were calculated for each group of animal by following Osuji *et al.* (1993) and McDonald *et al.* (2002). The apparent digestibility coefficients and digestible nutrient content of the experimental diets were determined according to McDonald *et al.* (2002). Feed conversion efficiency (FCE) was measured as proportion of average daily BW gain to daily DM intake (Ball and Pethick, 2006). Metabolizable energy intake (MEI) (kJ/kg BW^{0.75}) was estimated according to Luo *et al.* (2004) as: 533 + (43.2*ADG (g/kg BW^{0.75})).

3.3 *In vitro* gas production assay

Rumen fluid was obtained from two rumen cannulated Holstein-Friesian cows receiving silage mixtures and a regular concentrate. A sample of rumen content was collected before the morning meal in thermos flasks and taken immediately to the laboratory where it was strained through four layers of cheesecloth and kept at 39°C under a CO₂ atmosphere. For the assessment of the kinetics of gas production, technique proposed by Menke and Steingass, 1988 was followed. DM (200 mg) was weighed in triplicate into serum bottles kept at approximately 39°C and flushed with CO₂ before use. Buffer solutions and rumen liquor/buffer (1:4) were prepared as described above. Nearly 50 ml of rumen/buffer mixture was anaerobically dispensed in each bottle with or without addition of 2 ml of aqueous solution of PEG (25%, g/ml). Six bottles were used for each substrate, three for each treatment (with or without PEG). All the bottles were crimped and placed in the incubator at 39°C, being shaken at regular times. The volume of gas produced in each bottle was recorded at different incubation times (2, 4, 6, 8, 10, 12, 14, 24, 32, 48, 56, 72, and 96h of post incubation). In order to compensate for gas production in the absence of substrate three serum bottles containing rumen fluid inoculums with or without PEG were incubated as controls. Fermentation gas was analyzed using gas chromatograph(GC).

3.4 Organic matter digestibility, metabolizable energy and short chain fatty acids estimation

The *in vitro* organic matter digestibility (OMD), metabolizable energy (ME) value and the short chain fatty acids (SCFA) contents of diets were estimated with the following equations:

$$\text{OMD (\%)} = 0.9991 (\text{GP}) + 0.0595 (\text{CP}) + 0.0181 (\text{CC}) + 9 \text{ (Menke and Steingass, 1988)}$$

$$\text{ME (MJ/kg DM)} = 0.157 (\text{GP}) + 0.0084 (\text{CP}) + 0.022 (\text{EE}) - 0.0081 (\text{CA}) + 1.06 \text{ (Menke and Steingass, 1988).}$$

$\text{SCFA (mmol)} = 0.0222 (\text{GP}) - 0.00425 (\text{Makkar, 2005})$, where: GP is gas production at 24 h of incubation, CP, EE and CA are crude protein, ether extract and crude ash content of diets, respectively.

3.4 Slaughtering and carcass evaluation

At the end of experiment, the sheep were fasted overnight, weighed and slaughtered to determine carcass parameters. The slaughter weight, carcass weight, edible and non-edible offal for each animal were weighed and recorded. Dressing percentage values for each treatment was determined based on the empty body weight basis rather than on live weight at slaughter basis, otherwise the influence of digesta (gut fill) would exaggerate dressing percentage (Gibbs and Ivings (1993) and El-khidir *et al.* (1998)). Hence, dressing percentage was calculated on the bases of empty BW (absence of gut fill) as: $\text{dressing percentage} = \text{hot carcass weight} / \text{empty body weight} * 100$. Total edible offal component was taken as the sum of liver, reticulo-rumen, small intestine, kidneys, heart and tongue. Total non-edible offal component was computed as the sum of blood, spleen and pancreas, head, skin, testis and penis, gut fill and feet (USAID, 1982; Clotter, 1985).

3.5 Chemical analysis and nutritive value evaluation of feed and faeces samples

The leaves of *A. gummifera* and basal diet, hay, were ground and analyzed for DM, organic matter (OM), crude protein (CP), crude ash (CA), crude fiber (CF) and ether extract (EE) according to the standard procedures of AOAC, (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest *et al.*, (1991). Lignin was determined by solubilization of cellulose with H₂SO₄ (Robertson and Van Soest, 1981). Hemicellulose (%HC) was calculated from the difference between %NDF and %ADF. Determination of total condensed tannins (CT) was based on oxidative depolymerization of condensed tannins in butanol-HCl reagent using 2% ferric ammonium sulfate in 2N HCl catalyst (Porter *et al.*, 1988) with the modifications of Makkar, (2003) and using purified quebracho tannin as standard. Concentration of CT was expressed in g/kg DM, standard equivalent. All chemical analyses were carried out in triplicate. The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). The faeces samples were used for estimating DM by oven drying at 105 °C for 24 hours for chemical analysis. Non-oven dried but well-mixed faeces were directly used for nitrogen analyses. Total carbohydrate (CHO) content was estimated according to Ranjhan, (1997). On the other hand, protein efficiency ratio (PER) was calculated according to Ranjhan, (2001) procedure.

3.6 Statistical analysis

Data was subjected to analysis of variance according to completely randomized design (CRD) using general linear model procedure of statistical analysis system (SAS, 2010 version 9.3). Differences between treatment means were compared by Duncan's multiple range test and significant differences were declared when $p < 0.05$. The statistical model used for the CRD analyses of all variables was: $y_{ij} = \mu + t_i + e_{ij}$; where y_{ij} is the dependent variable; μ is the overall mean; t_i is the effect of treatment; e_{ij} represents random error that assumed normally and independently distributed. For the analysis of carcass parameters slaughter weight was added as a covariate in the model. Correlation analysis was used to test relationships between average CP concentration in the diets, *in vivo* and *in vitro* nutritive value parameters.

4. RESULTS AND DISCUSSION

4.1 Nutritional quality of experimental feedstuffs

The nutritional quality of basal and test diets included in the trial are presented in Table 1. Although *A.gummifera* leaves contained high amount of CT (72 g CT/kg DM), it's CP, IVOMD and ME content was higher by 357%, 112% and 113%, respectively than that of basal feed, respectively. Both of the feedstuffs contained high fibre (NDF and ADF) contents. Yet, no traces of CT were determined for the control diet, the trends in NDF, ADF and ADL contents were dissimilar to that of CP, IVOMD and ME contents of the feedstuffs.

