#### INTRODUCTION

In Sudan small ruminants, are raised in the traditional extensive grazing system, with limited resources. Goats (*Capra hircus hircus*) are important in food production, because they are highly prolific and easy adapted to land not suitable to other farming activities. Ruminants feeding is mainly based on grassland vegetation, residue of crop production and occasionally agro-industrial by-products. These conventional feed resources that constitute ruminant diets are composed mainly of forage material which becomes mature with progress of the dry season. The characteristic of such feed are low palatability, low digestibility on the range of 30-45%, low in macro-nutrients and in micro-nutrients (Foroughbakhch, *et al.*, 2013). In the prevailing long dry season, animals are subjected to more nutritional stress and loss of production. The poor condition of livestock in the tropics is more likely to be the result of inefficient digestion in the rumen and inefficient utilization of nutrients absorbed from low quality feed (Ramirez, *et al.*, 2009).

To increase animal productivity, it is necessary to keep animals in adequate levels of nutrition in both quantitative and qualitative terms. Ruminant animals, have evolved unique digestive system which involved pre-gastric fermentation of complex cellulose by microorganism to provide the host with energy and protein. When animals are fed on fibrous feedstuff only, they cannot achieve satisfactory organic matter intakes to obtain digestible nutrients for efficient performance. Protein being the most limiting nutrient in the diet of livestock, therefore supplementation of ruminant roughage diet with protein is necessary for optimum production. The use of suitable agro-industrial by-product or concentrate supplements is usually restricted by limited supply, soaring prices of protein supplement.

Therefore, searching for alternative feed resources for ruminants has become a priority in such circumstances. Numerous multipurpose trees and shrubs have been identified as important sources of browse for ruminants especially in the dry season (Foroughbakhch, *et al.*, 2013). They have the potential abilities to alleviate nitrogen and minerals deficiencies of ruminant diets on pasture/crop residues (Makkar, 2005). The protein content of legume tree leaves is usually high (12-30%) compared to low protein content (3-10%) present in mature grasses (Norton, 2003).

Fodder trees are available all round the year especially during the dry season, when grasses are dormant and fibrous. The nutrients content of browse trees are subjected to less variation than that of grasses, this enhance their value as dry season feed supplement. Fodder trees when fed with pasture or straw, can provide many of the nutrients deficient in the basal forage and create a rumen environment more efficient for microbial growth and digestion of basal forage (Bhatta *et al.*, 2005). The incorporation of fodder legumes up to about 35% of the ruminant diet does not seem to have any effect on the intake of fibrous feed materials.

Mesquite (Prosopis spp) is a multipurpose leguminous tree native to arid and semiarid regions of the world. The P. africana (Abu Soroug) is indigenous to Africa and grow naturally in the native forests of Sudan. Prosopis was introduced earlier to this country, as means to control desertification. Nevertheless, now mesquite trees are considered as weed in irrigated schemes and at river banks. As mesquite control programme becomes increasingly expensive (Holechek and Hess, 1994), it is rational to consider the potential uses and benefits of mesquite. The nutritive value of a ruminant feed is determined by concentration of its chemical components, as well as the rate and extent of digestion. *In vivo* as well as a number of *in vitro* techniques are available to evaluate the feeds and ranking the browse trees in terms of nutritive value (Labri, 1998). The value of mesquite as animal feed was reported from many regions in the world (Pasiecznik, 2004). The leaves from mesquite trees are unpalatable (Bhatta et al., 2002) where as pods and seeds are relish to ruminant animals. Small ruminants are accustomed to browse in the natural grazing and eat mesquite leaves and pods when other forage is limited. In Sudan Prosopis tress flowers year-round and the fruiting period, which peaks in December to June, coincides with climax of the dry season (ElTayeb et al., 2001). large quantities of mesquite pods are collected and displayed in the local markets as feed for livestock. Mesquite pods can be ranked as medium protein source, rich in minerals, sugars and fiber. These trees contain secondary plant compounds that may modify their nutritive value for the benefit of the ruminant animals by increasing the fraction of by-pass protein (Makkar, 2003). It was reported that when *Prosopis* leaves were fed as a sole diet, it had a negative effects such as reduced intake, reduced digestibility of nutrients and body weight gain in sheep and goats (Bhatta et al., 2002).

The pods lend themselves better to feeding livestock when dried, ground and turned to flour.

The pods of *P.* species were incorporated at levels up to 200 g/kg without compromising animal feedlot performance (Mahgoub *et al.*, 2004). There is a good evidence for nutritional and benefits of inclusion of Mesquite pods as a supplement to basal ration for ruminants.

Goats are predominantly browsers, browse constitute 60- 80% of their diet (Silanikove, 2000) and spend 90% of their eating time browsing. They have advantage over other forage eating ruminants, in that they prefer and digest well leaves and twigs of trees. Goats have a considerable ability to detoxify the secondary compound of the fodder trees and perform well even when consuming tanniferous tress (Silanikove, 2000). Animals fed *Prosopis* were in excellent condition throughout the trail without any signs of ill health and their blood values were within the reference range for animals of similar age group (Mahgoub *et al.*, 2008). It is reported that response to fodder supplement vary because they have different degradation characteristics in the rumen, as well as gas production. However depending on the level of replacement and associate effect of feed ingredients, supplementation of fodder may yield better intake improved blood metabolites and growth response. Blood profile tests, are expected to be sensitive measurements for animal nutritional status and reflect the variation in response to various types of diets such as browse.

The objective of this study were to investigate:

The dietry effect of *Prosopis* pods on the hematological parameters and serum biochemical parameters of Sudanese Nubian goat kids.

The growth performance of goat kids fed different levels of Prosopis pods.

The dietry effect of *Prosopis* pods on physiochemical properties of the rumen ecosystem.

To assess the nutritional value of *Prosopis* pods by chemical analysis of the whole pods or its fraction the sweet part and the seed, by *in vitro* gas production.

To study the rumen degradability (*in sacco*) characteristics of *Prosopis* pods.

# CHAPTER ONE LITERATURE REVIEW

## 1.1: Prosopis tree characteristics and distribution:-

Prosopis (Fabaceae) is a genus of about 44 species of spiny leguminous trees and shrubs found in subtropical and tropical regions of the Americas, Africa and southwest Asia. Prosopis juliflora (Sw.) is a small perennial tree native to arid and semi-arid regions of Mexico, South America and the Caribbean and has established itself as a weed, notably in Asia and Australia (Pasiecznik et al., 2004). Three species including P. cineraria are occurring in the Near East and Pakistan (Pasiecznik et al., 2004). In their native range of North South America, P. chilensis and p. pallaid are among the most important species producing palatable pods which can be used as food. *Prosopis Africana* is the only species native to Africa and spread in the Sahelian zone from Senegal to the Sudan, Uganda and Ethiopia. Prosopis was first introduced in Africa in 1822 in Senegal, followed by South Africa in 1880 and Egypt in 1900. In the Sudan the genus *Prosopis* was introduced to stabilize sandy dunes and to benefit their drought resistance capacity in the arid lands (Laxen, 2007). Mesquite (P. Juliflora) was first introduced into Sudan in 1917 from South Africa and Egypt and planted in Khartoum (Broun and Massey, 1929). The tree was then distributed in the dry land of central Sudan, in Sinar in year 1938, the distribution included western, eastern and northern Sudan. Late in 1947 and subsequently in 1965 mesquite was reintroduced into eastern Sudan, where it was planted in a green belt around Kassala (Abdel Bari, 1986). The mesquite was redistributed at the aftermath of drought periods of 1970s and period of 1980s. Several species of mesquite (P. chilensis, P. valutina, P. *qlandulosa var. terreyona, P.alba, P. pallida and P. articulata*) were introduced, in the period 1978-1986, with the objective of selecting suitable species for the different ecological zones. Some of the species selected, had their seeds multiplied and distributed in western Sudan around El Obeid, and various other locations (El fadl, 1997). In the initial introductory stages, the tree was appreciated due to its ability to grow where nothing else seemed to be able to grow. These trees are multipurpose producing excellent timber, gum, charcoal, fire-wood and bee honey, beside their nitrogen fixation capacity. Although in their new habitat the trees were less productive, less managed and more invasive.

The major contribution of genus *Prosopis* in animal feed is in terms of pod production as consumption of leaves is restricted to occasions when other feed are of limited supply (Pasieczik *et al.*, 2001).

The *Prosopis juliflora* is the main species widely distributed in Sudan, have aggressive growth making thick stands and became a weed in many locations (ELsidig *et al.*, 1998). It started to spread rapidly and its ability to survive cutting by coppicing made it uncontrollable. Some species of genus *Prosopis*, can thrive on all types of soils under variable climatic conditions (Pasiecznik *et al.* 2004). The genus is well adapted to the arid and semi-arid regions where it is valuable in rehabilitation of degraded dry lands. The tree can be grown successfully in the salty soils.

The Arabic name for *Prosopis* is mesquite, in English honey mesquite or iron wood. "Algarrobo" is the common name for trees of *Prosopis pallid* and *Prosopis juliflora* predominant in Peru, while the fruits are called "algarroba". The name "algarrobo" is also used in Spain for carob tree (*Ceratonia siliqua*) which sometimes causes confusion. As the Spaniards gave *Prosopis* the same name when they arrive to America, due to resemblance of the fruits. In North America *Prosopis* species are known as mesquite, their fruits seem considerably different from algarroba. The Hindi name is *vilayati khejra*, *Prosopis cineraria* is locally known as" *Khejri*" has long history of use in herbal medicine in arid region of India where *Prosopis juliflora* Swartz is known as *Vilayati babool*. *Kiawe* is the name given in Hawaii. *Algarobeia* is the name given in Brazil (Portuguese). In Middle East mesquite is known as *ghaf*. *Prosopis farcta* is a native of Asia, distributed from India to Iran (Pasiecznik *et al*. 2004).

#### 1.1.1: Prosopis pods characteristics:-

The genus *Prosopis* pods are edible and sucrose rich. The pods are several-seeded, strongly compressed when immature, thick when ripe, more or less constricted between seeds, 10 to 25 cm long, coiled or straight, brown or yellowish and every pod contains 10 to 30 seeds. The mature pod remain intact, they do not split open when ripe. The seed is compressed and oval or elliptic and 2.5 to 7 mm long with brown colour (Pasiecznik, 2001). At maturity the seed lies freely inside a cavity in the endocarp of the pod (Pasiecznik, 2001). Pods production start three years after tree planting, average annual pod yield of 8.7 ton/ha was reported from USA and average annual yield of 6 ton/ha from Brazil (Pasiecznik, 2001).

The average annual yield per tree is about 10-50 kg pods (Sawal *et al.*, 2004). The harvested crop could be stored for several years in wood-lined or brick—built houses. Stored pods are susceptible to insects attacks, require pre-storage treatment of house with appropriate insecticide (Pasiecznik, 2001). All American species are heavily attacked by seed eating insects, mostly bruchid beetles, which can destroy over 25% of seed produced, affecting most pods (Pasiecznik, 2001). The leaves of *Prosopis cineraria* are highly palatable and nutritious, but contain tannins 8-10% (Bhatta, *et al.*, 2013). The leaves constitute important feed resource especially during dry season.

## -: Nutritional characteristics of Prosopis pods :1.2

#### -: Chemical composition of the pods :1.2.1

The structure of mesquite pods was described by (Pasiecznik, 2004) consist of two main parts. The per-cap consist of the "exocarp" the outer covering smooth and thin- the "mesocarp" – thick, spongy and sweet pulp- and the "endocarp" or seed husk – hard and stony-contain all the sugar and most of the fiber. The second main part of the pod is the seed which contain most of the protein and the fat. The seed passes unchanged through the alimentary canal of animals browsing the whole pods. The nutrients composition and mineral concentration of mesquite pods show large variations between species, within species and between trails (Pasiecznik, 2001). These variation are due to factors such as soil, climate, stage of maturity and season (Chopra and Hooda, 2002). Protein content is relatively consistent between species with P. Juliflora recording the highest values (Pasiecznik, 2001) and sugar content exhibits some variation (Sawal, et al., 2004). The nutrient composition of fresh, ripe pods was found to contain (7-10%) moisture, and on dry matter (DM) basis contain (9 to 17%) crude protein, (CP) (1.2- 4.3 %) ether extract, (EE) (16-34%) crude fibre (CF), (47-61%) nitrogen free extract (NFE), (28 %) acid detergent fibre (ADF), (8 %) acid detergent lignin (ADL), (4-5%) ash, (0.14.029%) silica, (0.3-0.5%) calcium (C) and (0.40-0.44%) phosphorus (P) (Sawal, et al., 2004). The dietary fibre of Prosopis pod represents 16% to 30% of the pulp mainly insoluble; and the predominant nonstarch polysaccharide found in the pods are cellulose, hemicelluloses mainly galactomannan, lignin, and several others (Sawal, et al., 2004).

According to Meseret *et al.* (2011a) the pods of *P. juliflora* contain 12.10 CP, 7.31 EE 14.40 CF, 5.8 ash, 0.26 Ca and 0.14 P on dry matter basis.

According to Salah and Yagi (2011) the chemical analysis of mesquite leaves and pods revealed, 32 % and 12.5 % protein, 52.6 % and 53.0 % carbohydrate, 4.1 % and 2.5 % lipid, 18.5 % and 27 % crude fibre, 12.6 % and 4.9 % ash, 4.8 and 6.1 % moisture respectively. Bhatta *et al.*, (2004) reported that the chemical composition of *Prosopis cineraria* leaves were (g kg¹DM) 502.0, 930.0, 159.2, 567.2 367.2 140.0 and 188.6 for DM, organic matter (OM), CP, NDF, acid detergent fiber (ADF), cellulose and acid detergent lignin (ADL) respectively.

Mesquite pods was found to contain, about 930 g/g DM, 120 g/kg CP and 317 g/kg ADF, 420 g/kg NDF, 26 g/kg EE and 40 g/kg ash (Mahgoub *et al.*, 2005b). Mesquite pod was reported to contain CP 18.5, DM 88.4, OM 83.2, Ash 5.2, ADDF 29.8, NDF 51.8 and ADL 3.2% (Koech *et al.*, 2011). Abelelnoor *et al.* (2009) found that *Prosopis* pods contain 172 g/kg CP and lower protein content was reported by (Batisa *et al.*, 2002) to be CP 127g/kg on DM basis. The protein of the mesquite pods contains nearly all the essential amino acids in amount higher than that found in the leaves. The essential amino acids content of leaves from six species of *Prosopis* were similar to that of alfalfa. (Astudillo, *et al.*, 2000). Protein content and sugar contents varies between (7.3-12.7%) and (16.3-41.0%) of DM respectively (Sharma *et al.*, 1997). The crude protein and energy content of *P.juliflora* pods are comparable to those in barley grain (12.6 CP and 3.20 kca/kg ME) (Abdullah and Abdel Hafes 2004). The macro and trace mineral content ranges (mg/100 g DM weight) in leaves and pods were: P (250 and 164), K (116 and 191), Na (1.0 and 4.0), Ca (276 and 26), Mg (28 and 94) Fe (9.0 and 3.0) and (1.0 and 1.0) for Mn (Salah and Yagi, 2011).

The raw seed contain 10.9 % moisture and on DM basis, contained about 39 % protein, 6.1 % CF, 4.5 fat, 18.6 carbohydrates and 3.8 % ash (Sawal, *et al.*, 2004)

## 1.2.2: Digestibility of Prosopis pods:-

Digestibility range from (38 to 78) % were reported from African woody species (Simbaya, 2002). The digestibility of crude protein did not always match the high content which characterizes fodder trees and shrubs (Mahgoub *et al.*, 2005b). Mesquite pods as whole and pericarp fraction contained (68.8 and 65.6%) digestible DM; (5.6 and 2.6%) digestible protein; (2,880 and 2.675) kcal/kg digestible energy (2,682 and 2,466 kcal/kg) metabolizable energy; (2,642 and 2,432 kcal/kg) nitrogen corrected metabolizable energy respectively (Koech *et al.*, 2011).

The dry matter (DM) and organic matter (OM) digestibilities in Awassi lambs were highest on the diet contained 150 g/kg of pods when compared to the control and other dietary treatments that contained pods at 250, 350, and 450 g/kg. The digestibility coefficient decreased as the proportion of the *Prosopis* pods increased in the diet (Abdullah and Abdel Hafes 2004). The dry matter degradability (DMD) of *Prosopis farcata* furit was investigated (Ansari, *et al.*, 2013), fast degradation (a), digestible particles over the time (b), potential degradability (a + b) and effective degradability (ED) were measured at 57.05, 18.62, 38.63, 57.26 and 38.85% respectively at incubation time of 96 h. The results showed that *Prosopis* had desirable nutritive value.

#### 1.2.3: Browse trees as animal feed:-

Trees are a source of bypass protein for intestinal digestion, due to the presence of secondary plant compounds. The protein content in browse trees consist of both soluble and insoluble components and used as source of nitrogen to increase rumen microbial activity and source of bypass protein supplying amino acid (AA) to the lower gut of the host animal (Makkar, 2003). The seed and pods of Prosopis can sustain livestock in dry seasons when other feed is not available. The price of concentrate mixture was reduced by 30% when Prosopis pods replaced 40% of the mixture (Chaturvedi and Sahoo, 2013). Feed costs were reduced by 25% when *Prosopis* pod replaced up to 50% of concentrate diet of sheep, without affecting their growth (Sawal et al., 2004). However, when pods of some species (Prosopis pallida and Prosopis glandulosa) are fed as an exclusive diet for long periods, livestock, particularly cattle, can become malnourished and show ill-thrift symptoms. The use of tree legume fodder as a supplement has improved intake, digestibility and animal performance (Simbaya, 2002). In the pasture ruminant animals browse leaves, flowers and pods from the trees before drop or take the dry ones from the ground. Animals are either fed parts cut from the trees or could be stall-fed on collected pods (Pasiecznik et al., 2001). The pods either fed as supplement incorporated in a mixed ration or fed alone as concentrate with a basal diet. The feeding value of mesquite is attributed to the pods, efficient utilization is achieved when the pods are mature and subjected to processing. Mesquite pods are palatable, rich in digestible carbohydrate and could be good energy source (Sawal, et al., 2004). The leaves of species from Africa or Asia are of a relative value as source of animal feed (Bhatta *et al.*, 2004).