Table 1. Chemical composition (g/kg DM), in vitro organic matter digestibility (g/kg DM) and metabolizable energy (MJ/kg DM) of basal and test diet, *A.gummifera*, offered to sheep (g/kg DM)

Feedstuff	DM	CA	OM	CP	EE	CF	NFE	CT	NDF	ADF	ADL	HC	CHO
Hay	895	116	884	84	14	365	421	BDL	738	580	123	457	658
<i>A.gummifera</i>	889	50	950	300	10	380	260	72	786	651	189	462	457

DM, dry matter; *CA*, crude ash; *OM*, organic matter; *CP*, crude protein; *EE*, ether extract; *NFE*, nitrogen free extracts; *NDF*, neutral detergent fiber; *ADF*, acid detergent fiber; *ADL*, acid detergent lignin; *HC*, Hemicellulose; *CT*, condensed tannin; *BDL*, below determination limit; *CHO*, total carbohydrate

The high CP content in the leaves of *A. gummifera* (300 g CP/kg DM) could be due to the N-fixing ability of the species. As expected the CP content of *A.gummifera* was generally higher than that of tropical grasses (Skerman and Riveros, 1990). In the present study, CP content was particularly high (Table 1) and greater than data reported for foliages of other leguminous MPTS such as *Leucaena leucocephala* (193 g/kg DM) (Longo *et al.*, 2008), *Sesbania sesban* (Kaitho, 1997), *Acacia saligna* (114 g/kg) (Krebs *et al.*, 2007) and other non-leguminous MPTS (Kindu *et al.*, 2006; Yisehak *et al.*, 2009). The notion of present study is in accordance with the reports of Solomon *et al.* (2004); Kindu *et al.* (2006); Mekoya *et al.* (2008); Aynalem and Taye (2008); Yisehak and Belay (2011) in that many multipurpose trees and shrubs gain increasing significance as the nutritional value of grass

drops and fibrous crop residues. The CP content calculated for all the leaves of *A.gummifera* is much higher than the minimum CP level (70 gCP/kg DM=45.5 g DCP/kg) required for optimum functioning of rumen (McDonald *et al.*, 2002; Norton (2003) and for adequate intake of forages (Davies, 1982). The optimum concentration level of rumen bacteria is reached at a CP level in the diet of 130g CP/kg (=85g DCP/kg). The minimum CP content required for lactation and growth of cattle is 150 g/kg DM (Norton, 1994). The results of the present study also agreed with Getachew *et al.* (2000) in that the browse forages are better used as protein supplement than poor quality roughages such as hay, straws and stovers. Voluntary feed intake rapidly falls if CP content of forage is below 62 g/kg (Nasrullah *et al.*, 2003). Although it is well reported that feeds containing less than 7% CP cannot provide the minimum ammonia levels required by rumen microorganisms to support optimum activity, foliage of *A.gummifera* can be potential supplement for low CP feeds provided that CT would be extracted biologically.

The EE content of dietary feedstuffs (avg. <50 g/kg DM) is an indication of low energy level for the animal. Odedire and Babayemi (2008) reported that feedstuffs contained more than 50 g EE/kg DM considered higher energy level for an animal. Moreover, total diets (Preston, 1995) do not contain EE more than 100g/kg is acceptable. Fats and oils are extremely rich sources of energy, although because they impede microbial fermentation, ruminant diets should be limited to about 40 g EE/kg DM (Campbell *et al.*, 2006).

All of the dietary feedstuffs contained high NDF and ADF contents (avg.>600 g/kg). Minimum NDF content of a ration should be in the range of 270-300g/kg (Jolly and Wallace, 2007). The threshold level of NDF in tropical grass beyond which DM intake of ruminants affected is 600 g NDF/kg (Meissner *et al.*, 1991) suggesting that all the diets have not acceptable NDF values (above 600 g NDF/kg DM). Tree forages with a low NDF content (200–350 g/kg) are usually of high digestibility (Norton, 1994). The digestibility of plant material in the rumen is related to the proportion and lignification of cell walls (Van Soest, 1994). High ADL content can limit the voluntary feed intake, digestibility and nutrient utilization of ruminant animals (Khanal and Subba, 2001). Moreover, lignin as a percentage of lingo-celulose is highly correlated with the digestibility of cell wall fraction (VanSoest *et al.*, 1991).

The highest CT levels observed in *A.gummifera* (720 g/kg DM, quebracho tannin equivalent), consistently with the results pointed out in the Yisehak *et al.* (2010). It has been reported that CT values above 50 g/kg DM can become a serious anti-nutritional factor in plant materials fed to ruminants and are even toxic (Leng, 1997). Barry and Manley (1984) explain that forage containing more than 50 g/kg CTs is considered CT-rich forages. Higher tannin levels become highly detrimental as they reduce digestibility of fiber in the rumen by inhibiting the activity of bacteria (Chesson *et al.*, 1982) and anaerobic fungi (Akin and Rigsby, 1985), high levels also lead to reduced intake (Leng, 1997).

4.2 Feed intake and live body weight changes

The mean DM intake (DMI) during 90-days of feeding was higher for the lambs supplemented with either *A. gummifera*, T2 (622.10) and *A. gummifera* + PEG, T3 (761.30), as compared to the control diet, hay alone (T1, 482.51) ($p<0.001$) (Table 2). DMI was dropped significantly for the first week because tannin-containing diets were affected indicating that the response is more likely due to learned food aversion caused by post-ingestive consequences rather than decreased palatability, but for the subsequently weeks significantly increased trends of DMI were recorded for either both T2 and T3 when compared with T1 (Figure 1). Although the trends in DMI were increasing with increases in the number of feeding periods, the highest DMI (957.38 g/d) was recorded for T3 in week 12 followed by T2 (742.59 g/d) ($p<0.001$). DMI was not consistent for T1 throughout the feeding weeks thus in turn resulted in inconsistency of overall mean values across the feeding period.

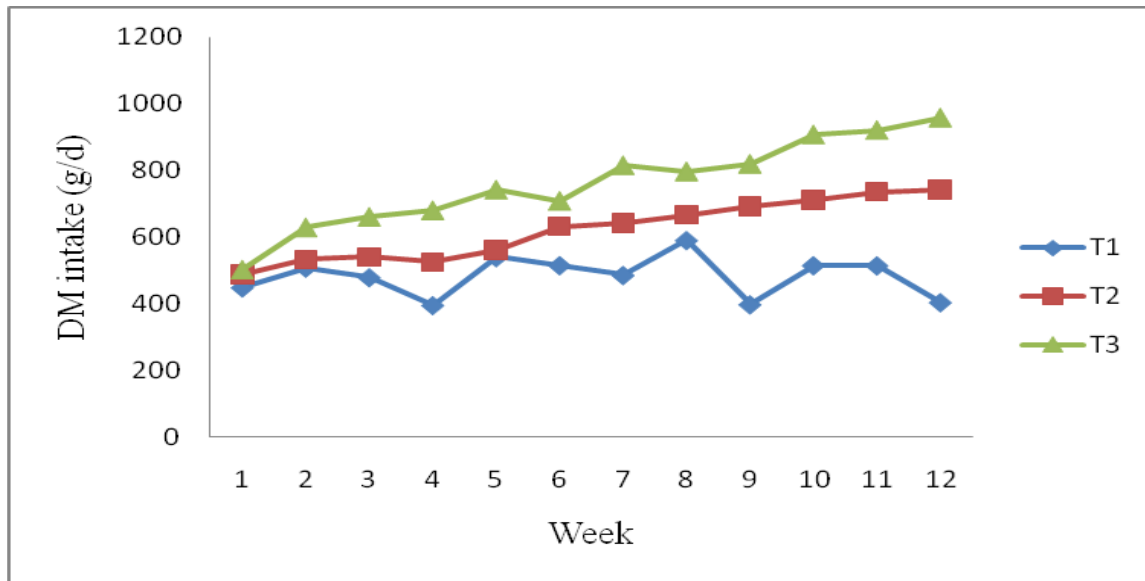


Figure 1. Average weekly dry matter intake of sheep (g/d)

Table 2. Average weekly dry matter intake of sheep during 90 days of feeding different diet groups

Week	Mean, DM intake(g/d)			SE	P
	T1	T2	T3		
1	447.90 ^b	487.00 ^b	502.20 ^a	5.13	*
2	508.00 ^c	532.61 ^b	628.68 ^a	11.00	**
3	478.71 ^c	539.45 ^b	660.33 ^a	16.00	***
4	394.18 ^c	524.41 ^b	679.71 ^a	28.21	***
5	539.64 ^c	559.86 ^b	741.56 ^c	19.06	**
6	515.43 ^c	632.11 ^b	707.97 ^c	16.61	***
7	485.48 ^c	641.10 ^b	814.72 ^a	28.08	***
8	591.18 ^c	667.05 ^b	796.25 ^a	17.70	**
9	396.73 ^c	691.75 ^b	819.11 ^a	38.75	***
10	515.08 ^c	710.94 ^b	907.05 ^a	33.54	***
11	514.80 ^c	736.37 ^b	920.34 ^a	34.70	***
12	402.95 ^c	742.59 ^b	957.38 ^a	37.36	***
M	482.51	622.10	761.30		

T1, treatment one; T2, treatment three; T3, treatment three; M, overall mean; SE, standard error of means; mean values within rows with different superscripts are significantly different ($p < 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Although animals were weighed throughout the trial, a loss in body condition was obvious in particular in the T1 ($p<0.001$) (Table 3, Figure 2). The average daily live weight change was significantly different among treatments in different weeks, where the highest and the lowest ADG values were achieved in T3 and T1, respectively ($p<0.001$).

Table 3. Average daily weight gain of sheep during 90 days of feeding different diet groups

Week	Mean ADG (g/d), treatments			SE	P
	T1	T2	T3		
1	0.80 ^b	10.82 ^a	14.21 ^a	1.70	**
2	2.12 ^c	8.72 ^b	16.89 ^a	1.52	***
3	4.23 ^c	9.46 ^b	17.91 ^a	1.84	***
4	-2.25 ^c	10.46 ^b	20.27 ^a	1.35	***
5	-1.38 ^c	12.55 ^b	22.71 ^a	2.30	***
6	-0.25 ^c	11.14 ^b	22.82 ^a	2.17	***
7	1.19 ^c	12.31 ^b	22.04 ^a	2.00	***
8	6.03 ^c	12.56 ^b	23.13 ^a	2.36	***
9	2.12 ^c	12.01 ^b	23.68 ^a	1.92	***
10	-0.19 ^c	12.01 ^b	24.30 ^a	2.21	***
11	1.52 ^b	12.31 ^b	26.27 ^a	2.31	***
12	2.08 ^c	13.10 ^b	30.24 ^a	2.50	***
M	2.46	11.24	21.46		

ADG, average daily gain; T1, treatment one; T2, treatment three; T3, treatment three; M, overall mean; SE, standard error of means; mean values within rows with different superscripts are significantly different ($p<0.05$); ** $p<0.01$; *** $p<0.001$

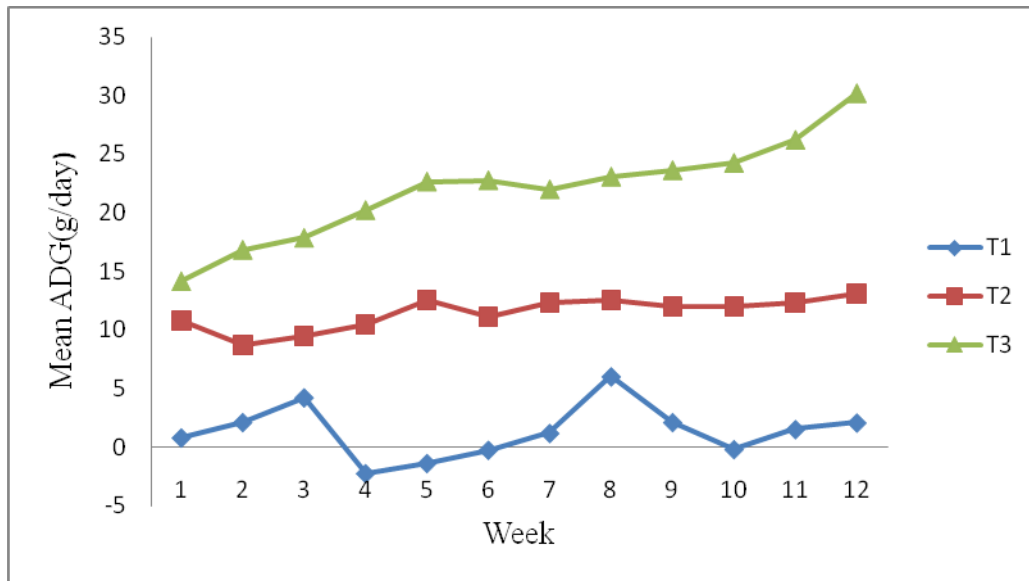


Figure 2. Average weekly liveweight gain of sheep (g/d)

4.3 Nutrient intake, utilization and growth performance of sheep during collection period

Average daily intakes of chemical nutrients, feed conversion efficiency, protein efficiency ratio, and live body changes of the lambs during faeces collection period are reported in Table 4. Lambs fed control diet in combination of *A. gummifera* and PEG showed an increase of 168.30% of DMI (1.06 kg/animal/day) as compared to control group (0.63 kg/animal/day). Similarly, NDFI of T2 (0.34 kg/animal/day) was numerically 142% higher than T1 (0.24kg/animal/day). Lambs fed a T1 diet had a low intake for most of the chemical nutrients as compared to supplemented groups ($p < 0.001$). Daily intakes for the rest of chemical nutrients followed the same trend ($p < 0.05$). The highest and lowest mean live weight gain of lambs was recorded for T3 (34.90 g/day) and T1 (3.00g/day), respectively. PEG supplemented sheep showed the highest LWG followed by T2 where as the lowest daily gain was recorded for T1 ($p < 0.001$). Sheep fed control diet were lowered by 5% and 12% LWG than T2 and T3, respectively. The lowest FCE (0.54) and PER (0.04) were determined for T1 whereas the highest values were recorded for T3 (3.55 and 0.21, respectively). In addition, the FCE in lambs fed T2 and T3 increased from the 362% and 710% over T1, respectively ($p < 0.001$).