The leaves from American species are unpalatable to animals, irrespective of high protein and mineral content. Species of mesquite pods had been used as source of feed for cattle, sheep, goats, camel, poultry, horses, rabbits and fish (Sawal *et al.*, 2004).

#### 1.2.4: Prosopis in sheep feed:-

In feeding experiment, Omani sheep were fed for 50 days on (Ghaf pod) *P.cineraria* at (0, 15, 30 and 45%) to replace *Rhodes* grass hay on the basal diet. Animal fed control diet without pods grew faster (135g/day) and had lower feed conversion ratio than those fed the pods.

Animals fed on 15 and 30% pods gain weight but those fed higher level of pods equal to 45%, lost weight (Mahgoub et al., 2005a). Sheep fed 15% Ghaf pods gain weight at rate of 90 g/day and their carcass attributes compared to those fed on diets without pods (Mahgoub et al., 2004). The researchers concluded that Prosopis pods could be included at a level up to 20% in the diet of Omani sheep and goats without adverse effect on their body growth. Awassi lambs were fed finishing diet, by inclusion of P. juliflora pods to replace barely at three levels (0, 100 and 200 g/kg), lambs fed 20% pods had highest organic matter intake as well as crude protein intake (Obeidat et al., 2008). Prosopis pods flour were used to replace sorghum in rams ration, their feed intake was not affected, but at 60% inclusion of mesquite resulted in low weight gain (Sawal, et al., 2004). A study was carried by Obeidat et al. (2012), Awassi ewes and their lambs were fed on diets containing P. juliflora pods at (0, 125, 250 g/kg) to replace barely grain for 8 weeks. High intake of NDF and low ADF digestibility were observed in animals fed on 250 g/kg of the pods compared with control. The ewes in the control group lost less of body weight but no difference in the final body weight among the groups. Ravikala et al. (1995) studied the effect of feeding Prosopis pods to lambs at rates of (0, 15 and 30%) of total ration. Feed efficiency was significantly lower in the 30% P. juliflora pods ration in comparison with other treatments. The pods containing rations were superior in enhancing the growth of the lambs within reasonable economical margins. Feed intake and digestibility were not affected when Prosopis pods was up to 50% of concentrate mixture for sheep (Shamra, 1997) but gain and CP digestibility was affected at 75% of the pods.

#### 1.2.5: Prosopis in goat feed:-

*Prosopis juliflora* pods have been tested in arid zone countries to feed goats. In Oman, a diet containing 20 % pods improved feed intake, feed conversion and body weight gain without compromising carcass yield or quality. However, intake and gain dropped sharply when pods where included at 30 % (Mahgoub *et al.*, 2005b).

In the dry lands of India, up to 35 % *Prosopis juliflora* pod flour was included in the diet of goats in late lactation, they maintained weight gain, blood parameters and milk yield (Mathur *et al.*, 2003). *Prosopis* pods supplement were fed in increasing amount to Galla goats kids at rate (100, 200 and 400 g/goat/ day) with hay as a basal ration. Results of the study concluded that *Prosopis* could be used to feed goats kids in proportion up to 200 g/goat/day to reach good weight gain and no adverse effect on feed intake and digestibility (Koech *et al.*, 2010 ). Reduced feed intake occured in animals fed diet containing 400g *Prosopis* pods (Koech *et al.*, 2011; Mahgoub *et al.*, 2004). Sudanese desert goats were fed diet consisting of crushed pods of *Prosopis*, cottonseed meal and wheat bran, it was found that feed intake, live weight gain and dressing-out percentage all decreased linearly with increasing proportion of the pods (Ibrahim and Gaili, 1985). The possibility of utilization of *P.juliflora* pods and sesame hulls to replace soybean and barely in the diet fed to finishing black goat kids was repoted by (Abdullah *et al.*, 2011). Milk production from ewes fed 250 g pods was more than other groups, but no difference in milk composition (Obeidat *et al.*, 2012).

#### 1.2.6: Prosopis in cattle feed:-

Mesquite pods in proportion up to 30 % were used in ration to maintain good dairy production (Talpada *et al.*, 2002). In order to be safe, cattle are fed rations containing no more than 40g of dry mesquite beans/100gDM, especially if the feeding period exceeds 60 days (Cook *et al.* 2008). Feeding ration containing up to 45% *Prosopis* pods, to replace rice polishing in the cattle could promote favourable growth and effective reproduction. (Pandya *et al.*, 2005). Replacement sugarcane molasses with *P.juliflora* pods at 0, 15, 30, 45 and 60 % was most effective in term of live weight gain at 30 and 45 percent inclusion levels (Sawal *et al.*, 2004). In semi- arid areas, steers on over-grazed pasture lost weight, but resumed gaining weight when

their diets were supplemented by mature pods of *P. caldenia* with digestible dry matter of 63% (Pasiecznik *et al.*, 2004).

Milk production was increased and no effect on milk flavour as a result of inclusion of pods up to 50 % in the diet. (Pasiecznik *et al.*, 2001).

## 1.2.7: Prosopis pods in feed of non-ruminant:-

Brown commercial layers (Bovans) were fed a ration of ground *Prosopis* pods at level of 0, 10, 20 and 30 % of whole ration. Layers fed on 30 % pods ration showed drop in egg production and improved yolk colour. Ration of 20 % *Prosopis* pods was found to be optimum for the layers performance, however 10% pods diet was economically fair (Meseret *et al.*, 2011a). Feeding broilers chicks with soaked *Prosopis* seed flour to substitute up to 50 % of the whole diet had a favourable effect on the performance of chicks and fair economical value. The experimental diets were compounded to contain (0, 25, 50 and 75 %) soaked *Prosopis* seed for partial substitution of sesame cake and sorghum grain (Zein Elabdin and Mukhtar, 2011). Substitution of soybean meal in broiler chicken by graded levels of decorticated fermented seed meal of *P. africana* did not affect the performance of the chicken but had economical benefit (Yusuf *et al.*, 2008). Broilers fed a diets containing 20 % *Prosopis* pods had the highest live bodyweight, body weight gain and the best feed conversion (Al-beitawi *et al.*, 2010).

The effect of feeding growing rabbits on *P. africana* pulp was studied by Adamu *et al.* (2011) where pulp replaced maize of the ration at levels of (0, 10, 20, 30 and 40%). The results suggested that up to 40% of pulp could be used in the ration, without impairment of the health status of the rabbits. The efficiency of *P. juliflora* pods in fish feeding was examined (Mabrouk *et al.*, 2008), different proportion (0, 20, 40, 60, 80 and 100 g/kg) of the pods were used to replace yellow corn in diets of Nile tilapia (*Oreochrmis nilotica*). The results indicated that diet contained 60 g/kg *P. juliflora* pods had best feed and nutrient utilization, and improved growth as well as whole body composition of the fish fry. *Prosopis* flowers are good bee forage and their nectar yield a superior honey (Orwa *et al.*, 2009).

# 1.3: Limitation to nutritive value of Prosopis:-

The main limitation to effective utilization of fodder legumes as feed for ruminants is the high content of tannins and other anti-nutrients such as saponins, cyanogens, mimosine, coumarins, etc which limit nutrient utilization (Makkar, 2003).

These compounds are also known to have other detrimental effects, which may range from reduced animal performance to neurological effects and increased animal mortality rates (D'Mello, 1992).

The pods are found to contain antinutritional factors saponin (317 mg 100 g), tannin (860 mg 100 g-1), phytic acid (181 mg 100 g-1) and total phenol (640 mg 100 g-1) (Cardozo *et al.*, 2010). The green pods of *Prosopis* and the leaves were unpalatable, with limited nutritive value and not relished by stock (Sawal, *et al.*, 2004). Moreover stock poisoning had occurred after ingestion of *Prosopis* pods previously exposed to rain. The ingestion of *Prosopis* pods by cattle for long period can result in fatal illness (Cook *et al.*, 2008). However, the intoxication was induced slowly, i.e., after ingestion of pods for (210-270) days in the trial study (Tabosa *et al.*, 2003). The presence of anti-nutritional factors are suggested as cause of the observed nutritional upsets and stock loss. The presence of alkaloids such as prosopine and prosopinine in certain parts of *P. africana* was demonstrated by Francis *et al.*, (2002) although their antinutritional effect in the ruminant was uncertain. *Prosopis* species, such as honey mesquite (*Prosopis glandulosa*) were reported to contain trypisn inhibitor, heat liable haemeaglutins which concentrated in the seeds (Zolfaghari and Harden, 1982).

#### 1.3.1: Effect of tannin on the nutritive value of browse:-

Tannins are a complex polyphenolic compound, possess sufficient hydroxyl and carboxyl that can form strong complexes with protein and other macromolecules under particular environment (Muller-Harvey, 2006). They are conventionally classified into two major groups, the hydrolysable and condensed tannins. Generally trees and shrub leaves contain both types of tannins. At very high level of browse intake, the intestinal wall may become damage leading to absorption of condensed tannins and causing toxicity (Walton *et al.*, 2001). The occurrence of systemic toxic effects of tannins implies that tannins or their degradation products are absorbed from the digestive tract. It is unlikely that molecules of condensed tannins are absorbed unmodified because of their usually large molecular size (Muller-Harvey, 2006). However, high tannin content can cause gastritis and damage to the intestinal mucous membranes, enabling absorption of condensed and hydrolysable tannins (Muller-Harvey, 2006). Degradation of HT release phenol which has toxic effect when absorbed.

Tannins are implicated in reducing the intake and the digestibility of feed, particularly the protein content by forming soluble and insoluble complexes mainly with proteins and enzymes (Hagerman, 2002). These complexes are resistant to both enzymatic and microbial degradation. Detrimental effects of tannins are commonly associated with reduction in the concentration of ammonia and volatile fatty acids in the rumen fluids which can automatically leads to the deficiency of nitrogen and energy (Silanikove *et al.*, 2001). It may also be noted that at lower levels (2–4%) of tannins, these could have beneficial effects on ruminant animals and reduce excess degradation of high quality protein in the rumen. (Provenza *et al.*, 2003 ). Tannin concentration greater than 5% in the browse diet adversely affect intake, digestibility and nitrogen retention and can be toxic, some adaptation to these compounds can be attained through a regular ingestion of tannins (Provenza *et al.*, 2003).

The total phenolics content of some common browse species range from 1.46 to 4.4% dry matter tannic acid equivalent (Dube *et al.*, 2001). *Prosopis* contain high level of tannins reaching more than 10% was reported by (Ibrahim, 1994). Tannin are present on the leaves of *Prosopis* species in high proportions to exert negative effects on DM and CP digestibility and lower nitrogen retention (Bhatta, *et al.*, 2004). *P. cineraria* has been observed to have tannins that have a very high protein precipitating capacity than tannins from other tree forage. *Prosopis* leaves have been reported to contain tannin at 2.2 % of DM, new leaves have higher tannin concentration than older leaves (Bhatta, *et al.*, 2002). Condensed tannin (CT) content of *Prosopis* leaves was negligible (Soltan *et al.*, 2012).

Digestibility of DM and nutrients and N balance could be affected by feeding different of *P. juliflora* due to the presence of anti-nutritional factors such as tannins and phenolic compound (Mahgoub *et al.*, 2004). The intake of tannin DM in *Prosopis* ration in sheep was 27 g/ day which represented 2.64 % of dry matter intake (DMI) (Bhatta *et al.*, 2005). High tannin level could be acceptable when diluted by other feed resources that contain appropriate level of protein necessary for biding free tannins. The most effective method to remove tannin negative effect is inclusion of polyethylene glycol (PEG) in the diet (Bhatta *et al.*, 2002). Feeding of 5g per day polyethylene glycol-6000 alleviated the negative effect of *Prosopis* tannin on protein digestion in kids (Bhatta *et al.*, 2002). A positive effect on rumen fermentation from browse when goat kids were fed complete feed block containing *Prosopis cineraria* leaves and polyethylene glycol was not demonstrated (Bhatta *et al.* (2005).

The amount of PEG required to offset the effect of ingested tannin was much higher in sheep than goats ( 50 g day<sup>-1</sup> versus 10 g day<sup>-1</sup>) (Bhatta *et al.*, 2005). In this regard, browsers are more efficient than grazers in utilizing tannin rich plant in general and goats are more efficient than sheep (Bhatta *et al.*, 2005). Three months feeding of *P. cineraria* leaves and *Cenchrus* species (50:50) basis with 1% urea has been adequate to maintain adult sheep (Ben Salem, *et al.*, 2005). Urea provided an extra N to the diet and deactived the tannins in the leaves and augmented the action taken by some rumen microbes in the tannins (Goel *et al.*, 2005b).

## -: Haematological components and their functions: 1.4

The changes in haematological, biochemical parameters are often used to determine stress due to nutrition and other factors (Antunovic *et al.*, 2009). Animals with good blood composition are likely to show good performance. Variations in blood parameters of animals are due to several factors such as altitude, feeding level, age, sex, breed, season, temperature and physiological status of animals (Mbassa and Poulsen, 2003).

#### -: **Blood** :1.4.1

Blood is a connective tissue composed of cells within fluid matrix (Jones and Allison, 2007). Plasma of cattle appears grossly clear and colourless and becomes yellow if animals are fed green feedstuff. Sheep plasma is clear colourless not affected by the diet. The serum is fluid obtained after removal of clotting factors (fibrinogen, prothrombin, calcium) from the plasma of the blood which is allowed to clot (Jain, 2000). Serum is clearer than plasma and is the preferred specimen in clinical testing as much of proteins are eliminated with clotting factors (Russell and Roussel, 2007). The main function of the blood are transportation, regulation and defense. Transport involve carrying oxygen and nutrients to the cells in the body and to remove carbon dioxide and waste from them. Transfer hormones from endocrine gland to the target organs. Defense function is concerned with immunity, phagocytosis and blood clotting. Blood maintains internal homeostasis by regulating body temperature, pH, water and electrolytes balance.

#### 1.4.2: Erthrocytic series:-

The red blood cells (RBCs) are produced in the bone marrow by the effect of erythropoietin formed primarily by the kidney (Jones and Allison, 2007).

Ruminants, particularly sheep and goats have small RBCs which may be erroneously classified as platelets (Jones and Allison, 2007).

The diameter of goats erythrocyte is 3.2 to 4.2 $\mu$  and the RBCs have only slight biconcavity. In blood smear they lack the zone of central pallor and do not exhibit rouleaux formation (in blood film stacking of RBCs like coins) except perhaps at the edge of a thick smear. Normal cell shape is quite variable to include triangular shape rod or pear shape and elliptical shape especially in young goats. Sickle cell can occur in Angora goats similar to those seen in deer (Jones and Allison, 2007).

The normal count of RBCs for goats vary with age with relatively low levels at birth (7 to 8 million/µl) which reach highest level at 8 months of age then gradually fall to stabilize at 8 to 18 million/µl by 1 year of age (Radostits *et al.*, 2003). Reduction in red cell count implies a decrease in the level of oxygen that would be carried to the tissues as well as level of carbon dioxide returned to the lung. The normal life span of circulating erythrocytes in the domestic goat is average of 125 days and up to 165 days in wild goats.

Haemoglobin in the red cells is responsible for carrying oxygen and carbon dioxide in the respiration process. In goats the FHb which has much affinity to oxygen is replaced by C Hb which has less affinity to oxygen at age of fifty days. Erythropoietin is a direct stimulus to shift of Hb to C type by developing erythrocyte in bone marrow (Jones and Allison, 2007). The concentration of haemoglobin is an indicator of erythrocytic normality and general well being of the animal (Radostits *et al.*, 2003).

The packed cell volume (PCV) express the percentage of blood composed of erythrocytes. The normal level of haemoglobin and packed cell volume for goats are 8-12g% and 24-48% respectively (Merck Veterinary Manual, 2012). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were introduced to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells.

The red blood cell indices, are important aid in characterization of anaemia and important to determine the state of RBCs, their variation from normal indicate the loss

in the integrity of RBCs which is caused by a number of factors (Jones and Allison, 2007).

The MCH expresses the amount of Hb in (pictograms) in an average erythrocyte of population of cells, while MCHC express the ratio of the weight of Hb to the volume of the erythrocyte in grams per deciliter (g/dl). The MCH normal value for goats are on the range of 5.2 to 8 pg and MCHC are in the range of 30- 60 g/dl (Merck vet Manual, 2012).

The MCHC was reported to be typical for all animals with the exception of camel to be 32 to 36 g/dl (Jones and Allison, 2007).

The mean corpuscular volume (MCV) is red cell index which expresses the average volume of population of erythrocytes in femtoliters (fl) (indicate change in the size of a single RBC). The normal MCV for goats are in the range of 16 to 25fl. Microcytosis indicates smaller size due to iron deficiency and consequently low Hb concentration. Macrocytosis indicate larger size and is usually caused by vitamine B<sub>12</sub> and folic acid deficiencies. The MCH is associated with haemoglobin concentration, increased during haemolysis and decreased in hypochromic anaemia of iron deficiency.

The MCHC is the most accurate of the indices, because it does not require RBC count, increased value indicate iron deficiency and reticulocytosis and increased value may be due to haemolysis.

Anaemia may arise from nutritional origin, and was defined as the presence of below normal red cell count, haemoglobin concentration, and/or packed cell volume, and increased MCV (Jones and Allison, 2007).

#### 1.4.3: Leukocytes:-

Leucocytes or white blood cells form a distinct greyish-white layer at the interphase of the plasma and red mass when blood is allowed to clot. White blood cells which are principal component of immune system (cellular or humeral immunity) are subdivided to granulocytes or agranulocytes (Dacie and Lewis, 2002). Granulocytes are differentiated to neutrophils, basophils and eosinophill, while agranulocytes, consist of lymphocytes and monocytes. The mean white cells count of the goat is generally reported to be 9000 cell/ul with a range of 4000 to 14000. The total number of white blood cells is dynamic because of their involvement in immune mechanism (Dacie and Lewis, 2002). However the total and differential count vary significantly with age. Leucocytes are less numerous in the blood than the erythrocytes..

# 1.5: Effect of feeding browse on the blood parameters:-

Pasture of low nutritive value and nutritional condition greatly affects the values of blood parameters of goats (Radostitis et al., 2003). Feeding of animals for extended periods on some tree fodder with high levels of anti-nutritional factors has been reported to produce harmful effect on animal health (Tabosa *et al.*, 2000). Feeding non-conventional feed to animals reduce the body mass growth rate and may also affect their health and their resistance against diseases (Mahgoub et al., 2008). The effect of feeding *Prosopis* in the blood profile of animals is attributed to presence of anti-nutritional factors and high sucrose content of the pods. There is some evidence that continuous consumption of mesquite pods as sole dietary component may lead to rumen impaction and some toxicological effects (Tabosa et al., 2000). Low packed cell volume (PCV) and RBC counts were observed in goats fed diet contained *Pterocarpus* erinaceus fodder tree leaves (Olafadehan, 2011a). The haematological and biochemical parameters in West African Dwarf goat fed a basal diet of Panicum maximum grass hay supplemented with Afzelia africana and Newbouldia sp, tree leaves were investigated by (Ikhimioya and Imasuen 2007). The results showed differences in the haemoglobin concentration, packed cell volume and red cells count among the treatments. The packed cell volume and haemoglobin concentrations were higher in goats fed diet 25 mixture of tree leaves :75 (Panicum maximum) hay and their values range was (21-35%) for PCV and (8.45 -11) g/dl for haemoglobin. Similar haemoglobin level up to 11g/dl was reported for goats fed Prosopis juiliflora pods (Misri et al., 2000). The haemoglobin concentration in goats fed supplement of leaves from *Prosopis* and other trees were not different indicating the absence of negative effect of tannin on iron (Fe) absorption (Bhatta et al., 2005). A study was conducted (Bhatta, et al., 2007) to assess the effect of feeding Prosopis cineraria leaves in complete feed mixture on blood parameters of lambs and kids. The results showed the highest values of haemoglobin concentration (Hb) and blood urea nitrogen concentration (BUN) in animals fed 25% followed by those fed 50% and 75% Prosopis leaves. Decrease in Hb concentration was also recorded in adult sheep fed *Prosopis* pods diet (Prasad *et al.*, 1997). Tannin in Prosopis pods may form complexes with iron and interference with its absorption (Mahgoub et al., 2005a). A concentrate mixture of *Prosopis* pods, wheat bran, date syrup and date tree branches was fed to

Omani sheep (Mahgoub *et al.*, (2005a). The sheep blood sample was analyzed blood for, red blood cell series, red blood indices and total and differential white cell count. The results indicated that, the blood values are within the normal reference range for the animal of similar age and not affected by feeding. Dairy concentrate contained 30% *Prosopis* pods was fed to cattle with no effect on red cell count, white cell count, Hb, blood glucose, macro mineral and iron level in the blood (Talpada *et al.*, 2002). The level of haemoglobin range (8.67 -11.05g/dl) in goat kids fed low and high protein diets containing *Prosopis* and the level of haemoglobin of the low protein diet was increased by addition of polyethylene glycol (PEG)-600 (Bhatta *et al.*, 2004).

#### 1.6: Serum biochemical constituents:-

Serum chemistry profile allows for evaluation of several body systems and assessment of metabolic and acid-base electrolyte disturbances (Russel and Roussel, 2007). Ruminant chemistry profile consists of the following analytes and enzymes: glucose, total proteins, lactate, urea nitrogen, creatinine, electrolytes (sodium, chloride and potassium), serum enzymes, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and Aspartate transaminase (AST), bilirubin, minerals (Ca, P and Mg), serum proteins (total protein and albumin) and muscle enzymes (creatinekinase CK, and AST). In addition, to estimation of albumin and globulin ratio (A G) ratio and calculation of globulin concentration. Tests for bile acids, ammonia, cholesterol, and non-esterified fatty acid can be run occasionally but not on routine basis. (Russell and Roussel, 2007).

#### 1.6.1: Blood glucose:-

Glucose is a blood parameter defining the energy metabolism and of special importance in late pregnancy and lactating animals. Glucose appears to be an essential metabolite for a number of tissues, in particular the red blood cells and the brain. There is continued *in vitro* glucose metabolism by the cells therefore serum should be separated from the clot very rapidly within 30 minutes to obtain accurate glucose values (Stockham and Scott, 2002). In ruminants glucose is made in the liver from propionate and their serum glucose concentration is lower than that of non-ruminant species and glucose level were lower for adult cattle compared with young (Hays, 2004).

The level of glucose were 60-100 mg per 100 ml of blood for different breeds of goat as reported by (Tambuwal *et al.* 2002). Hyperglycemia occurs with stress, excitement, pain and disease such as bovine neurologic diseases displaced abomasums and hypoglycemia is associated with neonatal malnutrition and pregnancy toxaemia in ruminants (Merck Veterinary Manual, 2012).

The sheep on tropical forest system were reported to have low serum glucose and triglyceride but normal concentration of total protein (Astuti *et al.*, 2008).

### 1.6.2: Plasma proteins:-

Plasma proteins are very complex mixture of simple proteins and conjugated proteins like glycol-proteins and lipoproteins. Protein constitutes 5- 10% of the plasma and consists of albumins, globulins and fibrinogens. Protein is an important factor for blood viscosity, acid-base balance, and supplying necessary enzymes (Keser and Bilal, 2008). The mean concentration of total goat serum protein is from 6.75 to 7.53 mg/dl (Merck Veterinary Manual, 2012). Serum total protein concentration reflects long term body protein status and is usually maintained until body protein stores are markedly depleted. Insufficient intake of dietary proteins reduces the synthesis of serum albumin as well as other protein fractions (Daramola *et. al.*, 2005). The blood profile, remained unaffected by protein sources indicating that homeostatic mechanism might not be influenced by different protein sources (Radostits, *et al.*, 2003).

Albumins are the most abundant protein fraction (52-62%) act as the transport system in the body and maintain osmotic pressure. Beside transport of amino acids (AA) to the tissues, albumin act as storage reservoir of AA. Albumin is synthesized in the liver and is catabolized by all active tissues. (Keser and Bilal, 2008). The level of albumin in plasma can be used to assess an animal protein intake, degree of hydration and liver function. Serum albumin level of 3.5g/100 ml was reported for West African Dwarf goat (Lazzaro, 2001). Globulins are protein molecules that are insoluble in plain water but soluble in salt water. Globulins are heterogeneous complex mixture of protein molecules identified as (alpha, beta and gamma) account for 29.5-54% of total plasma protein and involve in the transport and immunity (Radostits, *et al.*, 2003). A large proportion of globulin fraction consists of immunoglobulins, which are synthesized by lymphoid cells. Many of the other globulins are mainly synthesized by the liver, with small amount synthesized by other tissue. (Daramola *et. al.*, 2005). The

mean concentration of total goat serum protein is from 6.75 to 7.53 mg/dl (Zubcic, 2001).

#### 1.6.3: Blood urea:-

Elimination of nitrogenous waste, the ability to concentrate urine to conserve body water and regulation of acid base are vital functions performed by the kidneys. Damage to the kidneys has been implicated for renal failure and changes in serum urea N, creatinine, uric acid and mineral concentrations (Garg *et al.*, 1992). Blood urea nitrogen (BUN) concentration which is greatly associated with protein metabolism has been used to monitor nutrient status in ruminants (Hammond *et al.*, 1994). Blood urea nitrogen and protein intake should have a positive relationship indicating that BUN could be an indicator of protein intake (Sunny *et al.*, 2007). Generally BUN tend to be lower in ruminants kept diet of low protein or sever liver disease (Meyer and Harvey, 2004).

Urea is generated in the liver as a mean of the detoxification of ammonia, produced by protein catabolism in addition to that which originates from the diet. The increased catabolism of amino acids, when proteins of lower biological values are used as feed, has been implicated for high plasma urea N levels (Aderolu *et al.*, 2007).

### 1.6.4: Activity of serum enzymes:-

Enzymes are protein catalysts occur mostly in living cells and constantly and rapidly disintegrate with capacity to be renewed by synthesis (Russell and Roussel, 2007). The concentration of serum enzymes reflects enzymes that are either in transit from site of synthesis to the site of action or which have been released from damaged cells. Aminotransferases enzymes act as a catalyst in the metabolism of amino-acids and carbohydrates. Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage (Kaneko, *et al.*, 2008). Hepatic enzyme activity greater than normal doses not always reflect primary hepatic disease (Russell and Roussel, 2007). The serum activity of the enzyme increase because they essentially leak out of hepatocytes into the serum. When interpreting AST and ALT levels the rate of clearance from plasma should be considered as the half-life of these enzymes are approximately 2-4 days. As well as the extent of liver damage, is it mild leakage or extensive necrosis and chronic or acute.

The AST is present in many tissues and is useful in evaluating muscle and liver damage in small and large animals. AST is not liver specific in any domestic animal species and skeletal muscle is the second largest source of AST in animals. It is an absolute prerequisite to eliminate extra-hepatic tissue damage as a possible source of serum AST when evaluating the enzyme in relation to the liver. AST is present in both the cytoplasm and mitochondria of the hepatocytes and many other cells and will be elevated when membrane permeability is altered.

Serum enzyme (AST) is widely distributed, is found in many tissues and organs (extra hepatic) and is highly active in the liver and is involved in energy metabolism of the cells (Tambuwal, *et al.*, 2002).

The enzyme ALT is a cytoplasmic enzyme, and is considered to be liver specific in dogs, primates and some other small animal species. There is little hepatic ALT activity in large domestic animals and therefore not useful to assess liver diseases of ruminants (Kramer, 2000). The enzyme ALP is primarily bound to cell membranes. The physiological function of these isoenzymes is not fully understood although recent information suggests that one of the biological roles of ALP is detoxification of endotoxin. An elevated alkaline phosphatase concentration is generally due to cholestasis in most adult domestic animals. A mild elevation in immature animals is likely to be the result of normal bone growth (Russell and Roussel, 2007). This is the only enzyme affected by age, high values of ALP is observed in young fast growing goats. The activity of the enzymes (ALT), (AST) and (ALP) is conventionally used for diagnosing hepatic damage (Silanikove *et al.*, 1996).

#### 1.6.5: Blood lipid:-

Lipids serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss.

Dietary lipids for animals are composed of triglyceride, cholesterol and phospholipids. Lipids are originally insoluble in water, lipoproteins act as primary transport carrier for lipid in biological fluids (Murray *et al.*, 2003).

The storage of excess energy as fat is crucial to animals in which fat mobility plays important role. During periods of negative energy balance, tissue fat is mobilized to free fatty acids, non-esterified fatty acids and glycerol for use as energy in the liver.

#### 1.6.5.1: Serum lipoproteins:-

Specific proteins called apolipoprotein interact with lipids to form soluble lipid. protein complexes called lipoproteins. The different apolipoproteins are recognized by specific membrane receptors, and function as signals that regulate lipid entry and exit at target cells. Plasma lipoproteins are classified according to their densities, which is determined by the proportion of triglyceride, phospholipids, cholesterol and proteins.

As triglyceride have lower densities than proteins, the less lipids and the more protein contained in a lipoprotein complex, the higher its density. Lipoprotein consist of four major classes chylomicrons (CM), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Apart from chylomicrons originating from intestinal epithelial cells during absorption of lipids, lipoproteins are synthesized in the liver (Merck, Vet Manual, 2012).

## 1.6.5.2: Serum triglyceride:-

Triglyceride constitute more than 80% of the lipid in chylomicrons (lipoprotein) and help to transport the digested lipids to liver and peripheral tissue.

The (VLDL) and LDL contain 60% triglyceride are synthesized in the liver for export of triacyylglycerol to extra-hepatic tissues (Murray et al., 2003). Triglyceride eventually leave the liver as VLDL. In the post absorptive phase VLDL are the major transport form of triglyceride, mainly to adipose tissue. After loss of triglyceride in the adipose tissue from very low density lipoproteins (VLDL), then LDL are formed and after further metabolism HDL are also formed. The LDL represent the final stage in the extrahepatic catabolism of VLDL. The LDL (bad cholesterol) is the main carrier of cholesteryl ester and contain apolipoprotein B as its major protein constituent (contain little protein). High density HLP (healthy cholesterol) are involved in VLDL and chylomicron metabolism. It also facilitate transport of extra-hepatic cholesterol back to the liver or steroidogenic organs (adrenals ovary and testes) for synthesis of steroid hormone (Murray et al., 2003). Receptor (macrophage) mediate uptake of HDL from the blood and subsequently selectively remove cholesterol from HDL. The concentration of triglycerides-rich lipoprotein (CM, VLDL and LDL) in the blood is controlled by a variety of hormones. The values of serum triglyceride are usually considered as indicators of good nutrition. Triglyceride increase in the presence of an altered regulatory mechanism of lipid metabolism or due to degenerated hepatic function. The triglyceride concentration were found to be 0.30±0.24 Mm/l in Spanish goats with no effect of sex on their concentration (Zubcic, 2001).

#### 1.6.5.3: Serum Cholesterol:-

In ruminants cholesterol is naturally produced in the liver and intestinal wall. Cholesterol is the precursor of cholesterol ester, bile acids and steroid hormones and metabolism fat soluble vitamins and also required for normal cell function.

The amount of cholesterol from dietary sources and hepatic synthesis is under close homeostatic control with the rate of synthesis inversely proportional to absorption. The dietary cholesterol ester is utilized almost completely in the liver, and losses are in the form of bile acids and free cholesterol and its derivatives in bile. The plasma HDL-cholesterol concentration in ruminants was changed by varying dietary fat and protein levels (Beynen *et al.*, 2000). Most animal species are HDL mammals, meaning that most of the cholesterol is carried by HDL. These mammals include dogs, cats, horses and ruminants with exception of pigs, rabbits hamster, camel and monkeys and human are carried by LDL. Khan *et al.* (2013) reported that the of level of serum cholesterol is generally lower in goat breeds as compared to cattle breeds.

The reference serum cholesterol levels (1.7–3.5 mmol/L) for normal goats reported by (Merck, Vet Manual, 2012). Normal serum cholesterol indicates that the animal is healthy and not susceptible to heart disease, since high level of serum cholesterol is related to heart diseases (Chatterjea and Shinde, 2007).

#### 1.6.6: Serum bilirubin:-

After the protein and iron are broken away from haemoglobin, globulin and iron are conserved to be reused. The heme in the macrophage is changed into green pigment biliverdin and finally bilirubin (yellow) (Cheesebrough, 2004).

The bilirubin is taken by liver to make bile acids form cholesterol, secreted in the bile, into the intestine (urobilinogen in large intestine) part is taken by the liver via the portal circulation.

Total bilirubin in the blood stream may be partitioned into two forms namely direct (conjugated) and indirect (unconjugated) (Singh, 2004). In the blood, unconjugated bilirubin is loosely bound albumin (not water soluble, free or prehepatic) is transported to the liver. The hepatocyte conjugates the indirect bilirubin with glucuronic acid and

it is then referred to as direct or conjugated bilirubin. Conjugated bilirubin (bilirubin glucuronide) passes down bile duct in the bile, unlike bile salt it has no role in digestion. Direct bilirubin (conjugated) is water soluble and excreted into bile.

The direct bilirubin is not bound to albumin and is freely filtered by the glomerulae. The renal tubular epithelial cells readily reabsorb the filtered bilirubin in most animals. Bilirubin and its components may be helpful when evaluating liver function or haemolysis. These tests may be useful in distinguishing prehepatic from hepatic or posthepatic hyperbilirubinaemia.

An elevation of indirect bilirubin is a rather uncommon finding in small animals, but when it occurs, it is generally the result of acute and severe haemolysis. A healthy liver is capable of conjugating large amounts of bilirubin and that is why many haemolytic anaemias have normal bilirubin values.

Direct reacting hyperbilirubinaemia occurs as a result of impaired hepatic secretion of bilirubin and/or obstruction to bile flow. Obstruction to bile flow can be intra-hepatic, extra-hepatic or both. Most jaundiced animals have elevations in both indirect and direct bilirubin. Haemolytic disease may also result in an increase in direct bilirubin since a large proportion of the free bilirubin is conjugated. The serum total bilirubin ranges of (0.00- 0.9 mg/100 ml) of serum have been reported for domestic animals (Singh, 2004).

# 1.7: Dietary effect of browse on serum metabolites:-

## 1.7.1: Dietary effect browse on blood glucose:-

Feeding of goats on Prosopis pods up to 90% of the diet with alfa alfa had no effect on blood glucose which remained in the normal range (Cook, *et al.*, 2008). Although Kailas, (1991) observed a significant decrease in blood glucose level in goats fed on *Leucaena* leaves. Significant decrease in blood glucose level was found in goats given diets containing 50% CP replaced by *Leucaena* leaves.

The hypoglycemic effect reported for some browse also lead to decreased amino acid due release of sucrose and depression of fibre digestion (Tabosa *et al.*, 2004).

## 1.7.2: Dietary effect of browse on total proteins:-

The level of serum total protein, albumin and globulin were not affected in the goats fed mixture of tree leaves (Ajit Pal *et al.*, 2010). Goats were fed mixture of

sun-dried chopped fodder shrub (*Prosopis juliflora*, *Acacia* and *leucaena*) the results revealed that the test diet lowered the concentration of total protein, albumin and globulins in the goats but not beyond the reference value (Shaker, *et al.*, 2014) and in the lambs (Shaker, *et al.*, 2008).

## 1.7.3: Effect of dietary browse on blood urea:-

Adding *Prosopis glandulosa Torr*. in the goat diet by 30, 60 and 90% resulted in decreasing blood urea nitrogen concentration in all treatment as compared to control (Cook *et al.*, 2008). The authors also reported that goats fed on diet consisting of 60% or 90% mesquite pods had high level of creatinine, indicative of kidney damage.