Table 4. Nutrient intake, live weight gain, feed conversion efficiency and protein efficiency ratio of sheep supplemented with a tannin rich diet and polyethylene glycol during faeces collection period

Nutrients	Mean, treatments			SE	P
	T1	T2	T3		
DMI, kg/day	0.63 ^c	0.91 ^b	1.06 ^a	0.05	***
CPI, kg/day	0.084 ^c	0.092 ^b	0.171 ^a	0.01	***
EEl, kg/d	0.013 ^b	0.023 ^a	0.024 ^a	0.00	*
OMI, kg/day	0.58 ^c	0.73 ^b	0.81 ^a	0.04	**
MEI, MJ/day	690 ^c	794 ^b	949 ^a	25.51	***
NDFI, g/day	0.24 ^b	0.34 ^b	0.42 ^a	0.02	***
LWG, g/day	3 ^c	14 ^b	35 ^a	2.84	***
FCE, %	0.50 ^c	1.81 ^b	3.55 ^a	0.03	***
PER, kg ADG/g CPI	0.044 ^c	0.162 ^b	0.214 ^a	0.001	***

DMI, dry matter intake; CPI, crude protein intake; MEI, ME intake; NDFI, NDF intake; M, overall mean; LWG, live body weight gain; FCE, feed conversion efficiency(% g LW gain/g DMI); PER, protein efficiency ratio; SE, standard error of means; *P<0.05; **p<0.01; ***p<0.001

In the current trial the DMI from air dried leaves of *A.gummifera* had significantly improved intake of basal feed over 144 % (T2/T1*100, Table 4). The treatment with PEG had a remarkable improvement in DMI over T1 by 168% (p<0.05). This figure is relatively greater than DMI recorded for Arsi sheep, central Ethiopia, fed to tanniniferous fodder tree leaves(125% improvement over hay based feed), *Acacia angustissima*, 63gCT/kg DM(Asfaw *et al.*, 2006) and Santa Ines sheep supplemented with different level of *Leucaena leucocephala* (101g/kg DM) (Longo *et al.*, 2008). Abou El Nasr *et al.* (1996) reported lower DMI by sheep supplemented tanniniferous fodder, *Acacia saligina*, which were not supplemented with PEG. These differences might be better palatability of air dried *A.gummifera* leaves that consecutively resulted in better DMI. The second reason might be better digestibility of *A.gummifera* leaves and also sheep in Jimma is better adapted to *A.gummifera* diet. The low DMI in lambs received T2 without PEG as compared to T3 might have been principally associated with the inhibitory effects of the high CT on digestion (Reed *et al.*, 1990; Chriyaa *et al.*, 1997; Degen *et al.*, 1997), with palatability having a minor influence on DMI.

The lower DMI of lambs received control diet alone (ha), T1, at all experimental periods as compared other treatment groups could be due to rumen fill that limiting feed intake (Van Soest, 1994) and preventing maximum intake of the hay. The CP content of the hay is not adequate enough to boost microbial growth in the rumen. Tesfaye *et al.* (2008) and Mesfin and Ledin (2004) showed that for dried roughages(hay, straws and others) supplemented with protein and energy sources, the daily DMI improved much more than feeding non-supplemented basal diets. The increased total DMI with *A.gummifera* leaf supplementation could be attributed to a higher intake of CP that led to better efficiency in the utilization of the fiber in the total diet. Moreover, increased availability of nutrients due to the supplementation of the *A.gummifera* with PEG might have promoted the observed higher total DMI intake in the supplemented sheep. The increase in MEI observed in diets containing the *A.gummifera* could also be due to the combined effect of the higher total DMI and digestibility associated with forage legumes intake with PEG. The positive effect of optimum level of leaves of forage legume supplementation on the dried roughages intake observed in this study could be the result of reduced rumen retention allowing for greater feed intake (Melaku, 2004).

Supplementation of *A.gummifera* leaves alone at the levels of 30% (refers to T2 and T3) didn't anticipate toxicity problems and death of sheep within 13 weeks of feeding. In the reports of Sillanikove *et al.* (2001) the antinutritional effects of CTs may be caused by toxicity or feed aversion leading to be an inverse relationship between tannin concentration in browse and voluntary feed intake by herbivory. Attempts reported for reduction of CTs such as drying/heat treatment, wilting and leaching (Szyszks *et al.*, 1983). Increased trends in DMI of a CT rich diet was observed throughout the experimental period, this might be due to adaptation of animals to the specific diet even if the test diet is rich in CT content. Better performances of sheep fed *A.gummifera* with PEG, mainly due to drenching of PEG to sheep completely triggered both feed intake and growth performance of sheep. This is because PEG has the ability to bind phenolic compounds instead of protein and displace protein from performed phenolic-protein complexes (Jones and Mangan, 1977). The improved DMI in sheep in which PEG was added in to the diet was due to the high affinity of PEG to release protein from tannins. PEG also mitigated the adverse effects of CTs on voluntary feed intake and growth rate (Makkar, 2003). Sillanikov *et al.* (2001) reported CT deactivating abilities of PEG in

tanniniferous browses. He also reported improvements in feed intake, body weight gains and reduced toxicity of CTs in sheep supplemented with PEG. The negative effects of CTs on nutrient intake have been reported by many authors. CTs react preferentially with polyethylene glycol (PEG) and the supplementation of PEG has been largely used to eliminate and to measure the effects of CT (Waghorn *et al.*, 1999).

The lower BW gain by the sheep in T1 throughout the experimental period could be attributed to low nutrient intake of CP and ME as compared with those treatments supplemented with *A.gummifera*. The highest daily BW gain and total BW change recorded for T3 over other treatments could be due to increased nutrient density as a result of higher protein in the *A.gummifera* and a reflection of increased total feed DM and nutrient intake. It was also shown that supplementation of leaves of protein rich plants with nutritionally poor roughage diets markedly improved the BW gain of ruminants (Solomon *et al.*, 2004). Getachew *et al.* (1994) also reported higher BW gain in sheep supplemented with the forage legumes. This therefore indicated that the difference in ADG might be partly related to higher intake of N and energy and this observation is consistent with Aregheore (2004; 2006).

Feed conversion efficiency, the term efficiency implies a ratio of outputs to inputs, found to be significantly higher in sheep received T2 and T3 as compared to T1 ($p < 0.001$). The higher the FCE, the higher the quality of the feed and the higher the growth performance and carcass quality of animal (Arthur *et al.*, 2001; Schenkel *et al.*, 2004). Highest DMI due to supplementation of *A.gummifera* with PEG resulted in better growth performance in lambs indicating that CT rich diets biologically deactivated by PEG can be a sustainable feed source. The higher FCE recorded for sheep on the high level of PEG could be due to the higher content of energy and protein in the diet of sheep in this treatment, deactivation of CTs in turn allows animals to utilize potential nutrients from tannin rich diet. Tesfaye *et al.* (2008) also showed the additive effect of combining supplementation of a forage legume (*Leucaena leucocephala*) with nutritionally poor roughages (eg. straws) enhancing nutrient utilization in sheep. An improved trend of FCE was observed in groups of sheep supplemented with a CT rich protein source alone or protein source with a tannin complexing that might be increased proportion of forage legumes in the total DM consumed as well as digested. This is due to better protein utilization as a result of forage

legume inclusion that caused higher BW gain. In agreement with the results of this study, Klopfenstein and Owen (1981) indicated positive associative effects in terms of BW gain and DM intake when crop residues were supplemented with dried leaves of leguminous plants. FCE has been shown to improve in response to an increase in dietary protein and energy (Aktas, 2001; Gorgulu and Ozturkan 1992; McClure *et al.*, 1994).

4 Apparent digestibility coefficients and digestible nutrients

Apparent digestibility coefficients (the weights of nutrients digested as proportions of the weight consumed) of DM, OM, CP, EE and NDF were higher for T3 as compared to T2 and T1 (Table 5 and 6). The highest digestible nutrients(g/kg, the composition of feed calculated in terms of digestible nutrients) of OM, CP, EE and NDF was also recorded for sheep supplemented with either *A.gummifera* alone or *A.gummifera*+PEG as compared to the control group ($p<0.05$)(Table 6). Supplementation of PEG increased apparent digestibility of all the chemical nutrients determined in T3 ($p<0.01$). Inclusion of *A.gummifera* increased DMDC over T1 by 117.31% other hand PEG inclusion in to T2 increased DMDC by 111.48%. Similar trend was observed for digestibility coefficients of other nutrients.

Table 5. Apparent nutrient digestibility coefficients in sheep fed a grass hay-based diet with or without *Albizia gummifera* leaves with or without polyethylene glycol

Nutrients	Mean digestibility coefficients (%)			SE	P
	T1	T2	T3		
Dry matter	52 ^c	61 ^b	68 ^a	0.02	***
Organic matter	56 ^b	66 ^a	67 ^a	0.02	***
Crude protein	47 ^b	50 ^b	66 ^a	0.03	***
Ether extract	50 ^b	60 ^a	64 ^a	0.02	**
Neutral detergent fiber	47 ^b	53 ^{ab}	56 ^a	0.01	***

T1, treatment one; T2, treatment three; T3, treatment three; means in the same row with different letters point to statistical significances; standard error of means; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

The higher digestibility coefficients in sheep received T3 and T3 (Table 5) corresponded to a higher digestibility of nutrients (g/kg DM, Table 6) compared to control group.

The lower DM digestibility coefficient for control diet, T1 (48% indigestibility) as compared to the treatments with *A.gummifera*(39%) or *A.gummifera*+PEG (32%) may be explained in part by high fiber content and relatively low metabolisable protein in the control diet (Table 5). The lower digestibility of CP in the T1 as compared to the other treatments might not only be related to the lower CP content of the basal diet, but also to the lower digestive utilization of nitrogen or higher microbial synthesis in the lower gut (Hassen and Chenost, 1992). There was improvement in CP digestibility in T2 as compared to T1, on the other hand, lower CP digestibility in T2 as compared to T3 might be explained that phenolic compounds reduce protein digestion by forming complexes with dietary protein and digestive enzyme in T2 while energy digestibility in the rumen is reduced by inhibition of microbial enzymes (Cheeke and Palo, 1995). The improvement in CP digestibility in T3 is mainly due to tannin binding effects of PEG. The results of present study is in accordance with the reports of Makkar (1993), Hagerman and Butler (1991) and Kumar and D'Mello (1995) that feeding CT rich diets with PEG positively reduces anti-nutritional effects of CTs in ruminants. In the study of Weigand *et al.* (1995), high levels of CTs depressed the digestibility of NDF. Therefore, higher content of soluble phenolics and CTs in sole MPTs were the major contributing factors for lowered digestibility of N and NDF in the sole MPTs as compared to wheat bran and Nug cake (*Guizotia abyssinica*) supplemented animals. The reports of present study is also in agreement with reports that indicated depression in fiber and N digestibility due to CTs (Reed *et al.*, 1990; Salawu *et al.*, 1999) when tanniferous MPTs were used as feed supplement. Low digestibility coefficients observed in this study is also in agreement with the findings of Solomon (2004) in sheep.

4.5 Carcass parameters of a sheep

The mean values of edible and non-edible carcass characteristics of slaughtered lambs are presented in Table 7. The values of the most edible carcass components such as hot carcass, kidney, liver, heart, kidney fat, omental fat and total ribs were increased in animals supplemented with tannin rich diet with PEG as compared those received a

control diet($p<0.05$). Moreover, carcass weights and dressing percentages were increased as PEG inclusion level increased ($p<0.05$). The highest carcass weight (16.25kg) and dressing percentage (42.81%) were determined for T3 but the lowest values were measured to T1. Similarly, most non-edible carcass traits such as head, feet, testicle, scrotal fat, lung, spleen, pancreas, penis, and subcutaneous fat weights were increased in lambs supplemented with either *A. gummifera* or *A. gummifera* with PEG.