Dietary supplement of freshly cut forage of *P. cineraria* and other browse had shown no difference in haemoglobin concentration among the grazing rams, but blood urea nitrogen level was lower in the *Prosopis* fed group (Bhatta *et al.*, 2005). The absence of changes in serum urea, uric acid or mineral concentration and absence of damage in kidneys in *Prosopis* fed animals was reported by (Garg *et al.* 1992). Serum urea concentration was considerably lower (15.1 mg/100 ml) in goats fed browse leaves than those fed wheat straw (Silanikove *et al.*, 1996).

The effect is most likely related to the severe limitation in protein availability in goats fed tannin-containing leaves. The effect of freshly cut leaves of *P. cineraria* supplemented with polyethylene glycol-6000 or without supplement on the blood metabolite was investigated by (Bhatta *et al.*, (2002). The Hb and BUN (g/dl) were higher in group fed polyethylene glycol with *Prosopis* leaves compare to fed leaves with active tannin.

#### 1.7.4: Effect of dietary browse on blood lipids:-

Cholesterol concentrations were 99.01 and 83.96 mg/dl for Shami goats fed berseem hay (control) and mixture of trees containing *Prosopis* leaves. Animals fed tree mixture had low cholesterol level compared to control (Shaker *et al.*, 2014). Cholesterol level ranged between 32.33 to 44.33 mg/dl in rabbit fed graded levels (0, 10, 20, 30, 40%) of *Prosopis Africana* pulp, no differences between groups were reported (Adamu *et al.*, 2011).

Cholesterol level was 47.0 mg/100 ml in goats fed browse leaves and there was no difference between the leaves and wheat straw (Silanikove *et al.* 1996).

The concentration of triglyceride in sheep fed *M. oleifera* and *A. hetrophyllus* leaves were higher than those fed other tree leaves (Astuti *et al.* 2011). Omidi *et al.*, (2012) found a significant reduction

in LDL-cholesterol along with a rise in HDL cholesterol concentration in ostriches fed prosopis farcta. This finding may reflect the beneficial effects of *P. farcta* extract on cardiovascular health or reduction of chest pain. Also Narasimhacharya *et al.* (2010) found that *Prosopis juliflora* leaf possess antihyperlipidemic properties and reverses the hypercholesterolemic conditions in hypercholesterolemic male albino rats.

### 1.7.5: Effect of dietary browse on serum enzymes:-

The level of alanine aminotransferase (ALT) were found to be higher in animals fed nonconventional (date tree parts) diet with higher level of polyphenol and condensed tannins (Mahgoub *et al.*, 2008).

The level of alkaline phosphatase (IUL<sup>-1</sup>) activity ranged from (129.27 to 177.96) in goats fed leaves mixture of (*Leucaena leucocephala-Morus alba-Tectona grandis*) or traditional protein (soybean or ground nut cake) with no evidence of any effect of dietary treatment or period of measurement (Anbarasu *et al.*, 2002).

In the mentioned study blood enzymes such as Alkaline phosphatase (ALP), ALT and aspartate aminotransferase (AST) did not differ throughout the experiment though the diet contained anti-nutritional factors such as tannins. The observed level of alkaline phosphatase was within the normal range as reported by (Kaneko *et al.*, 2008). The concentration of various blood biochemical constituents, and the activity of alkaline phosphate, AST and bilirubin were not affected by leaves supplement, except for ALT (Raghuvansi *et al.*, 2007). There were no consistent differences between goats fed tannin rich leaves and wheat straw in any serum metabolite except urea. The metabolites studied were protein, albumin, uric acid, cholesterol, urea, GGT, ALP, AST, Na and Cl (Silanikove *et al.*, 1996).

The report from (Tabosa *et al.*, 2000) indicated that the anti-nutritional factor in *Prosopis juliflora* pods diets caused damage tissue in the goats. Diet contained mixture of *prosopis, acacia and leucaena* resulted in elevation of serum AST and ALT in Shami goat (Shaker *et al.*, 2014).

## 1.8: Rumen environment:-

# 1.8.1: Rumen pH:-

Ruminal pH represent physicochemical measure with relevance to fermentation in the rumen, that take place during daily feeding cycle. The ruminal pH is the net effect of rates of production and absorption of acidic fermentation products and the addition of buffers to the rumen via saliva. The degradation of protein releases ammonia and readily increase the alkalinity and increase rumen pH. The ruminal pH varies considerably during the course of the day, and is particularly driven by the amount of fermentable carbohydrate in each meal.

Shifts of 0.5 to 1.0 pH unit within a 24 hour period are common (Oetzel, 2003). The variation in rumen pH is influenced by the type of diet, type of ruminant species and the feeding frequency (Franzolin et al., 2010). The variations in ruminal pH may be more critical for rumen fermentation than a low, but less variable, pH as suggested by (Wales et al., 2004). The mean ruminal pH values are not dramatically affected by large dietary changes but the lowest (nadir) pH values are greatly affected by diet (Oetzel, 2003). The changes in rumen fermentation started immediately after the pH started to decrease and the largest effect is reached within the first 12 h of suboptimal pH (Cerrato et al., 2007). Low rumen pH for prolonged periods each day can negatively affect feed intake, microbial metabolism, and nutrient degradation (Cerrato et al., 2007). Among microbes ciliated protozoa and fungi are more sensitive to pH than bacteria. At low pH microorganism is unable to regulate intracellular pH, maintain enzyme activity, growth and have successful attachment to cellulose substrate (Allen et al., 2006). Generally rumen fluid pH is high on range of 6.0 to 7.2 when forage are fed but can decrease to 5.0 when the proportion of concentrate in the diet is increased. The diet of best balance of fiber and starch, digestion occurs at rumen pH of around 6.35 (De Veth and Kolver, 2001a). Increasing the amount of physically effective fiber in the ration is the most efficient nutritional measure to alleviate the pH decline after a meal (Allen et al., 2006). Animal-to-animal variation in ruminal pH as well as ability to handle an acid load was reported by (Oetzel, 2003).

Buffering capacity in the rumen arise from ingested feed and added dietary buffer and saliva. Urea secretion and ammonia absorption have significant importance in regulation of rumen pH (Dijkstra *et al.*, 2012). Minerals salts buffers (bicarbonate, carbonate and phosphate of sodium, potassium calcium and magnesium) and fiber are important in that they help control rumen pH (Dijkstra *et al.*, 2012). Cattle are generally able to maintain ruminal pH within physiological limits by their own regulation of intake, endogenous buffer production, microbial adaptation, and VFA

absorption. Adaptive responses are invoked when rumen pH drops as result of fermentation of glucose to lactate instead of VFAs by *Strep. bovis*. Acid-tolerant ruminal bacteria have the ability to reduce accumulation of intracellular acids and help to moderate the effect of high pH (Oetzel, 2003).

## 1.8.1.1: Effect of feeding browse on rumen pH:-

Generally feedstuffs affect the ruminal acid-base via their pH, buffering capacity and stimulation of saliva secretion. The exogenous buffering value of feed is important, forage have inherent buffering capacity, legumes are superior to grasses (Levic *et al.*, 2005). The rumen pH was found to be above 6.2 during the 6 h collection period and did not drop below that level in goats which browsed tree shrubs supplemented with urea-molasses blocks (Gasmi-Boubaber *et al.*, 2006). The rumen pH was optimum for microbial activity when penned sheep fed *Acacia cyanophylla* and polyethylene glycol received feed in blocks (Gasmi-Boubaber *et al.*, 2006).

The effect of feeding complete diets containing graded level of *Prosopis cineraria* leaves on rumen fermentation in lambs and kids was studied at 6 hours post feeding (Bhatta, *et al.*, 2007). Rumen pH were significantly different between treatment groups ranged 6.17- 6.77 for animals fed 25 and 75% *Prosopis* leaves receptively. The effect of feeding *P. juliflora* pods at 0, 15 and 30% levels in concentrate mixture to lactating cows on rumen pH and other rumen metabolites was studied (Talpada and Shukla, 1987). The results revealed that the rumen pH values were 7.2, 7.05 and 7.22 in 0, 15 and 30% *Prosopis* level, respectively. The average ruminal pH values were almost similar in all the three treatment groups. Rumen pH and concentration of VFAs as well as ammonia nitrogen were not affected when *Prosopis* pods comprised 75% of concentrate mixtures for sheep (Sharma, 1997).

## 1.8.2: Rumen ammonia production:-

The hyper-ammonia–producing bacteria (*Prevotella spp*), a small group of bacteria sparsely populate the rumen (less than 0.01% of the rumen bacteria population), but are characterized by very high metabolic activity (Hart *et al.*, 2008). This bacteria responsible for 50% of protein decomposition in the rumen and ammonia production.

Oba *et al.*, (2004a) reported that, only 30%  $NH_3$ -N produce in the rumen was incorporated into microbial N and much of the remainder was absorbed across the rumen wall. In the normal rumen pH ammonia is present as the  $NH_4$  ion. Ammonium

ions must leave the rumen against the potential gradient which is positive for the blood, but are assisted by the concentration gradient (Oba *et al.*, 2004a). The rate of absorption is affected by pH or the concentration of other substances in the rumen.

In the liver ammonia is metabolized to urea, much of it re-enter the rumen secretion across the rumen epithelium or with saliva (Oba *et al.*, (2004a). However, recent studies have demonstrated that some detoxification of ammonia to urea can occur in rumen epithelial cells and duodenal mucosal cells (Oba *et al.*, 2004a). Conversion of urea to ammonia in the rumen occurs very quickly. Urea is secreted into the blood and can be measured as BUN. The urea not recycled to the rumen is excreted in urine. It has been estimated that at least 70% of nitrogen daily intake passes through urea pool of the body. Urea is usually secreted into the rumen in amounts beyond the needs of microbes except when the dietary N intake is low as with fibrous feed. (Reynolds and Kristensen, 2008).

The pool size of ammonia nitrogen in the rumen is small and subjected to large variations in ammonia concentration even in high protein diet. The variation is due to the extent of protein breakdown, metabolic activity of rumen micro flora, rate of passage to omasum, rate of absorption from the rumen and rate of uptake by bacteria (Hristov et al., 2005). Rruminal ammonia concentration is affected by species, breed, and age of the animal, ration composition, concentrate/roughage ratio, time and frequency of feeding. Despite of variation, ammonia level must be above the critical level for considerable period of the day. The level of ammonia that supports maximum digestibility in the rumen, and therefore the largest population of micro-organisms, will vary among diets. The critical level for ammonia has been reported to be 50 to 250 mg of ammonia nitrogen per liter of rumen liquor (Cajarville et al., 2006). Ammonia level may increase 2 h after feeding to 20 or 30 mmol/l relevant to rumen degradable N intake, then decrease rapidly due uptake by microbe (35 to 65% of decrease), flow to omasum (10% of the decrease) or absorption across the rumen epithelium (Reynolds and Kristensen, 2008). It cannot be assumed from the level of protein in a diet that rumen ammonia levels will be adequate.

If the protein is heavily protected from rumen fermentation rumen ammonia levels can be low and protein passes intact into the abomasum and duodenum, where it is digested by enzymatic hydrolysis. Amino acids so produced are deaminated in the liver and the ammonia produced is converted to urea which may be secreted into the rumen (in saliva or across the rumen wall). The recycled N to the rumen contribute to

stabilize microbial growth even when N supply is not well synchronized (Bach *et al.*, 2005).

# 1.8.2.1: Effect of feeding browse on rumen ammonia:-

The most important aspect of browse plants is that, unlike grasses during dry season, they are usually protein-rich. Feeds containing less than 1.3% N (8% CP) are considered deficient as they cannot provide the minimum ammonia levels required (Simbaya, 2002). All forage tree legumes have N contents higher than this value and may be judged adequate in protein. At a higher level of supplementation with browse trees, the principal effects of tannins in ruminal fermentation is a reduction in proteolysis of dietary protein and, in the concentrations of ammonia in rumen fluid (Mueller-Harvey, 2006). However animals supplemented with browse induce higher NH<sub>3</sub>-N concentration in the rumen and this could be as a result of the quantity and solubility of protein in browse (Isah *et al.*, 2013).

The effect of feeding diet containing *Prosopis cineraria* leaves on rumen fermentation was evaluated (Bhatta, *et al.*, 2007). The diet contained 25% *Prosopis* leaves produced the highest concentration (27.0 and 28.0) of ruminal ammonia in lambs and kids, compared to diets contained 50 and 75% of leaves.

Sheep was fed 30 and 40% of *Prosopis* pods showed an increase in total ruminal N and higher urinary N output (Chaturvedi and Sahoo, 2013). The effect was correlated to the high concentration of soluble N in the pods. *Prosopis* leaves produced the highest rumen ammonia concentration (30.9 NH<sub>4</sub>-N mg/100ml) than the other browse and Tifton grass hay (Soltan, *et al.*, 2012). The low concentrations of NH<sub>3</sub>-N in rumen fluid found with other browse (acacia and leucaena) could be attributed to the inhibition of rumen protein degradation and deamination processes by condensed tannins.

Significantly low concentration of ammonia nitrogen in goats fed *Ficus infectoria* (pakar leaves) as compared to control (green oats) might be due to reduced proteolytic activity in the rumen of pakar leaves fed animals (Singh *et al.*, 2011).

## 1.8.3: Rumen volatile fatty acid production:-

Total VFAs produced in the rumen are primarily acetate, propionate and butyrate and to a lesser degree branched chain VFA and occasionally lactate. Their production depends on the level of intake of ruminally fermentable OM. About 70-

80% of the ruminant energy supply is from the volatile fatty acids, generated in the rumen. The molar proportions of acetic acid, propionic acid and butyric, in ruminal fluid are from 45 to 70%, 15 to 40%, and 5 to 20% respectively (Loncke *et al.*, 2009). Immediately after feeding, acids production exceed loss and the concentration increases, with time the situation is reversed and concentration falls. (France and Dijkstra, 2005).

The majority of VFAs produced in the rumen are absorbed across the rumen wall, although a proportion of (10-20% in sheep and up to35% in dairy cow) pass the rumen to be absorbed into the omasum and abomasums. However at extreme energy intake (4 times maintenance) have been found to enhance the fraction of VFA passing unabsorbed into the omasum. The absorption rates of VFA from the rumen is affected by ruminal pH and osmolality, type and concentration of VFA. In the rumen wall an extensive metabolism of butyric acid occur and small amount of the other acids (Kristensen and Harman, 2004a).

The fermentation pattern is determined by the composition of the microbial population, the basal diet, particularly the type of dietary carbohydrate. In addition to the type of dietary carbohydrate, factors such as the physical form of the diet, level of intake, frequency of feeding and the use of chemical additives may also affect the fermentation pattern (Nagaraja *et al.*, 1997).

Acetate is considered to be reflective of cell wall fermentation and Propionate is associated with soluble carbohydrate (starch) fermentation. High forage diet favor the growth of acetate producing bacteria and the acetate: propionate: butyrate molar proportion would typically be in the range 70:20:10 (France and Dijkstra, 2005).

Under certain circumstances concentrate diet favor the growth of protozoa and result in an increase in butyrate rather than propionate. Ruminal acetate is used as substrate for lipogensis, and propionate is a precursor of hepatic gluconeogenesis. Fermentation to acetate evolve H and increase methane production but propionate decrease methane formation. Propionate formation retained more of the energy of fermentation in the in useful products, but excessive propionate can cause undesirable consequence on production (France and Dijkstra, 2005).

## 1.8.3.1: Effect of feeding browse on volatile fatty acid:-

Total VFAs concentrations were 12 and 9% higher in rumen liquor of cows fed *P. juliflora* pods at 15 and 30% level on concentrate part of ration, respectively but the differences were not significant. The results showed that peak concentrations of TVFA

in group fed (0% pods) and 15'% pods was at 2 hours post feeding while in the group fed 30% was at 1 hour. This was attributed to rapid fermentation of diet contained high *Prosopis* pods that was also high in nitrogen free extractive (NFE) (Talapada and Shukla., 1987).

The total ruminal VFAs produced were 68.97, 65.96, 65.72, 68.37 and 62.93 mmol/l for Tifton hay, and leaves of four trees, *Acacia*, *Atriplex*, *Prosopis* and *leucaena* respectively The decreases in the acetate: propionate ratio, increased propionate without decrease in total VFA was found in ruminal fermentation of *Prosopis* and *Leucaena* leaves (Szumacher-Strabel and Cieślak, 2010; Soltan *et al.*, 2012).

The effect of replacement of *Prosopis juliflora pods* (PJP) at 0.30 and 0.40 in the concentrate mixture fed to sheep along with Cenchrus grass (CG) was studied (Chaturvedi and Sahoo, 2013). The total VFA concentration in the rumen showed periodic fluctuation higher values were in all of the groups at 4 h post-feeding and dropped at 8 h to values as it was during pre-feeding.

The total VFA concentration was not different between the groups, but they were significantly higher in Prosopis fed groups. The observed reduction in pH and increased in total VFA at 4 h post feeding in group fed 30% and group fed 40% (PJP) compared to the group fed 0% of *Prosopis* pods was attributed to the presence of fermentable carbohydrates in *Prosopis* pods. (Chaturvedi and Sahoo, 2013).

#### 1.8.4: Rumen protozoa:-

# 1.8.4.1: Characteristics of rumen protozoa:-

Rumen protozoa are composed of two types, flagellate and ciliate, the latter are more important in rumen function. On the basis of cell morphology, ciliated protozoa, grouped into five families containing 24 genera, new species are still reported yearly.

Two groups are distinguished within the Ciliates: the Holotrich and Entodiniomorphs (Oligotrich) protozoa. The existence of flagellate in the normal rumen is not abundant but they become extremely numerous in the absence of ciliated protozoa (Dehority, 2005). Protozoan population is equal or exceeding that of bacteria, at a concentration of about (10<sup>5</sup>-10<sup>7</sup>) per ml of rumen fluid.