Table 6. Weights of carcass components of lambs fed a grass-based diet with or without *Albizia gummifera* leaves with or without polyethylene glycol

Traits	Mean, treatments			SE	P
	T1	T2	T3		
(a)Edible carcasses					
Hot carcass weight (kg)	13.55 ^c	14.30 ^b	16.25 ^a	0.30	**
(Empty body weight)					
Liver (g)	221.72 ^b	245.33 ^b	269.64 ^a	0.59	**
Heart (g)	91.06 ^c	99.67 ^b	107.55 ^a	1.60	**
Empty gut(kg)	3.025	3.20	3.51	0.12	NS
Kidney without fat(g)	138.09	141.68	149.70	2.10	NS
Heart without fat(g)	78.05 ^b	85.01 ^a	86.05 ^a	0.95	*
Tail fat(g)	283.37 ^c	307.08 ^b	380.67 ^a	9.16	***
Ribs fat(g)	2.5 ^c	6.3 ^b	13.5 ^a	1.02	***
Pelvic fat (g)	3.65 ^c	15.10 ^b	22.61 ^a	0.80	***
Dressing percentage	28.73 ^b	33.68 ^b	42.81 ^a	1.70	**
Intestinal fat(g)	129.08 ^b	167.68 ^a	172.21 ^a	4.34	***
(b) Non-edible fat					
Skin(kg)	3.56 ^b	4.03 ^a	4.10 ^a	0.06	NS
Spleen(g)	49.12 ^c	52.00 ^b	57.56 ^a	0.87	***
Testicle(g)	220.54 ^b	237.86 ^b	306.29 ^a	9.83	***
Head(kg)	2.25 ^c	2.60 ^b	2.80 ^a	0.05	**
Blood(kg)	1.763	1.78750	2.05950	0.03	NS
Lung(g)	426.13	436.68	456.63	12.22	NS
Full gut(kg)	7.50 ^c	8.67 ^b	11.67 ^a	0.37	***

T1, treatment one; T2, treatment three; T3, treatment three; means in the same row with different letters point to statistical significances; standard error of means; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; NS, non significance difference($p>0.05$)

Relatively the highest dressing percentage values in T3 over T2 in the present study can indicate that treating CT rich diets with PEG positively improves the growth performance and weight addition of sheep. On the other hand the higher dressing percentage values in T2 over T1 implies that supplementation of a sheep with a dried protein source feed such as leaves of *A.gummifera* has a potential to improve edible carcass parameters through improved feed intake and digestibility of the basal diet. The differences might be related to the energy protein ratio of diets supplied to animals, which might have constrained the growth of some these organs.

The results observed in this study agree to Gebremeskel and Kefelegn, (2011) who reported nutritionally poor and dried roughage feeding lambs supplemented with a protein source had significantly higher hot carcass weight, and dressing percentage than the non-supplemented lambs. Moreover, by increasing the nutritional densities of the diet, it is possible to obtain heavy and fleshy carcasses (Alexandre *et al.*, 2010). The dressing percentage values calculated from the empty body weight basis were higher than on live weight at slaughter basis, implying the influence of digesta (gut fill) on dressing percentage. Expression of dressing percentage based on empty weight basis rather than live body weight at slaughter basis can be a less exaggerated and realistic. Pralomkarn *et al.*, (1995) also indicated that dressing percentage increased as feed intake increased. Gibbs and Ivings (1993) and El-Khidir *et al.*, (1998) reported that gut content constitutes a large portion of the body weight and contribute 4 - 14% of fasted live weight in sheep. It has been reported that the dressing carcass coefficient for sheep generally falls between 40% -50%, (Gatenby, 1991).

Kirton *et al.*, (1972) reported that proportion of carcass offal can be affected by the nutritional status and BW of animals, and therefore, the differences observed between the control and supplemented treatments in proportion of carcass offal could be traced to dietary origins. Wester *et al.*, (1995) also reported the effect of protein and energy nutrition in visceral organ mass in lambs. Low-energy diets (such as those provided by forage-based feeding), might lower growth of liver and kidneys compared with high-energy diets (such as those supplied by concentrate-based feeding) (A´lvarez-Rodríguez *et al.*, 2009). The nutrients produced by fermentation of PEG-containing diets are important reasons in changes in liver weight (Ortigues and Doreau, 1995). Similarly (Atti

et al, 2003) reported that the higher weight of testis, liver and other organs in sheep given PEG as compared to those fed without PEG, thus it would be remarkable to confirm these findings on animals given PEG in CT rich feeds or in other feeds. Because it is well documented that testis weight is correlated to spermatozoa production (Mahouachi, 1985).

4.6 *In vitro* gas production

In vitro gas production kinetics of the three dietary groups (treatments) over a period of 96 h is presented in Table 8. There was highly a significant variation in the gas volume produced at every interval of time ($p < 0.001$). The least value of total gas produced was observed at 2 h of incubation while the highest was found in at 96h of post incubation ($p < 0.001$). The gas production was significantly higher ($p < 0.001$) for the diet treated with PEG (T3) and lower for T1 ($p < 0.001$). Moreover, *A.gummifera* supplementation without PEG (T2) has resulted higher gas production than gas produced at T1 alone. The highest volume of 54, 68 and 104 ml/200 mg DM gas was recorded at the end of 96 h incubation ($p < 0.001$). The overall gas values recorded for 2 and 96 hrs of incubation were 5.14 and 75.33 ml, respectively.

Table 7. *In vitro* gas production value (ml/200mg DM) measured for different dietary feedstuffs (treatments)

Treatments	Incubation times (hr), mean												
	2	4	6	8	10	12	14	24	32	48	56	72	96
T1	3.9 ^c	11.8 ^c	16.1 ^c	20.1 ^c	20.4 ^c	24.6 ^c	27.9 ^c	30.5 ^c	33.1 ^c	46 ^c	46 ^c	53 ^c	54 ^c
T2	4.9 ^b	14 ^b	19 ^b	28.2 ^b	32 ^b	35.4 ^b	39 ^b	57 ^b	57 ^b	66.1 ^b	67 ^b	69 ^b	68 ^b
T3	6.6 ^a	18 ^a	24 ^a	29 ^a	33 ^a	38 ^a	42 ^a	46 ^a	66 ^a	67 ^a	99 ^a	100 ^a	104 ^a

T1, treatment one; T2, treatment two; T3, treatment three; ^{a, b, c} Means a column with different superscripts differ ($p < 0.05$); SE, standard error of mean; *** $P < 0.001$

A.gummifera supplementation (T2) significantly increased gas production as compared to gas values measured for control diet (T1) ($p < 0.001$). In contrast, the highest gas values were measured for T3, i. e, when T2 was incubated with the addition of PEG. The results of present study is in accordance with Seresine and Iben, (2003) and Tedonkeng *et al.*, (2004) who reported increases in gas production in tropical taninniferous feedstuffs

attributable to PEG addition. The PEG supplementation had also a significant ($p < 0.001$) effect on the estimated parameters such as IVOMD and ME content of feed resources. The results of present study are also in agreement with the findings of Kamalak *et al.*, (2005), Getachew *et al.*, (2001), Getachew *et al.*, (2002b) and Seresinhe and Iben (2003) who reported that PEG supplementation increased the gas production and volatile fatty acid production of tropical tanniniferous feedstuffs. PEG also can liberate protein from the preformed tannin-protein complexes (Barry *et al.*, 1986). The increase in the gas production in the presence of PEG is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. McSweeney *et al.*, (1999) reported that addition of PEG caused a significant and marked increase in the rate and extent of ammonia production. The mechanism of dietary effects of CTs may be understood by their ability to forming complex with proteins. CTs may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Also, CT can adversely affect the microbial and enzyme activities (Singleton, 1981; Lohan *et al.*, 1983). The improvement in gas production, IVOMD and ME with PEG emphasizes the negative effect that CTs may have on digestibility. PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin-PEG complexes (Makkar *et al.*, 1995b). PEG can also liberate protein from the preformed tannin-protein complexes (Barry *et al.*, 1986).

As part of the use of analytical methods to the quantification of tannins, recently there has been interest in the use of tannin-binding agents such as PEG for the quantification and neutralization of tannins and their negative effects on animals. Based on this principle, the relative increase in gas production as a consequence of the addition of PEG represents the quantitative effect of tannins: the higher the biological activity of tannins on rumen microbes, the higher the increase in gas production as a result of the neutralization of tannins by PEG. In our present study addition of PEG to the incubation medium was translated by a significant increase (as percentage of the control) of the volume of the gas produced, the fermentation rate and the gas produced at 24 h of incubation (Table 8). The results of this experiment also supported the fact that PEG can be added to tannin-containing plant material in *in vitro* fermentation systems to demonstrate the nutritional importance of tannins on IVOMD and to measure nutritive value of the forage after

neutralization (Getachew *et al.*, 2001; McSweeney *et al.*, 1999). This result is also in agreement with findings of Rubanza *et al.* (2005) who found that PEG supplementation resulted in the increase in IVOMD of leaves from Acacia species. Rubanza *et al.* (2003) also found that PEG supplementation resulted in the increase in ME of leaves from browse fodders. According to Blummel and Becker (1997) the *in vitro* gas production technique is a useful tool in determining the nutritional value of forages because the volume of gas produced by forage species reflects the end result of the fermentation of its substrate to volatile fatty acids (VFA), microbial biomass and neutralization of the VFA, thus demonstrating the nutritional value of such forage.

4.7 Organic matter digestibility, Metabolisable energy content and short chain fatty acids

The results of calculated parameters: ME (MJ/Kg DM) and OMD (g/kg DM) is shown in Table 9. Both ME and OMD contents were found to be highest in T3 followed by T2 as compared to T1 ($p < 0.001$). Highly significant difference was observed between dietary groups in relation to these parameters ($p < 0.001$). The values of SCFA was highest in T3 (1.346 mmol) as compared to T1 (0.932 mmol) ($p < 0.001$). PEG inclusion to tanniniferous diet had a higher SCFA value as compared to treatment composed of a tannin rich diet without PEG ($p < 0.001$).

Table 8. Least square means for organic matter digestibility, metabolizable energy and short chain fatty acids of different dietary groups with PEG or without PEG

Parameter	Mean, treatments			SE	P
	T1	T2	T3		
OMD, g/kg	615.25 ^c	648.58 ^b	742.41 ^a	13.52	***
ME, MJ/kg	8.20 ^c	10.16 ^b	11.60 ^a	0.34	***
SCFA, mmol	0.932 ^c	1.112 ^b	1.346 ^a	0.042	***

^{abc} Means within the same row with differing superscripts are significantly different GP, Gas production (ml) at 24 h incubation, OMD, Organic matter digestibility, ME, Metabolisable energy (MJ/kg DM), SE, standard error of mean.

The values of *in vitro* OMD, ME and SCFA estimated in this study between T1 and T2 treatments are comparable to reports for range forages with variable CT contents (Babeyemi, 2007; Murillo *et al.*, 2011). However value estimated for PEG treated diet was found to be higher than reports of the above mentioned authors. This might be due to differences in plant species as well as influences of PEG on diets with CT.

The SCFA value found in the present study (avg.1.13 mmol) is higher than SCFA estimated for feed stuffs (0.95 mmol) by Akinfemi *et al.* (2009). The lowest SCFA value in T1 as compared to T2 and T3 might be attributed to a lower absolute gas production which is based mainly on carbohydrate fermentation (Sallam *et al.*, 2007). PEG inclusion might have a significant contribution in SCFA that are estimated from fermentation gas. According to reports of Njidda and Nasiru (2010), about 94% of the variation in the *in vitro* gas production of browse leaves was explained by SCFA produced, which mainly comes from carbohydrate fermentation. Akinfemi *et al.* (2009) suggests that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation; while contribution of fat to gas production is negligible.

4.8 The correlation between crude protein content of dietary groups, and some *in vivo* and *in vitro* nutritive parameters

The result of the correlation analysis indicated that CP content was positively correlated with all the determined parameters of *in vitro* and *in vivo* ($p < 0.01$). Moreover, the relation between some *in vivo* and *in vitro* parameters had showed positively significant variation ($p < 0.01$).

Table 9. The correlation coefficient(r) between protein content and some *in vivo* and *in vitro* nutritional parameters

	CP	GP	DMI	ADG	OMI	CPI	EEI	NDFI	OMD	MEI
CP	1	0.71**	0.82**	0.60**	0.80**	0.67**	0.80**	0.80**	0.72**	0.87**
GP		1	0.86**	0.55**	0.79**	0.65**	0.79**	0.79**	0.85**	0.84**
DMI			1	0.69**	0.87**	0.64**	0.87**	0.87**	0.68**	0.81**
ADG				1	0.56**	0.36	0.56**	0.56**	0.47**	0.60**
OMI					1	0.93**	1.00**	1.00**	0.54**	0.68**
CPI						1	0.93**	0.93**	0.39	0.49*
EEI							1	1.00**	0.54**	0.68**
NDFI								1	0.54**	0.68**
IVOMD									1	0.93**
MEI										1

CP, crude protein; GP, gas production; DMI, dry matter intake; ADG, average daily gain; OMI, organic matter intake; CPI, crude protein intake; EEI, ether extract intake; NDFI, NDF intake; IVOMD, *in vitro* organic matter digestibility; MEI, ME intake; * $P < 0.05$; ** $p < 0.01$

The positive as well as strong correlation between CP content, *in vivo* and *in vitro* parameters can be an indication that CP in the present study is in the level permissible for optimal feed intake, growth performance and rumen function. McDonald *et al.* (2002), Solomon *et al.* (2004); Mekoya *et al.* (2008), and Njidda and Nasiru (2010) reported found a positive correlation between CP of tannin containing forages, digestibility and *in vitro* gas production, however Rittner and Reed. (1992) found for the 72 West African fodder trees and shrubs that *in vitro* protein degradability was negatively correlated with extractable CTs. However, the behavior of some species deviated greatly from that indicated by the correlation studies. Wood and Plumb (1995) also found strong correlations between CP and the inhibition by tannins from Bolivian fodder tree leaves of *in vitro* fermentation (gas production) by rumen microbes. PEG supplementation would be best option in such scenarios that would freed proteins from tannins and establish a positive correlation.