The most important factors affect the protozoa population are the type of the host, its geographical location and nature of the ration and feeding habit of the host (browser or grazer), the level and frequency of feeding and interspecies protozoal antagonism. In general, protozoal concentrations were higher in concentrate selectors, followed by the

intermediate or opportunistic mixed feeders and lowest in the grass and roughage eaters (Dehority, 2005). The number of protozoa in the rumen changes during the diurnal cycle and in animals fed once daily the numbers are highest during the feeding period and begin to decline thereafter. Generally maximal protozoal concentrations are attained immediately prior feeding, reaching minimum shortly post feeding. Because of their adherence to food particle and sequestration, protozoa have regarded to be virtually completely recycled within the rumen. (Dehority, 2003; Karma, 2005).

## 1.8.4.2: Role of the rumen protozoa:-

The ruminal protozoa contribute significantly to the digestion of plant cell wall polymers and their absence from rumen may have negative effect on the extent of fiber digestion. Protozoa slow the rate of fermentation by engulfing starch granules, preventing their bacterial attack and modulate the rumen pH (Russel and Rychlik, 2001). Protozoa contribute to the supply of protein to the intestine, approximately 11% of total crude protein flow.

Protozoa engulf large number of chloroplasts and bacteria as their nitrogen source. Rumen ciliate are proteolytic, producing ammonia and amino acids as end products. Part of peptide and amino acid formed by the action of protozoal enzymes are used for protozoa protein synthesis. Ciliated protozoa cannot utilize urea for their body protein synthesis (Walker *et al.*, 2005). It is apparent that removal of protozoa from the rumen (defaunnating) prevents the recycling of N between bacteria and protozoa, increasing the efficiency of N metabolism and enhances flow of microbial protein from the rumen (Dehority, 2003). Protozoa detoxify toxin of poisonous plant and eliminate some toxins out of digestive tract and stabilize the number of Streptococci to reduce the produced harmful lactic acid (Franzolin and Dehority, 1996a). Methanogenic ruminal bacteria have observed attached to ciliated protozoa, imply a possible indirect role in methane formation (Dehority, 2003).

# 1.8.4.3: Effect of feeding browse on protozoa:-

Rumen fungi, proteolytic bacteria and protozoa are more resistant to tanninniferous browse as compared to other microbes (McSweeny *et al.*, 2001). The effect of four legume browses namely *Prosopis*, *acacia*, *atriplex and leucaena* on rumen protozoa was studied (Soltan *et al.*, 2012). The results showed that all of the browse diets had a lower total counts of protozoa compared with control (Tifton hay), and *Prosopis* leaves showed the least number of protozoa followed by *Leucaena* (2.21

and  $2.56 \times 10^5$ /ml) respectively. These worker proposed that the presence of antiprotozoal factor other than tannins are responsible for the reduction of protozoa count when goats were fed *Prosopis* diet. Odenyo *et al.* (1997) included *A. saligna* as a supplement to maize stover of sheep and observed an associated decrease in protozoa numbers from  $1.60\times10^5$  to  $0.62\times10^5$  cells ml<sup>-1</sup> rumen fluid. There was a reduction in the rumen protozoa count in goats fed pakar leaves (*Ficus infectoria*) rich in tannins, compared to control group fed green oats (Singh *et al.*, 2011). There was a reduction in the number of rumen protozoa in Holstein-Friesian dairy fed *Vaccinium vitisidaea* was observed by Cieslak *et al.* (2012) and suggested that antiprotozoal effect occurred with Acacias tannins. Amira *et al.*, (2014) investigated the influence of spineless cactus on rumen microbial population profile changes. There was a significant reduction in rumen protozoa count with *A.cyanophylla*, *Opuntia* and *A.nilotica*.

#### 1.8.5: Rumen bacteria:-

## 1.8.5.1: Characteristics of rumen bacteria:-

Rumen bacteria constitute more than 50% of microbial population, in 300 to 400 species and number of  $(10^{10}-11^{11})$  cells per ml of rumen liquor. These bacteria are mainly non-spore forming anaerobes and are not found to exist outside the rumen environment. Ruminal bacterial groups, include those involved in breaking down of cellulose, hemi-cellulose, starch, sugar, organic acids or protein and bacteria that producing lactic acid or methane. It was found that the most common rumen microorganisms are gram-positive cocci, rods and spirilla (Edwards et al., 2004). The major bacterial group contains three important species of bacteria, two are involved in cellulose digestion (fiber digester or cellulolytic bacteria), the third group of bacteria is starch digesters (amylolytic bacteria) (Firkins and Yu, 2006). Ruminal bacteria are either specialist or generalist in their use of energy substrate (Mould et al., 2005). For example, a specialist starch digesting species predominates when diets are rich in concentrates and methane producers predominate when low quality forages are fed. However, most rumen bacteria are generalist which degrade a wide range of polysaccharide, or minor group utilize sugar and a variety of bacterial end products (Mould et al., 2005). The principle starch digesting bacteria are S. bovis, R. amylophilus the starch digestion is regulated by protozoa. Roughage-rich diet induce a ruminal microbial population in which fiber digester and methane producing bacteria predominate (Wright *et al.*, 2004b). Rumen bacteria especially cellulolytic species are essential for rumen function and wellbeing of the host (Singh *et al.*, 2011). Methanogens however, have morphological resemblance to bacteria are placed in a new domain, called Archaean and they are responsible for regulating the overall fermentation, methane keeps the partial pressure of hydrogen in the rumen low (Janssen and Kirs, 2008).

Regular changes in the numbers of both bacteria and protozoa in the rumen of grazing sheep are correlated with seasonal changes in the availability of green pasture. The diurnal variation in the numbers of rumen bacteria is related to the changes in the amount of ruminal digesta and available carbohydrate. The concentration of rumen bacteria is greatest just before feeding and is reduced by diluting effects of feed and water consumption and saliva flow, and then increased by bacterial growth in response to the increased supply of feed. This diurnal variation may depend on the nature of the diet. Longer periods without food lead to large reductions in the number of rumen bacteria, but numbers rapidly increases when the animal is fed. The diurnal variation in the concentration of rumen bacteria and protozoa revealed less fluctuation of bacterial count relative to protozoal count (Edwards *et al.*, 2004).

# 1.8.5.2: Effect of feeding browse on rumen bacteria:-

The impact of tree-based browse on the ruminal bacterial community varies by browse species, likely due to differences in browse composition. Hung et al. (2013) reported that Leucaena leaf pellet (LLP), supplementation significantly increased rice straw intake and total intake. There was also an increase in the population of fungal zoospores, amylolytic bacteria, proteolytic bacteria and cellulolytic bacteria with an increased level of (LLP) supplementation while the population of methanogenic bacteria decreased. The effect of tree leaves supplementation on rumen microbe's activity in sheep was evaluated (Singh and Kundu, 2011). The total viable bacteria and cellulolytic bacteria were significantly higher in rumen of sheep fed Dicanthium annulatum-Leucaena leucocephala (16.05)and 21.46) than Dicanthium annulatum- Hardwickia binata diet (8.53 x  $10^8$ /ml and 9.18 x  $10^7$ /ml).

The effect of *Acacia* pods meal on the rumen bacterial diversity in cattle was investigated by (Angeles *et al.*, 2013). Bacterial diversity analysis showed that

feeding Acacia pod meal resulted in the proliferation of tannin-resistant bacterium *S. ruminantium* and loss of cellulolytic bacteria A. ruminis.

Legumes containing condensed tannin (e.g. Lotuses) are able to lower methane (based on g/kg DMI) by 12-15% mainly through a direct toxic effect on methanogens (Beauchemin, *et al.*, 2008).

## -: Nutritive value of feedstuffs :1.9

Feed value is the potential of feed to supply the nutrients required by an animal both quantitatively and qualitatively in order to support a desired type of production. The nutritional value of ruminant's feed is commonly estimated from the characteristics of their chemical components, as well as from their fermentation pattern in the rumen, and from undegraded feed components, especially protein passing to the small intestine (Hamid *et al.*, 2007). The nutritive value of forage is affected by factors of plant origin, animal origin and environment related. Feed value is influenced also by of feed composition, physical nature, (odour and taste), intake level, associate effect when given in ration, and the physiological status of animals (Hamid *et al.*, 2007).

Chemical composition comprise information in both nutritive and anti-nutritive factors, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and tannin content of tree foliage and it is needed for comprehensive assessment of their nutritive value. The digestibility of plant material in the rumen is related to the proportion and lignifications of plant cell wall (NDF). Tree forage with low NDF content (20-35%) are usually of high digestibility and species of high lignin content are often of low digestibility. Fodder trees compared to grasses have high CP, NDF and mineral and low in ADF and DM digestibility (Kaitho *et al.*, 1998).

When digestibility is combined with intake data, one can make an accurate prediction of overall nutritive value. Feed intake is relatively more important than digestibility in determining the nutritive value. Feed intake in ruminants consuming fibrous forage is primarily determined by level of rumen fill, which in turn, is directly related to the rate of digestion and passage of fibrous particle from the rumen.

Utilization of feedstuff such as *Prosopis* pods in complete feed depend on its nutritive value. The most important information about the nutritive value of tree fodders is the level of supplementation which optimizes nutrient intake. This level will vary with the quality of tree fodders and of basal diet, and will vary with different plant parts and

fodder tree species. An ideal forage supplement should maintain or increase the basal diet rather than substitute for it, a phenomenon that has been frequently observed in animals fed on legumes (Anbarasu, *et al.*, 2002). It was observed that increased supplementation of tree fodder levels reduced nutrient digestibility due to increased passage rate and therefore recommended low level of tree leaves supplementation (Melaku *et al.*, 2004).

#### 1.9.1: Concept of rumen degradation:-

The large rumen capacity and longer retention time for feed in forestomach are two main attributes to achieve digestive function. The rationale for degradation, is that forage consists largely of carbohydrate in form of fiber and this can only be digested by microorganism in the rumen. Chemical component of diets of ruminants can be separated into two structural fractions of nutritional significance (Van Soest, 1994). Cell contents include substances that are soluble and potentially highly digestible in the intestine like non-structural (NSC) or non-fiber carbohydrates. However, these substances tend to be rapidly and extensively fermented before reaching the gastric-intestinal region.

Ruminal digestion is dynamic process that start by the entry of food in the rumen fluid and output of fluids, microbes and undegraded food (Van Soest, 1994). Cell wall component such as (NDF) are more slowly and less completely digested.

Digestion of the cell wall fraction is almost exclusively by microbial hydrolysis and fermentation. Food enters the rumen partly in degradable form, and partly in an undegradable form. If the undegradable food particle are sufficiently reduced in size, they move to the abomasums and small intestine for digestion and absorption. The factors affecting forage digestion are how fast it digests and how long it is retained in the tract.

The forage dry matter is classified into three parts according to its digestion characteristics: soluble-instantly digested dry matter or cell soluble (fraction A), slowly digested NDF (fraction B) and indigestible NDF (fraction C). Fraction B is also defined by its rate (kd) of degradation (Van Soest, 1994). The food fraction that is hydrolyzed in the rumen to provide energy (fermentable carbohydrate) or a peptides mixture, amino acid and ammonia (NNP and protein) for growth and synthesis of microbial protein is referred to as degradable fraction. The ruminal degradation of food nutrient, is influenced by several factors, such as, the characteristics of the diet, related to amount of potentially degradable nutrients, the food intake level, the food

retention time in the rumen and food exposure to microorganisms and rumen environment (pH, NH<sub>3</sub> concentration) which affects the activity and survival of microorganisms (Ørskov and Ryle 1990). The main factor influencing the rate of fermentation of feeds is the structure of the carbohydrate fraction, especially the extent of lignification of the cell wall (Nagadi *et al.*, 2000).

The effective degradability (the digestibility that can be expected *in vivo*) also it depends on the rate of degradation, potential degradability and fractional outflow rate from the rumen (Hvelplund and Weisbjerg, 2000).

Protein in feeds is, to a large extent, degraded in the rumen. Degradation is one of the most important quantitative factors determining the nutritional value of feed protein, the supply of ammonia, branched chain fatty acid to ruminal microbe, and the passage of undegradable protein to intestine (Hvelplund and Weisbjerg, 2000). The rate and extent of digestion are critical variables in the description of the digestion process. A first order mass action model often describes the ruminal degradation of protein. An important characteristic of this model is that it considers that (CP) of the feed consists of multiple fractions, which differ greatly among themselves in relation to degradation rates, and that the ruminal disappearance of protein is the result of two simultaneous activities degradation and passage (NRC, 2007). Several methods have been used to divide CP into rumen degradable protein (RDP) and rumen undegradable protein (RUP). These methods include in vivo and in situ evaluations, and a variety of in vitro methods.

Regarding carbohydrate, non structural carbohydrate like starch is subjected to digestion by enzymes from the animal and the ruminal microorganisms (Russell, *et al.*, 2009). Starch digestion from cereal grains or other concentrate feedstuff, is complete in most ruminants. However, the degree and form of ruminal digestion of starch will be different, depending on the raw material, its processing and presentation (Nocek and Tamminga, 1991). Starches from various ingredients of the ration for ruminant are characterized by different degraded parameters (Zhang *et al.*, 2006). The carbohydrate degradation kinetics are very complex, it needs information in the characteristics of carbohydrate and protein degradation of seeds and grains use in animal rations, as well as the factors that affect them.

The system of Sniffen *et al.* (1992) is subdivided into four fractions of carbohydrates depending on their degradation rate:

A = very quickly rate of sugars degradation.  $B_1 = intermediate$  rate of degradation (pectin and starches).  $B_2 = fibrous$  carbohydrates of slow degradation and potentially degradable. C = indigestible fiber fraction.

There are many factors linked to food, as nitrogen and carbohydrate sources characteristics, can be considered as elements that will affect the rate of degradation in the rumen. There are other factors linked to animals that influence the nutrients rumen degradation. Such as structure of the protein molecule, solubility of protein in the rumen, protein and starch location and structure. The rate of ruminal degradation of the protein matrix, determines the rate of starch hydrolysis, because the starch surface, which is in contact with the amylases, increases as the matrix degrades. The cereals such as maize and sorghum have a lower proportion of soluble proteins (albumins and globulins) and a greater proportion of reserve proteins (prolamin and glutelin) that are less soluble and slow in degradation. Starch degradability from legumes is high, due to the starch type, its interaction with the protein matrix, and the lowest ratio of amylose-amylopectin (Zhang *et al.*, 2006).

#### 1.9.2: In vitro gas production:-

In vitro rumen system are the most accurate methods for estimating the digestibility of feed stuffs, because they utilize microorganism and enzymes which are sensitive to undetermined factors that influence rate and extent of digestion (Van Soest, 1994). Tilley and Terry, (1963) *in vitro* dry matter digestibility (IVDMD) described a two-stage method for predicting the *in vivo* digestibility of good quality temperate roughages, in which a feed sample is digested for 48 hours in a buffered rumen liquor taken from rumen-fistulated animals, followed by pepsin digestion in an acidic solution. Fiber digestion by rumen microbe had been completed within 48 hours (although this is not the case for poor quality tropical roughages), but the residue contained unchanged feed protein and microbial protein. To digest these material, 48h incubation in acid pepsin solution was used. This method produces end-point digestibility estimate. A modification of the Tilley and Terry (1963) system, replaced the second-stage pepsin digestion with neutral-detergent extraction which dissolves all microbial matter (Van Soest, 1994). The values obtained are estimates of true digestibility.

In vitro digestion techniques Menke *et al.*, (1979), where gas evolved during fermentation by rumen microbe is collected and used as measure of the extent of

fermentation. The gas production during fermentation (measure the rate constant b) as indicator the rumen degradation rate as well as end point of degradation (measured by dry matter appearance during 96 h gas production and acid pepsin treatment).

While the IVDMD assay gives digestion after 48h incubation time with rumen microbes and acid pepsin treatment. Feed samples were incubated in a buffered medium containing fresh rumen fluid to provide rumen microbe, essentially very similar to the first stage of the Tilley and Terry method. However incubation was conducted in large glass syringe to trap the gas evolved, where as in the Tilley and Terry (1963) method gases are allowed to escape to the atmosphere. Blummel and Ørskov (1993) modified the technique to monitor gas production at regular intervals up to 72 incubation.

Gas production data were analyzed using the same equation used to interpret nylon bag data (Ørskov and MacDonald, 1979), using gas produced up to 6 h of incubation as indicator of parameter (a) the rapidly fermented fraction. A range of regression equations linking gas production, various compositional parameters and metabolizable (ME) or net energy-lactation have been reported by (Menke and Steingass, 1988). The main products of carbohydrate fermentation by rumen microbe are VFAs: acetate, butyrate and propionate) the gases carbon dioxide and methane and microbial biomass. Gas produced when substrate is fermented to acetate and butyrate. The gas that is released with generation of propionate is the only indirect gas produced from buffering, therefore relatively lower gas production is associated with propionate production. Carbon dioxide is also released from the bicarbonate buffer used to neutralize VFAs, hence giving indirect indicator of VFAs production. VFAs accumulated in the fermentation medium can be analyzed to investigate the VFAs production profile. Non-carbohydrate component, such as protein produce little gas when they are fermented. Moreover, ammonium formed by degradation of protein can combine with carbon dioxide to form ammonium bicarbonate which stay in the medium, hence reducing the amount of carbon dioxide released as gas.

Some of carbon and other elements of the feed will also be incorporated into microbial biomass and so will not be released as gas. Thus the quantity of gas produced per gram of feed sample fermented can vary. Although gas production is a nutritional wasteful product but provide a useful basis from which methane, metabolizable energy, organic matter digestibility, short chain fatty acids and molar VFAs may be predicted (Makkar, 2005).

According to France *et al.*, (2000), the gas production is directly proportional to the degradation rate of the substrate. The gas produced in vitro is closely related to digestibility and therefore to the energetic value of feedstuff for ruminants.

The gas production technique and its variants are superior to digestibility and degradability technique because they monitor fermentation non destructively and account for contribution from soluble and insoluble feed fractions while providing information on the dynamics of forage fermentation. Additionally when nutrient content is not limiting, gas production measures microbial growth. Gas production is the only in vitro technique suitable for study of the degradation of soluble material such as urea molasses block.