5. CONCLUSION AND RECOMMENDATION

There is a clear justification for building up feed resources, since there is a major gap exists between the requirements and supplies of nutrients for small ruminants. Nutrient deficiencies (particularly protein, energy and minerals) and parasite infestations are common in ruminant livestock raised by smallholders. Thus the use of tanniferous feeding resources for livestock farming is widely adopted as a replacement to concentrate diets could represent a strategy to face the rising production costs. Therefore the study was conducted in order to determine the effects of PEG supplementation with CT rich tree leaf on feed intake, and growth performance of lambs.

The results obtained in this study suggested that *A. gummifera* had a significant effect on chemical composition, gas production and estimated parameters of the leaves. From the feeding trail and chemical analyses it was indicated that *A. gummifera* leaves are a source of good quality protein and high fiber concentration that has around 786g NDF per kg DM and *Albizzia gummifera leaf* meal contained 50 g CP per kg DM, indicating that it has potential as a browse source for ruminants fed low quality roughages, since high protein content in browse improves digestibility of low quality feed and lead to an overall increase in intake of digestible dry matter, suggesting that CT of *A.gummifera* had adverse effect on feeding value. The *A. gummifera leaves* also had reasonable IVDMD digestible nutrient and ME. This demonstrates the high nutritive value of the browse foliage *A.gummifera* when used in livestock feeding. However, *A.gummifera* also contained high amounts of tannins, 72g kg⁻¹ DM which can impede intake and digestion in ruminant animals. That is why *A.gummifera* leaves were supplemented with PEG, because the possible detrimental effect of CT in this leaves reduced by applying these tannin deactivating substances on daily ration of lambs. Intake of browse over critical periods like dry season result in increased survival and productivity of livestock thus based on analysis of chemical parameters and IVDMD, the foliage of *A.gummifera* has potential to be used as source of fodder with a proper feeding management system.

The beneficial effect of feeding Sheep with 30% of *A.gummifera* and PEG to lambs improved nutrient intake such as CP and ME intake, and resulted better growth performance and feed efficiency, However, unsupplemented and control one decreased the

protein intake, increased the faecal protein loss and decreased the protein retention of lambs in this experiment. This can be agreed with the theory that CT influences feed intake. It can then be suggested that CT has post-ingestive effects that affect body weight gain, because there was a relationship between CT concentration and protein retention and protein loss by lambs. Furthermore, gradual adaptation and dilution of *A.gummifera* with PEG has contributed to the strategic utilization of the plant material.

Supplementation with 40 g of PEG 4000 increased diet OM and CP digestibilities by lambs. *In vitro* diet dry matter, organic matter and crude protein digestibilities have a good capacity to predict *in vivo* diet DM, OM and CP digestibilities. Thus, the correlation coefficients between *in vitro* and *in vivo* parameters were generally high. From these results it can be interpreted that the amount of CTs affects the formation of CT-protein complexes and prevents rumen degradation of proteins. The depression of *in vitro* microbial fermentation of tannin-containing browses could partly be due to the low degradation of feed proteins that limits the availability of ammonia nitrogen for microbial growth, but the larger effect could be attributed to the inhibitory effect of tannins on microbial cells/enzyme activity. The use of PEG in supplementation strategies would be of immense advantage in improving the nutritive value of browses. However, this binding capacity does not guarantee that proteins will be digested in the lower gut. This explains that the CT remains effective throughout the digestive tract. Lambs fed PEG with condensed tannin rich leaf gained more body weight compared to those on control and unsupplemented one. It is concluded that supplementation of lambs with PEG of *A. Gummifera* avoided BW loss and promoted BW gain, and thus may be a viable management tool to enhance growth during seasons of feed scarcity. Moreover, supplementation promoted dressing percentage and increased the proportion of edible offal. However, body weight gain is the function of feed intake because in the present study CT significantly relates to feed intake in all treatments. It is widely accepted that CT reduce the feed intake which can reduce the nutrient uptake by lambs which can consequently affect the body weight gain. Therefore, the pre- and post-ingestive effects were speculated as the mechanism that regulates the body weight gain of lambs fed CT rich browse, however it is not only CT affected the body weight of lambs but other phenolic compounds in browses may have influenced the results.

The PEG inclusion had a significant effect on the gas production and estimated parameters such as OMD and ME of tree leaves. The PEG inclusion increased the gas production, OMD and ME contents of leaves. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility. The total condensed tannin in leaves was negatively correlated with gas production and some estimated parameters. Leaves from *A. gummifera* with a considerable amount of CP had a high rank value in terms of ME. Therefore leaves from *A. gummifera* may have a high potential value for small ruminant animals in terms of rumen and whole digestibility. Leaves from *A. gummifera*, require energy and protein supplementation when they are the sole feed consumed by ruminant animals. A special attention should be given to efficient integration of multipurpose fodder shrubs and trees as fodder bank in feeding calendars of sheep and goats especially during dry season. Moreover, that the *A.gummifera* tree leaves has relatively potential nutritional value for ruminant nutrition and the value is more pronounced when its tannin content is biologically inactivated using a tannin-binding agent. The *in vitro* gas production technique coupled with the use of PEG appears to have promising potential for the assessment of phenolic-related ant-nutritive effects in feeds. The *in vitro* gas production can be used to determine the quality nutritive across of the seasons of year and may indicate deficiencies in the energy content of diet consumed by grazing cattle. It is suggested that understanding structure-activity relationships of tannins is particularly important, with the development of new methods of quantification that relate chemical structure to biological activity.

The improvement in nutritive value of *A. gummifera* leave due to PEG supplementation seems to be higher than expected. However, before large scale implementation, further investigations are required to the profitability of the supplementation since the success of PEG supplementation will depend on cost: benefit ratio, moreover the issue of anti-nutritive factors (other than tannins) has not been addressed in this study thus in view of the tendency for *Albizia* species to contain highly biologically active secondary compounds, this is an area that would merit further investigation on the species at the study area. Also the effects of season and moisture limitations on tannin contents in *A. gummifera* species are not clear. It is, therefore, recommended that more studies be done to determine the effects of PEG 4000 supplementation on productivity of ruminants fed albizzia browse during different seasons.

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