The technique is important in evaluation of feedstuff posses secondary metabolites. Gas production is positively related to microbial protein synthesis *in vivo* digestibility and intake (Blümmel and Ørskov, 1993). The technique involves ruminally canullated animals which are expensive to maintain. Uniform diet should be fed to exclude the impact of inoculate on the results. The requirement for several incubation vessels for each feed at each sampling time.

The gas production technique as an index of the nutritive value is hampered by the dependence of total gas production on sample size, sample form and the composition of the end product of fermentation. Leguminous tress are likely to provide an excess of N for rumen microbes relative to fermentable carbohydrate and source of easily fermentable fiber.

*Prosopis juliflora in vitro* characteristics, indicate pods are highly fermentable, but the leaves are particularly poorly fermented. Leaves, rate constant b 0.042 h<sup>-1</sup>DMD 30% and IVDMD 45%. The pods rate constant b 0.05 h<sup>-1</sup>, DMD 77% and IVDMD 90%. The nutritive value of six species of mesquite (*Prosopis*) including *P. chilensis* was investigated (Koech, *et al.*, 2011). Chemical analysis indicated that all of them are suitable sources of forage. However, in vitro digestibilities were negatively correlated with content of phenolic compounds. Species with high concentration of phenolics (*P alba and P chilensis*) are significantly less digestible than other species with lower phenolic content. Depression effect in gas production of *Prosopis juliflora* was observed by Abdulrazak *et al.* (2001) due to the presence of condensed tannin. The enzymatic digestibility with cellulose plus protease was only 22-26 % in leaves

with high phenolic content, but is 39-47% in leaves with low phenolics. The parameters of in vitro gas production for *Prosopis* pods with seeds were 213, 0.0644, 0.4084, 64 and 201 for b, k, L, gas 24 h and gas 48 h respectively (Juárez *et al.*, 2013).

Where b = gas volume corresponding to the complete digestion of the substrate (asymptote) (mL/gDM).

c=constant rate of gas production of the potentially degradable material (/h).

L = colonization time.

## 1.9.3: In sacco nylon bag method:-

The in sacco method, is essentially in vivo technique, in which the synthetic fiber bags, containing test feed incubated in the rumen for fistulated animals and measurement of material lost from bags for different lengths of time to evaluate feedstuff for DM and N degradation and particle outflow rate. The method can estimate the rate and extent of forage digestion by plotting the disappearance of feed sample over time (Ørskov and McDonald, 1979).

The *in sacco* method is used to estimate protein degradability of the feed in the rumen and becomes basis to estimate nitrogen requirement of ruminant in several feed systems.

Parameters from the nylon bag technique have been correlated with in vivo digestibility, dry matter intake, digestible dry matter intake and growth rate. This method provides a means of ranking feed according to the rate and extent of degradation of DM, OM, N or other nutritional parameters (Broderick and Cochran, 2000). The method requires fistulated animals and cannot produce information about the product of digestion. There are many sources of variation in the results obtained from different laboratories, with main sources of variation coming from basal diet, type of samples and animals, replication, incubation conditions, washing technique and correction for microbial contamination (Broderick and Cochran, 2000). Microbe can adhere to feed samples giving rise to under-estimation of protein degradation. Other limitation are the need to measure or assume outflow rates, data which are laborious especially when feeding level can be very low. Hence in practice outflow rates often have to be assumed. The reliability of the method for evaluating feeds with large, soluble component or feed of fine particle size is doubtful, as component of

samples are lost from the bags, without necessary being degraded. The method is not suitable for assy of anti-nutritive factors such as tannins, because anti-nutrive factors may be washed out of the feed and diluted to neutralize their effect (Preston, 1995). The technique is very important for adequate evaluation of the feed and for comparison of obtained results and it needs standardization (Broderick and Cochran, 2000). Two studies were conducted to investigate the roasting effects in the composition, in vivo digestibility and in situ degradability of fiber fraction (NDF and ADF) of *Prosopis* pods (Andrade-Montemayor, et al., 2009). The results revealed that roasted mesquite pods modify their nutrient content, modify NDF degradation kinetics, increased the fractional degradation rate and effective degradability (Ed). The nutritive value of *Prosopis farcta* fruit was evaluated using in situ nylon bag technique in fistulated male cattle (Ansari nik et al., 2013). At 96 h incubation the dry matter degradability (DMD), fast degradation (a), digestible particles over the time (b), potential degradability (a+ b) and effective degradability (ED) were 57.05, 18.62, 38.63, 57.26 and 38.85 percent respectively. In this technique, the disappearance of the material sample is equal to the degradability.

The results showed that *P. farcta* furit (dwarf mesquite) has desirable nutritive value and can be used as a part of the forage diet for ruminants. The reduced dry matter degradability of *P. farcta* fruit in various stages of incubation in the experiment were due to high ADF and NDF of the sample and presence of secondary plant materials including lignin, tannin and saponin. The effective degradability of dry matter of all plants was in range of 0.33- 0.62 (Ramirez, *et al.*, 2009).

The increase in the CP of the plant has a positive impact on DM disappearance in the rumen, because Ed increase by increasing the protein content of plant species. Riasi *et al.* (2008) attributed the reduced dry matter degradability of feedstuff to factors such as low ash and high NDF content. The *in sacco* dry matter digestibility of *Prosopis* seed pod meal was higher than that of the hay, 74.5 and 56.8, respectively (Koech *et al.*, 2011). This result was attributed to the high crude protein present in *P. juliflora* compared to that present in hay. *In situ* ruminal and intestinal digestibility of dry matter, crude protein and neutral detergent fiber from as collected and dried (80°C for 2h) mesquite pods was determined using two steers fitted with ruminal and duodenal cannulae (Batista *et al.*, 2002). Drying at 80°C for 2 h reduced the in situ soluble CP fraction and increased the slowly degradable CP fraction and its rate of degradation. However, ruminal degradability of both CP and DM of mesquite were not

affected by drying. Post-ruminally digestibility of rumen un-degraded CP and NDF were low and unaffected by drying. Total tract digestibility of DM, CP and NDF were similar for as collected and dried mesquite pods, averaging 68, 78 and 9% respectively. It was concluded that the rumen is the main site of digestion of mesquite pods and that drying had no adverse effect on their intestinal or total tract digestibilities.

-: Goats :1.10

#### -: Digestion function in goats: 1.10.1

As a general rule of thumb, small ruminants will consume between 3 to 5 percent of their body weight on a dry matter (DM) basis as feed. Very large proportion of digestion occurs in the rumen and involves 84 % of organic matter, 92 % of cellulose and 89% of hemicelluloses (Hadjigeorgiou *et al.*, 2003). The goat has a superior digestion capacity attributed to some physiological features that are common in the intermediate feeders group of ruminant, like large salivary gland, the large absorptive area of their rumen epithelium and the capacity to rapidly change the volume of the foregut in response to environmental changes (Silanikove, 2000). Goats produce much saliva about (6-16 L), rich in urea, which is important to enrich poor forage by nitrogen and recycling urea. Even though nutrient requirements are greater in relation to the unit body weight in small ruminants, rumen capacity is significantly less, retention time is shorter and flow rate is significantly faster than in

The goats have higher digestive capacity, the rumen concentration of VFAs in goats is higher than sheep regardless of the type of the consumed diet. The difference in concentration of VFAs is more pronounced as the forage quality decrease. Goats have higher rumen ammonia concentration than sheep when fed low quality roughages and more efficient of recycling of urea in the rumen (Devendra, 1990). Goats prefer diet dominate by browse, which constitutes 80% of their diet. Goats have greater preference for high tannin than sheep and cattle, because of their ability to secrete mucin or proline rich saliva to reduce the stringency of tannins and spare the protein for digestion in the intestine and posses bacteria that degrade tannins (Clauss, *et al.*, 2005). Goats are tolerant to travel long distance for forage and tolerate stressful conditions of feed shortage.

large ruminants (Huston and Pinchak, 1991).

#### -: Goat production :1.10.2

In the tropics goats slow growth rate is reflecting the poor nutrition of the animals, but also an effect of the genotype (Peacock, 1996). The growth rate of goats is often lower after weaning, Lambert, *et al.*, (1996) found that the post weaning growth rate of Australian Cashmere kids grazing improved pastures was poor, especially in autumn period despite adequate quantity and quality of pasture.

Sheep and goats require higher feed quality in order to fulfill their nutrient requirements, to be able to express their genetic production potential. Nutrition studies in tropics show a generally poor response to improved nutrition by tropical breed of goats.

Goats production depends solely on grazing and browsing, supplementation or feedlot operation are limited. Growth rate in goats is dependent on the mature size of the particular breed. Dhanda *et al.* (1999a) found that male kids from larger breeds (Saanen) grew faster than kids born to smaller breeds. The rate of growth can vary from around 50 g/day for the small tropical breeds (Barbari) to over 200 g/day for large breeds (Alpine) and the south African Boer breed (Cameron, *et al.*, 2001). The initial weight of Nubian goat kids at 2-3 month age and final weight gain after 90 days were 9.28 and 13.46 Kg respectively. The total weight gain was 4.18 kg and feed conversion ratio was 10.31. (Babiker *et al.*, 1990).

Regarding reproductive performance, Nubian goats can give birth 3 times every two years, with 50% of twinning or triple kids (Arab Organization for Agricultural Devolopment, AOAD 1990). Puberty can be reached by female Nubian kids within 6 months of age, and the gestation period cover146 days (AOAD, 1990). Age at first kidding is 12 months and kidding intervals 228 days, birth weight is 2.3 – 2.6 kg for males and 2.1 – 2.4 kg for females (Gall, 1996). The large built of Desert goats suggests that they probably have good potential for meat production. Meat from younger animals is preferred than that from older animals, because it is more tender and juicier (Gaili, *et al.*, 1972). The meat from goats becomes progressively darker and less tender with increasing age (Dhanda *et al.*, 1999a). Meat from desert goats has superior processing properties (Babiker *et al.*, 1990). Goats compared to sheep, are lean animals which deposit their fat around the viscera. The dressing percentage of goats normally varies between 44 and 55% but in goats fed *Prosopis* pods, it was 44.4 -47.0% (Mahgoub *et al.*, 2005). Goats have higher visceral fat content than sheep,

which could be related to poor feed conversion efficiency in goats (Naude and Hofmeyr, 1981). The goat meat could be more healthier and nutritious, but the less fat content interfere with its keeping quality.

# CHAPTER TWO MATERIALS AND METHODS

Four experiments were conducted in this study. Experiment (1) was conducted to study the effect of feeding diets containing graded levels of *Prosopis* pods haemto-biochemical parameters of Nubian goats kids. Three rumen fistulated Kenana steers, were used in experiment (2) to obtain ruminal liquor to determine the effect of feeding *Prosopis* diets on the rumen ecosystem and rumen pH and rumen metabolites. Experiment (3), the nutritive value of *Prosopis* pods and parts (sweet and seed) was determined in *in vitro* gas production, the inoculum used in it was obtained from the cannulated steers. Three rumen fistulated castrated Kenana calves, were used in an *in sacco* experiment (4) to determine rumen dry matter and protein degradability and

degradation characteristics of *Prosopis* pods and seeds. Experiment (5) the growth performance of kids on *Prosopis* pods diets was evaluated.

## Experiment (1) blood profile growth of goat kids :2.1

## -: fed prosopis pods

## -: Description of the study site :2.1.1

The study was conducted in Sudan University of Science and Technology (SUST) Farm at Hilat Kuku, Khartoum North, Sudan in October 2012. The site is at latitude 15° 76′ 11.3"N and longitude 32° 33′ 51.35" E. The area has tropical arid climate with a mean annual rain fall of (100- 200 mm) and average daily temperature between (17.9- 44.1°C).

## 2.1.2: Experimental animal management:-

Twenty four male Nubian kids of age less than (6 months) and with an average initial body weight (13.1±0.37) were used in the experiment. The animals were purchased from Hilet-Kuku Livestock Market and selected according to the characteristic of Nubian goats of the Sudan. The animals were ear tagged, blocked by body weight and were randomly divided into four equal groups in a complete randomized block design.

The animals were randomly assigned to the dietary treatments. Goat kids were individually housed in pens equipped with a feeder and water trough. Prior to their arrival to the farm the animals were dewormed, hair clipped, sprayed with insecticide against ectoparasites and they were given a prophylactic dose of antibiotic.

An adaptation period of 14 days was allowed for the goats to become adapted to pen living and routine feeding and allot time for proper diet adjustment before the start of the study. At the beginning the adaptation period all the animals were fed berseem hay and Abu70 fodder and a diet without *Prosopis juliflora* pods (PJP) (the control diet). On the second week of the adaptation period, the experimental diets were gradually fed to the animals. The experiment continued for seven weeks.

### 2.1.3: Experimental diets:-

The whole *Prosopis* pods were collected from trees grown in Elqutainha area - White Nile state. The pods were sun dried by spreading thinly on plastic sheet and turning thoroughly to facilitate uniform drying for save storage. Dried pods were

cleaned from dirt and were chopped to shorter lengths of approximately (1- 2 cm) and further ground in a hammer mill and stored on plastic bags for use in feeding experiment. Experimental diets (Table 1) contained different amounts of *Prosopis* pods mixed with other feed ingredients. Experimental treatments were diets contained *Prosopis* pods (0%), 10%, 20% and 30% and fed to the animals in group T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. The prepared *Prosopis* pods were mixed with the other ingredients of the diets. Experimental diets were formulated to be isonitrogenous isocaloric and met the animal requirement for growth and body weight gain according to the National Research Counsil (NRC) (2007). The animals were fed once a day at 0800 h and had free access to water and commercial mineral block. Amounts of feed offered were adjusted every 3 to 4 days to obtain a daily refusal of 5 to 10%.

:(Table (2.1 Ingredients of the experimental diets on (fresh basis) % fed to Nubian Goats kids

Ingredients	Dietary treatments				
Prosopis pods	0	10	20	30	
(Sorghum (feterita	30	30	30	28	
Wheat bran	20	15	5	8	
Molasses	13	8	8	5	
Groundnut cake	10	10	15	17	
Berseem Hay	25	25	20	10	
Limestone	1	1	1	1	
(Salt (Na Cl	1	1	1	1	
Total	100	100	100	100	

Table (2.2):
Proximate analysis on dry matter basis of the experimental diets.

parameters	0	10	20	30
DM	90.66±.01	90.77±.010	90.87±0. 01	90.93±.01
СР	16.90±0.1	16.86±0.02	16.9±0.1	16.87±0.1
CF	12.14±0.1	12.98±0.10	13.28±0.1	13.55±0.10
EE	2.77±0.01	2.72±.010	3.24±0.01	03.50±0.01
NFE	50.08±0.69	50.08±1.06	49.58±1.0	50.18±0.91
Λ -1-	0.77+1.00	0.12+1.00	7.07+0.05	00.02+0.00
Ash	8.77±1.00	8.13±1.00	7.87±0.95	06.83±0.90
MEMJ/Kg	10.86± 1.05	10.9±1.0	10.9±1.00	11.10±1.0

DM: Dry matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract and NFE: Nitrogen free extract Calculated ME MJ/KG metabolizable energy. PJP: Prosopis juliflora pods

## 2.1.4: Haematological measurements:-

## 2.1.4.1. Blood collection:

Blood samples were collected in the morning prior to feeding weekly throughout the experimental period. Five ml blood sample were drawn by puncture of the external jugular vein using a disposable syringe and sterile needle. Immediately 2 ml of blood were placed in tubes containing anticoagulant ethylene diamine acetate (EDTA) to determine the haematological parameters as described by (Dacie and Lewis, 2001).

#### 2.1.4.2:Total red blood cells:-

Erythrocyte or red blood cells (RBCs) were counted on Neubauer haemocytometer (Hawksely and Sons Ltd., England) using light microscope at 40×10 magnification after diluting the blood sample to 200 times with a diluting fluid of formal citrate (3g sodium citrate, one ml conc. formaldehyde, 100 ml distilled water) before counting. The red cell diluting pipette was filled up to mark 0.5 and immediately RBC diluting fluid was drawn up to mark101 according to the procedure described by (Dacie and Lewis, 2001). A drop of blood was placed on the counting chamber by holding pipette at an angle of 45°. The haemocytometer was allowed for 2-3 min to settle the RBC in the chamber. All the erythrocyte in the 4 corners of the chamber and the centre square were counted, the total was multiplied by 10⁴. The result of the test was expressed as number of cells per unit volume, specifically cells/μL.

## 2.1.4.3: Haemoglobin concentration:-

Haemoglobin (Hb) conentation was determined by cyanomethaemoglobin method as described by van Kampen and Zijlstra (1961). The haemoglobin concentration was measured in g/100 mL of blood.

## 2.1.4.4: Packed cell volume:-

Packed cell volume (PCV) was determined using whole blood samples. into Microhaematocrit tubes were filled with the blood, sealed then centrifuged in microhematocrit-centrifuge (Hawksley and Sons, Ltd., England) at 2,000 rpm for 4 to 5 minutes (Hawaks, 1965).

The PCV (erythrocyte mass) was read off on the scaling instrument provided with the centrifuge. The values were expressed as percent.

### 2.1.4.5: Erythrocyte sedimentation rate:-

Determination of ESR was by Westergrens method in which, the distance (in mm) that the erythrocyte sediment during a given period of time when blood to which anticoagulant has been added in an ESR tube placed in a vertical position.

Blood was taken in the ESR tube exactly up to 0 mark and placed vertically in ESR stand, the RBCs – under the influence of gravity- settled out from the plasma. The rate at which they settled was measured as the number of millimetres of clear plasma at the top of the column after one hour (mm/hr). First reading was taken after 5 minutes (zero hour) and the values after one hour and two hours were recorded. The final result was obtained from reading at 24 hours.

## 2.1.4.6:Clotting time:-

Microscope glass slides and stop watch were used to measure clotting time. One drop of blood from each sample was placed in glass slide, then was shaken manually time required for clot formation was recorded.

#### 2.1.4.7: Red blood cell indices:-

Blood indices were calculated according to Dacie and Lewis, (2001). The three cardinal RBS (Hb, red cell count and PCV) measurements were used to drive red cell indices by using the following formulae:

Mean cell volume (MCV) fl (femtoliters) = 
$$(\underline{PCV \times 10})$$
  
RBC( $10^6 \text{ul}^{-1}$ )

Mean cell haemoglobin (MCH) pg (pictogram) =  $\frac{\text{Hb(g/dl)} \times 10}{\text{RBC}(10^6 \text{ul}^{-1})}$ 

Mean cell haemoglobin (MCHC) % =  $(Hb(g/dl) \times 10)$ PCV(%)

#### 2.1.4.8: Total white blood cell count:-

White blood cells (WBC) were counted in a haemocytometer (Hawksely and Sons Ltd., England) using light microscope at 10×10 magnification after diluting blood samples to 20 times with diluting fluid (Turks fluid) (1.5% glacial acetic acid solution and few crystals of gentian violet) before counting. The white cell diluting pipette was filled according to the method describe by (Jain, 2000).

Enumeration of the white cells was achieved by counting all leukocytes in the four leukocyte counting squares, the total was multiplied by 50.

The number of WBC is expressed as thousands per mL of blood.

White blood cells (WBC  $10^3/\mu$ L) = Nx2.5x20= Nx5

N= number of cells counted in 4 squares

Total white blood cells count (TWBC) =  $\underline{N}$  x200

4

### 2.1.5: Determination of serum metabolite:-

Three ml of blood were placed into a plain tube without anticoagulant, left at room temperature to clot and, then serum was separated immediately by centrifugation at 4000 rpm for 15 minutes (EBA20- Hettich zentrifuge- Germany) and stored at -20 °C in plastic container for the analysis of blood serum metabolites, enzymes and chloride (Blum *et al.*, 1983). Unicam-8625 UV Spectrophotometer- UK.

## 2.1.5.1:Determination of serum glucose level:-

Glucose concentration was determined immediately by enzymatic colorimetric method using commercial kit (Plasmatic Laboratory Products Ltd England).

Test principle: Glucose level was measured according to the method described by (Giterson *et al.*, 1971). Serum glucose concentration, principle of the test: Glucose is oxidized according to following reaction:

Glucose  $\rightarrow$  (glucose oxidaze)  $\rightarrow$  Gluconic acid  $+H_2O_2$ 

 $H_2O_2$ +Phosphate+4amino-antipyrine  $\rightarrow$  (Peroxidaze ) quinoneimine red+ 4  $H_2O$ 

Protocol : Dilute enzyme reagent (GOD+ POD+4-amino-autipyrine was mixed with buffer (phosphate buffer, pH 7.5+ phenol. None- haemolytic serum was mixed with reagent solution then the mixture was incubated at 37°C for 15 min. The absorbance was read at wavelength 492 nm against the reagent blank in spectrophotometer (Corning 252, England) and calculation as follows:

Serum glucose (mg/dl) = (A sample) × standard con100ml/dl (A standard) Where A=absorbance

## 2.1.5.2: Determination of total protein:-

Total protein was determined according to biuret method. Stored serum samples were analyzed for total protein (TP) by spectrophotometer method using commercial kit (Linear Chemicals, Barcelona, Spain).

The method is based on biuret reaction in which a chelate is formed between  $Cu_2^+$  ion and peptide bonds of protein in alkaline solution (NaOH) to form a violet colored complex. The intensity of the color produced is proportional to the concentration of the protein in the sample. The Rochelle salt (K-Na- tartarate) contained in the biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue color.

The absorbance of sample (A sample) and the standard was read at 540 nm in the spectrophotometer against the reagent blank. Total protein is stable in serum for week at room temperture for at least one month refrigerated at  $2-8^{\circ}$ C for up to 2 months at  $-20^{\circ}$ C.

$$C (g/dI) = \frac{T-B}{S-B} \times 7.5$$

Where:- T: Titration, B: Blank, S: Standard and 7.5 Factor.

#### 2.1.5.3: Determination of albumin:-

Albumin determined according to the method of Doumas (1971). Serum albumin concentration was determined by spectrophotometer method using commercial kit (Linear Chemicals, Barcelona, Spain). Test principle is based on indicator, 3, 5, 5, 5. (Tetrabromocresol green, BCG), an anionic dye and the protein at acid pH 402 with formation of a colored complex. The intensity of

colour produced is proportional to the concentration of albumin in the sample, serum was mixed with a buffered BCG reagent and the mixture was incubated for 10 min at room temperature. The absorbance of the sample (Asample) and of the standard (Astandard) was measured against the reagent blank at 630 nm in the spectrometer. Serum albumin concentration (c) was calculated as follows.

 $C(g/dI) = \underline{T-B}_{X5}$ S-B

Where: T: Titration, B: Blank, S:Standard and 5: factor

#### 2.1.5.4: Determination of urea:-

Serum urea concentration was determined by spectrophotometer method using commercial kit (Linear Chemicals, Barcelona, Spain). As described by Young (1995).

Test principle: Ammonia and carbon dioxide are produced from hydrolysis of urea by urease.

Urea+  $H_2O$ ---urease = Ammonium +  $CO_2$ 

Ammonium ions react with phenol and hydrochlorite to give a coloured complex.

Protocol: The serum was mixed with a buffered urease solution and the mixture was incubated for 10 minutes at room temp. The hydrochlorite solution was added and mixed and the content were incubated at room temp for 15 min.

The absorbance of the sample (sample A) and of the standard (standard A) was measured against the reagent blank at 630 nm in the spectrophotometer. Serum urea concentration (C) was calculated as follows.

Calculation:  $C(mg/dl) = (\underline{Sample A})_{\times}$  concentration of the standard (standard A)

#### 2.1.5.5: Determination of cholesterol:

Cholesterol serum cholesterol was measured by enzymatic method using a kit (Quimica Clinica Aplicada, S.A., Spain).

Principle: cholesterol esterse was first converted to cholesterol and fatty acid by enzyme cholesterol esterase. Cholesterol is oxidized with O<sub>2</sub> to form cholesten-3-one + HO2. Lastly, the hydrogen peroxide is reacted with 4- aminoantipyrine and p-HBS

to yield quinoneimine (red dye) and water. The absorption measured at 520 nm, is proportional to the concentration of cholesterol in the sample. The kit comes with control solution containing a cholesterol standard (200 mg/dl which can be used to calibrate the assy. The absorbance of sample (A sample) and standard (A standard) were read in spectrophotometer at a wave length of 520 nm and serum cholesterol concentration (C) was calculated as follows:

C (mg/dl)=  $\underline{AS}_{\times 200}$ AST

Where: AS: Absorbance of the Sample. AST: Absorbance of Standard

## 2.1.5.6:Determination of total and direct serum bilirubin:-

Serum total bilirubin concentration was measured according to Van Roy *et al.* (1971) using a diagnostic kit of Merck Mega, Darmstadt, Germany.

Bulirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which is red in neutral and blue in alkaline solution whereas the water-soluble bilirubin glucouronides react directly, the free indirect bilirubin reacts only in the presence of an accelerator.

Sulfanilic acid + NaNO₂ → Dionized Sulfanic acid

Bilirubin + Dionized Sulfanic acid  $(\rightarrow Hcl) \rightarrow Azobilirubin$ 

Protocol: None-haemalytic serum was mixed with diazotized sulfanilic acid after the addition of accelerator (caffeine + sodium benzoate + sodium acetate). A blue azobilirubin is found in alkaline Fehling solution (potassium sodium tartrate + sodium hydroxide solution).

The direct bilirubin was read exactly 5 minutes after the addition of serum at wavelength 548 nm. The absorbance of the sample was read against the sample blank as follows:

Calculation: Abs= absorbance:

(Abs of unknown – Abs of blank)  $\times$  conc.of calibrator(mg/dl) = (Abs of calibrator-Abs of calibrator blank)

= Bilirubin (mg/dl conc. direct bilirubin =  $A \times 14.0$  mg/dl=  $A \times 240$   $\mu mol/L$ 

(Where A= absorbance)

N.B Direct bilirubin determination shoud have its own procedure and calculation.

## 2.1.5.7: Determination of triglyceride:-

Determination of triglyceride test involve enzymatic hydrolysis by lipase of triglycerides to glycerol and glycerol derived by hydrolytic action of lipase on glycerides.

Triglycerides <sup>i</sup> (lipoprotein lipase) glycerol+fatty acids

Glycerol +ATP  $\rightarrow$  (GK) G-1-P+ADP

 $G-1-P + O_2 \rightarrow (GPO) DAP+H_2O_2$ 

 $H_2O_2+4$ -AAP + ESPA  $\rightarrow$  (POD) Quinoneimine dye +  $H_2O$ 

 $H_2O_2+4$ -AAP+ ESPA  $\rightarrow$  (POD) Quinoneimine dye +  $H_2O$ 

Triglyceride were first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids.

Glycerol was phosphorylated by adenosine 5-triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5-diphosphate (ADP) in reaction catalyzed by glycerol kinase (GK). G-1-P was oxidized by glycerol phosophate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxade(  $H_2O_2$ ). Peroxide (pod) catalyzed the coupling of  $H_2O_2$  with 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (ESPA) to produce a quinoneimine dye that shows an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to triglyceride concentration of the sample.

#### Calculation:

Serum triglyceride conc.=  $(Asample-ABlank) \times conc.of$  standard (Astandard-ABlank)

#### 2.1.6: Determination of serum enzymes:-

## 2.1.6.1: Determination of alkaline phosphatise:-

The serum activity of enzyme Alkaline phosphatase (ALP) was measured photometrically using commercial kit (Plasmatec Laboratoy Products Ltd, Spain)

(Young, 1995). The test based on the catalytic activity of ALP on hydrolysis of  $\,^p\,$  -nitrophenylphosphate to liberate p-nitrophenol and inorganic phosphate. The rate of

*p* -nitrophenol formation is proportional to ALP activity present in the serum.

Non- haemolysed serum was added to a chromogenic substrate and mixture was incubated at 37°C for 5 min. A colour developer was then added and mean absorbance change per min at 405 nm/ min was utilized for calculation of enzyme activity as the following:

Enzyme activity (IU)=A405/minute × 2764

The enzymatic activity was then read off on the standard curve.

ALP (IU/L) = 
$$(\Delta Abs/min \times TV \times 1000)$$
  
(18.75×LP×SV)

Where:

 $\Delta Abs/min=Averg$  absorbance change /min

1000 = conversion of IU/mL to IU/L

1.025= (TV) Total reaction volume (mL)

18.75=Millimolar absorptive of p-nitrophenol

0.025= (SV) Sample volume (Ml)

1 = LP Light path in cm

## 2.1.6.2:Determination of enzyme aspartate aminotransferase:-

Serum AST activity was measured by a spectrophotometric method (Younig, 1995) using commercial kit (Linear Chemicals, Barcelona, Spain). The test is based on catalytic activity of the enzyme with transfer of an amino group from aspartate to

 $^{\alpha}$  -ketoglutarate in a reversible reaction. The end products formed in this reaction are oxaloacetate and glutamate. 2,4, Dinitrophenyl hydrazine was added to form hydrazone of keto acid. These hydrazone subsequently react with sodium hydroxide to form a colour which can be read by the spectrophotometer (Unicam-8625 UV - UK.) at wave length of 505 nm. The enzymatic activity was then read off on the standard curve.

## 2.1.6.3: Determination alanine aminotransferase:-

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT). The serum enzyme activity was measured by a spectrophotometric method using a kit (Linear Chemicals, Barcelona, Spain) (Young, 1995). The test is based on activity of the enzyme to catalyze the transfer of amino acid group from alanine to

 $^{\alpha}$  - ketoglutarate in reversible reaction. The end product of the reaction was glutamate and pyruvate. The transaminase activity is proportional to the amount of pyruvic acid formed over a definite period of time and was measured by reaction with 2, 4, dinitrophenyl hydrazine (DNPH) in alkaline solution (NaOH). The absorbance was read by spectrophotometer at wave length 505 nm. The enzymatic activity was the read of on the standard curve.

#### 2.1.7: Goat kids growth measurements:-

Measurements for performance of goats in the experiment (1) were recorded. Daily feed intakes for goats were determined, and the feed allowance was adjusted daily according to the amount of feed consumed, with 50 g/kg refusals as the target, to avoid feed selection. Feed intakes were determined by weighing the offered feed and the residual feed. The animals were weighed every week in the morning before feeding using suspended weighing scale. Data on total feed intake and live weight gain was used to calculate the feed conversion ratio (FCR). The general health condition of the animals was closely observed.

Feed intake = <u>Offered- residual</u> x100% Offered

## 2.2: Experiment (2) rumen activity study:-

In this experiment the effect of *Prosopis* diet in rumen activity was studied by measuring pH, ammonia concentration, volatile fatty acids, bacterial and protozoal numbers in rumen liquor.

## 2.2.1: Experimental animals, design and treatment:-

Three castrated local Kenana steers at 3- 3.5 years old fitted with ruminal canulae as described by Brown *et al.*, (1968) were used to study rumen activity. Animals were individually fed the experimental diet to maintenance level and have free access to clean water and mineral block throughout the study.

Four rations, were formulated using mixture of ingredients, sorghum grain, Berseem hay, molasses, wheat bran and ground nut cake. *Prosopis* pods were included

in at 0, 10, 20 and 30 in ration fed to Kenna castrated calves to make four treatment groups ( $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ ). Ingredient and chemical composition of the experimental ration is shown in table (1.). No hay was offered; concentrate was the sole diet given to animals. The trial was designed according to a Latin square design with four treatments and four experimental periods. Each period lasted ten days. The adaptation period was 6 days to allow calves to adapt to experimental diet; followed by 10 days of sample taking.

### 2.2.2: Rumen liquor samples:-

Rumen fluid samples were collected at 0, 2, 4, 6 and 8 h-post feeding. Approximately 100 ml of rumen fluid was taken from the middle part of the rumen using a 60-ml hand syringe at the end of each period. Rumen fluid pH was determined after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. The filtered rumen fluid was sub- sampled into 10 mL aliquot which was preserved with 2 mL of metaphosphoric acid (25% wt/v) and another 10 mL aliquot of rumen fluid was preserved with 1.5 mL of 1% of sulphuric acid. Samples were put into plastic bottles and were stored at - 20°C, pending analysis of rumen ammonia nitrogen (NH³-N) and volatile fatty acids.

## 2.2.2.1:Measurement of rumen pH:-

A sample of about 60 cc of rumen liquor was taken via rumen cannula by using a 50 mL syringe and a 50cm –long plastic probe attached to the syringe. The pH of the fluid was determined immediately at 0, 2, 4, 6 and 8 hours using Hanna HI-98107 pocket manual pH meter (Hanna Instrument pH, Italy).

The sample should be evaluated as soon as possible after collection to minimize the effects of cooling and air exposure on protozoal activity and pH. For accurate reading, pH meter was immersed in the container shaken well until the reading stabilized in pH meter and then the liquor pH was recorded.

## 2.2.2: Ammonia (NH3) determination:-

As described by Conway *et al.*, (1957) using Conway unit. The method is based on diffusion of ammonia in Conway dishes induced with saturated potassium carbonate solution and subsequent titration against 0.01 N sulphuric acid using methyl red as the indicator. In the outer chamber of each unit 2 ml. Of saturated potassium Carbonate (K<sub>2</sub> Co<sub>3</sub> were put, while in the inner chamber 2 mL of mixed indicator were pipette (40 gr. Boric acid + (0.02 gr. Methyl red + 0.06 gr. BCG in 100 ml ethanol)

complete to 2000 ml with distilled water D.W). Covers were then replaced leaving small opening on the upper side of outer chamber through which 0.5 ml of sample was added by a 0.5 ml pipette. The opening was then closed and the tilted unit set up right while shaking gently 2- 3 times so as to ensure the through mixing of the sample with the saturated potassium carbonate in the outer chamber. The unit were then left for six hours. (or 40 minutes at 60 c ). Weight were put on the top of each cover and the units were carefully removed and the content of the inner chamber titrated against 0.01 N sulphuric acid solution, using Conway micro-burette.

```
NH3 Rumen liquor = (\underline{T \times N \times 100})
(Volume of Sample)
```

where:

T = titration. N = normality of acid.

## 2.2.2.3. Volatile fatty acids determination:-

For volatile acid determination as described by Kroman *et al.*, (1967) the strained rumen liquor was deproteinized by adding 10 ml of 0.1 normal HCl to the 10 mL of the sample in 50 ml volumetric flask.

The flask was then shaken thoroughly and filled to the mark with distilled water after a lapse of 5 minutes. The precipitate portion was then filtered, 0.5 ml from the filtrate was pipette in Markham micro distillation apparatus and 1 ml of orthro-phosphoric acid was added. The distillation was continued until 50 mL distillation were collected in 100 ml conical flask receiver.

The distillation was then recovered and 3 drops of 0.04% phenol red indicator was added. Then the nitrogen was bubbled through the distillate to remove any carbon dioxide and then titration was made against 0.01 sodium hydroxide from a burette.

The amount of milliequivalents of V.F.As in 100 ml of sample were calculated in the following manner:-

Calculation for V.F.As

 $V \times N \times 100 = ml Eq.\%$  per 100ml.Of sample

Where:- V = Volume of NaoH, N = Normality of NaoH.

#### 2.2.2.4: Total Protozoal Count:-

Protozoal count was preformed according to the procedure described by (Galyean, 1989). A large composite sample of rumen content was taken by hand and the fluid squeezed through four layers of cheesecloth into plastic container.

The subsamples were placed in plastic container and preserved by adding an equal volume of formal saline. Formal saline solution was prepared by mixing of 100 ml formaldehyde 35%, 900 ml distilled water and 0.80 gram sodium chloride.

To the mixture 0.60 g of methyl- blue was added and the solution was stored in dark place. One ml of rumen liquor was diluted further by adding four ml of formal solution to give 1:5 dilution. Further dilution (1:10) were made as needed to give 30- 50 cells per field. After gentile mixing a micropipette was used to fill haemocytometer (Neubauer improved Marienfeld Germany) chamber with diluted sample. The haemocytometer was already covered with cover slip.

Counting was done under microscope using 10×10 ocular × objective lens. Counting was performed in the four corner squares of the haemocytometer and was made in 30 microscopic field.

The haemocytometer is designed so that the number of cells in one set of 16 corner squares is equivalent to the number of cells  $\times$  10<sup>4</sup>/mL.

v = volume of hemocytometer chamber or v sample (0.1mm $^3$  or  $10^{-4}$  ml). Calculations:

The number of protozoa was calculated using Giri et al., (2005) formula:

number of protozoa =  $\underbrace{NAD}_{av}$ 

Where:

N is the average number of protozoa/ microscopic field. A is the area on slide on which the sample is spread (area of cavity of haemocytometer).

D is dilution factor, a is area of microscopic field and v is the volume of diluted sample of rumen liquor (SRL) in the counting chamber.

### 2.2.2.5: Bacterial count:-

To count bacterial population, in samples of rumen fluid procedure recommended by (ISO 2003) was followed. A 25g represntive portion from each sample was incubated aseptically into strile stomacher bag containg 225 ml of buffer peptone water (HIMEDIA, M 614) (pre-enrichment medium) to obtain 1:10 sample dilution. The samples were homogenized for one minute at 260 rpm in stomacher circulator unit 400 (sewero UK). Serial dilutons were made in peptone water (LAB, 104) up to  $10^{-6}$  by using (ISO,6887-1:1999) procedure. According to (ISO 4833:2003) procedure one ml was pipette from each dilution into separate

appropriately marked petri plates. About 20 ml of agar media (HIMEDIAI,IM 091) was added after colling to (44-46°C) to each plate.

The diluted sample and agar media were mixed gentley and uniformly. The agar was left to solidify, petri plate were inverted and incubated for  $48\pm2$  hrs at  $35\,^{\circ}$  C. All colonies in the plates were counted and reported as colony forming units.

## 2.3: Experiment (3): In vitro gas production:-

In vitro dry matter and organic matter digestibility of Prosopis samples were determined using procedure by (Menke and Steingass, 1988). The samples were Prosopis from white Nile state (Elgetainha) and their respective fractions specifically, the seed and the pulp (mesocarp). Rumen liquor was collected from three rumen fistulated Kenna steers, before morning feeding in thermos flask and send immediately to laboratory, homogenized in laboratory blender and filtered through four layers of cheese cloth. All laboratory handling of the rumen fluid was carried out under continuous flushing with Co2 and at 39°C. Five different solution were prepared as media (anaerobic artificial saliva) and were mixed with rumen liquor at (  $^{1:2\,v/v}$  ) and kept at continuous flushing of Co<sub>2</sub>. The buffer solution used have previously been described by Menke et al., (1979). Air dried Prosopis pods samples were ground to pass 1mm screen using laboratory mill and subsamples of 200 g were obtained. About 200g of feed sample were measured and carefully transferred into pre-warmed syringe after removing of the plunger. The plunger was returned by pushing the substrate upwards the syringes. Calibrated plastic syringes were used into each 30 ml of prepared inoculums (10 ml rumen fluid and 20 ml artificial saliva) were dispersed into the substrate through silicon tube. The silicon tube in the syringe was then tightened by metal clip. All syringe including the blank were crimped and placed in the incubator at 34°C, shaking them at regular time. Each substrate was incubated in triplicate in three different runs in order to generate 9 measurements per substrate sample. Each run included in triplicate, a blank (syringe incubated with inoculum alone). The gas volume was recorded after 3, 6, 12, 24, 48, 72 and 96 hours of incubation. To prevent gas volume in the syringe from exceeding 60 ml, the pistons were moved back to 30 ml piston after 12 h of fermentation. The initial volume of material in each syringe was also recorded before the commencement of incubation of the samples.

### 2.3.1: Estimation of gas production:-

At the end of incubation, the content of each syringe were centrifuged at 12000 rpm and the residue were dried at 80°C until constant weight.

The IVDMD was assessed by difference between initial and final amount of samples (correcting for blanks). The following equation was used to estimate the volume of gas produced at any incubation period:

$$GPt = \underbrace{[(SVT-SV0)-(BVT-BV0)\times0.200G]}_{ACW}$$

Where GPTt = volume of gas produced at time "t". SVt= syringe reading for the sample at "t". SV0 = syringe reading for the sample at the beginning of the incubation. BV0 = mean of the three replicate of blank readings at the beginning of the incubation BVt= mean of three replicates of blank at time t. ACW= actual weight of the sample incubated on dry matter basis. The equation of McDonald (1981) was used to describe the course of gas production.

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

"a" is the gas production from immediately soluble fraction.

"b" the gas production from insoluble fraction

"c" the rate the rate of fermentation (the gas production constant for fraction "b"

"t" incubation time.

Y gas production in time t.

## 2.3.2: Estimation of dry matter digestibility and metabolizable energy:-

The metabolizable energy and organic matter digestibility of samples can be estimated by 24 hours gas production and chemical composition-

The organic matter digestibility OMD OF Forage was calculated using equation of Meneke *et al*, (1979) as follows :

OMD (%) =14.88 +0.889 GP+0.45CP +XA

Where:

GP is 24h net gas production (ml/200 mg)

CP = crude protein (%)

CXA = Ash content (%)

ME (MJ/Kg DM) was calculated using equations of McDonald (1981) as follows

ME (MJ/Kg DM) = 2.20 + 0.136GP + 0.057 CP + O.OO29CP2

Where = GP is 24 net gas production (ml/200mg) CP=crude protein

ME = Metabolisable energy. MJ = Mega Jole. Kg DM = Kilogram.dry .matter.

## 2.4: Experiment (4). In sacco degradability:-

The dry matter and crude protein degradability of whole pods and seeds collected from Elqutianha in the semiarid region of the Sudan where, the earliest introduction of *Prosopis* occurred.

#### 2.4.1: Animals and experimental design:-

Three steers of local breed (Kenana) aged 3 - 3½ years, were fitted with rumen cannulae. The animals were described before in experiment (2) rumen activity. They were fed on ration consist of fodder Abu 70 as a roughage, and concentrate (wheat bran, sorghum grain and ground nut cake) along with *Prosopis juliflora* pods (PJP) to meet nutritional requirement for 22 days. Initial 10 days were adjustment phase, followed by 20 days for data collection. Water and mineral lick were offered. The animals were used to study degradability of *Prosopis* whole pods and seeds and as donor to collect rumen fluid for *in vitro* gas production experiments.

## 2.4.2: Feed Sample preparation:-

Samples of whole *Prosopis* pods (PJP) and *Prosopis* seed( PJS) were prepared to be use in rumen degradability experiment. Prosopis pods were collected from, White Nile state (Elqutainha). The whole pods were subjected to repeated drying and were ground in laboratory mill to pass (>2.0 mm) screen. The seeds were recovered by hand separation of the pods and were ground in the same mill screen. The four feed samples were oven-dried overnight (24hrs) at 70° C for 24 h prior to weighing into bags.

### 2.4.3: Feed Sample incubation:-

*In Sacco* technique was conducted to examine the degradation kinetics of *Prosopis* pods and seeds. Nylon bags measuring 10×23 cm with an average pore size of 45 μm were incubated in the rumen to determine DM and CP degradation. The bags were numbered with permanent marker, oven dried at 65 c for 30 minutes and weighed after being cooled to room temperature in a desiccator to measure the weight of the empty bag (Osuji *et al.*, 1993). Three grams (weighed precisely to three decimal points) of each of Prosopis pods and seeds were placed in each bag. Suspension of the bags in the rumen was accomplished by tying of the bags, into plastic tubing with

nylon string. All samples in the nylon bags (bags/animal/period/fraction) were prepared in duplicate and incubated in the rumen of each animal for (0, 3, 6, 12, 24, 48, 72 and 96 h). The bags were placed in the rumen in reverse sequence.

After incubation, all the bags were withdrawn from the rumen at the same time and immediately placed under running cold tap water until the rinse water become clear. This was done to wash off ingested food particle adhering to the bag as well as stop further terminative processes. The bags with sample residues were then oven dried at 65°C for 48 h, cooled in desiccator and weight of the bags plus residues measured, recorded and the contents were transferred to plastic bags. The zero-hour washing losses that is, losses due to non incubation, were determined by soaking 3 g of each sample in triplicate in warm water (37 °C) for 1 hour which was followed by washing and drying the bags as done with the incubated sample residues.

Residue in the bags were analysed for DM and CP concentration (Association of Official Analytical Chemist AOAC- 2002). Dry matter, organic matter and N were divided into three fractions as follows: 1) the soluble fraction a determined as loss during washing process, 2) the potentially degradable fraction b determined as difference between initial DM, OM and N content after washing and the amount of DM, OM and N recovered after 96 h incubation. The indigestible fraction c determined as amount of DM, OM and N residue recovered after 96 h incubation. Digestibility was calculated at 48h of incubation. Rate of disappearance was determined by subtracting the indigestible residue i.e. the 96 residue from the amount in the bag at each point and then regressing the natural log (In) of that value against time. The extent of digestion was determined at 96h of incubation. Dry matter disappearance were estimated as follows:

Dry matter disappearance =  $(SWa - BW) \times DMa - (SWbW) \times DMb$ ) (SWa-BW)×DMa)

Where:

SWa = Weight of the original sample + nylon bag

BW = Weight of empty nylon bag

SWb = Weight of the sample + nylon bag after incubation

DMa = Dry matter of feed sample

DMb = Dry matter of residue samplee

The DMD and ND data were fitted to the equation proposed by Ørskov and McDonald (1979) to drive degradation parameters

$$Y = a + b (1-e^{-ct})$$
  
 $Y = a + b (1-e^{-c}(t tl))$  Mc Donald (1981) model with lag

#### Where:

Y = degradability at time (t) the potential disappearance of DM at time t

a = intercept. The rapidly degradable fraction

b = potentially degradable fraction. The potentially, but slowly degradable fraction.

c = rate of degradation of b

tl = lag time

e = the natural logarithm

t = time after incubation.

Calculation of the ruminal (DM, CP) degradability:-

Degraded dry matter percentage was calculated by the formula:

(W of sample – W of residue after incubation  $\times$  100 (Weight of sample incubated)

Residual samples after incubation for each period were separately mixed, pooled and made ready for analysis.

Degraded protein was calculated according to the formula:-

<u>CP of sample - CP of residue after incubation</u> × 100 CP of sample incubated)

The degradation kinetics of the different fractions was described by curve-linear regression of DM or CP loss from the bags with time by the equation of Ørskov and McDonald (1979).

$$P = a + b (1 - e^{-ct})$$

Where:

P = potential degradability (percentage)

a= the soluble fraction (percentage).

b= the potentially degradable fraction (percentage)

c= the rate of degradation of b (percentage /hour)

t= time (hour). relative to incubation.

e=2.7182 (natural logarithm base

The effective degradability of the samples was calculated using the equation of Ørskov and McDonald (1979), at three rumen outflow rates (r)  $(0.03,0.05 \text{ and } 0.08 \text{ h}^{-1})$ 

Pe =  $\frac{(a+b)}{(c+k)} \times c$ 

P (ED): the effective degradability of DM.

Where:

pe= Effective degradability for response variables %

a= highly soluble and slowly degradable fraction.

b= insoluble and slowly degradable fraction.

c=rate constant for degradation.

k= rate constant of passage.

## 2.5: Chemical analysis:-

Samples of *Prosopis* pods, seeds and sweet (pulp) as well as samples of experimental diets were ground to pass 1mm sieve and stored for further analysis. The determination of their proximate components, DM ash, CP, CF and EE were done according to AOAC (2002). Dry matter was determined by drying the feed sample at  $105c^{\circ}$  over night and Ash by igniting the sample in muffle furnace at  $525 c^{\circ}$  for 8 hrs. Nitrogen (N) content was determined by the Kjeldahl method (AOAC 2002). Crude protein C.P was calculated as (N × 6.25). Crude fibbers C.F and ether extract (E.E) were determined by the methods described by the (AOAC, 2002).

Condensed tannin content of *Prosopis* pods was evaluated using the vanillin assay (Price *et al.*, 1978). The method is based on condensation of vanillin reagent with proanthocyanins in acidic solution. An aliquot of 0.5 the Prosopis pod extract was placed in centrifuge tubes and 20 mL of 1% HCl in methanol was added to each sample. Then, the tubes were placed in a water bath at 30°C with constant shaking for 20 min. After incubation, the samples were centrifuged. Aliquot of supernatants were placed in two separate assay tubes, one for the sample determination and other for blank determination. Samples and blanks were incubated for exactly 20 min after adding 5 mL of vanillin reagent (0.5 g of reagent and 200 mL of 4% HCl methanol) to the samples and 4% HCl in methanol to the blanks. Afterwards, the absorbance was measured at 500 nm using UV-Vis spectrophotometer (Thermo Aquamate). The results were expressed as milligram catechin equivalents per 100g of the pods. All chemical tests were carried out in triplicate.

## 2.6: Statistical analysis:-

The data obtained were subjected to one way analysis of variance to examine the difference on the DM, CP degradation kinetics of the among Prosopis pods and seeds, the in vitro gas production of the pods, sweet (pulp) and seeds of Prosopis. As well as the feed lot performance of goats, effect of the ration on the rumen ecosystem parameters and blood constituents. Significant differences among the parts were assessed using Least Significant Differences (LSD) test according to Gomez and Gomez, (1984). The Statistical Package for Social Sciences Program (SPSS) computer program version was used for the analysis.

## CHAPTER THREE RESULTS

## -: Haematological parameters :3.1

The goats kids did not show any signs of illness from inclusion of different level of Prosopis pods in their diet. Animals were assigned to diets contained 0, 10, 20 and 30% Prosopis pods and designated as  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  respectively. Haematological indices of goats kids fed different *Prosopis* pods is presented on (Table 3.2). There were no significant differences (P< 0.05 ) among the tested parameters at different dietary treatments. The average WBC ranged from (9.66±1.66 to  $10.86\pm1.50$ ) thousand/mm³ with highest count in  $T_3$  and the lowest in  $T_4$ . The average RBC was high  $T_4$  and lowest in  $T_1$ . The haemoglobin concentration (g/100) was not significantly affected by treatments and it was higher in  $T_3$  followed by  $T_1$ ,  $T_4$ . Packed cell volume falls within the range of  $27.17\pm1.51$  to  $29.77\pm2.73\%$ . The ESR mm/h range was  $3.97\pm2.12-5.5\pm1.71$ . None of the erythrocyte indices had shown any significant (P< 0.05) change with the diet.

## 3.2: Serum parameters:-

The values of some serum metabolites and enzymes of the experimental goat kids fed different level of *prosopis* pods are presented in (Table 3.2). Except for total protein, albumin and cholesterol, the other serum metabolites of goats kids were significantly different (P< 0.05). Serum glucose concentrations were significantly different (p<0.05) among dietary treatments. The highest glucose concentration of  $(67.20\pm7.61\ \text{g/dl})$  and the lowest was  $(60.10\pm2.29\ \text{g/dl})$  on  $T_4$  30% and  $T_10\%$  respectively. The highest urea level was  $(37.18\pm3.50\ \text{mg/dl})$  for  $T_4$  and the lowest was  $(32.4\pm6.7\ \text{mg/dl})$  for  $T_2$ . Triglyceride value decreased as the level of prosopis in the diet was increased was significantly (P< 0.01) higher in the control  $(37.33\ \text{mg/dl})$  was lower(19.30 mg/dl) in the group  $T_2$  fed 20% prosopis. Total billirubin values decreased

with increased Prosopis in the diet and were significantly (P< 0.01) higher in the control group. Direct billirubin values were significantly (P< 0.05) higher in  $T_4$  (0.27 mg/dl) and the lowest value was in  $T_3$ (0.16 mg/dl). The activity of the serum enzymes AST was significantly highest (p<0.05 ) in  $T_4$  and was lowest in  $T_1$ . The activity of enzyme ALT was not significantly increased with increased level of the pod in the feed.

:(Table (3.1 Haematological parameters of Nubian Goat kids fed graded .levels of Prosopis pods

<b>Inclusion</b>	level	of	Proso	pis	pods%
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Significance	30	20	10	0	<b>Parameters</b>
NS	9.57±1.32	10.86±1.50	10.23±1.89	09.66±1.66	(WBC(×10 <sup>3</sup> /mm <sup>3</sup>
NS	13.50±0.93	12.53±1.68	13.34±3.59	12.20±0.51	(RBC(×10 <sup>6</sup> /mm <sup>3</sup>
NS	29.77±2.44	27.73±3.50	27.17±1.51	29.77±2.73	(%) PCV
NS	09.00±1.21	09.25±0.81	08.93±1.42	09.20±1.56	(Hb(g/dl
NS	01.40±0.15	01.25±0.08	01.25±0.16	01.24±0.10	Clotting time
NS	05.50±0.17	03.97±0.21	04.56±0.83	04.56±0.33	(ESR (mm/h
NS	25.75±2.13	25.10±2.71	24.44±2.02	24.48±1.39	(MCV(fl
NS	08.16±0.89	08.04±1.40	07.89±0.83	07.92±0.97	(MCH (pg
NS	05.50±0.17	03.97±0.21	04.56±0.83	04.56±0.33	( ESR (mm/h

WBC white blood cells. RBC red blood cells. PCV packed cell volume. Hb haemoglobin ESR. Erothrocyte sedmintation rate. MCV mean corpuscular volume. MCH mean corpuscular haemoglobin MCHC mean corpuscular haemoglobin concentration.

Sign significant level NS: Not significant at (P< 0.05)

Table (3.2)
Serum biochemical parameters of Nubian goat kids fed different levels of Prosopis pods

uniterent levels			of Prosopis p	ods (%)	
parametres	0	10	20	30	Sig
(Glucose (g/dl	60.10±2.29 <sup>a</sup>	60.33±3.32 <sup>a</sup>	65.93±3.84 <sup>b</sup>	67.2±7.61 <sup>b</sup>	**
(Total protein(g/dl	6.57±1.20	6.53±0.99	6.62±1.30	6.64±1.05	NS
(Albumin (g/dl	3.29±0.35	3.19±0.48	3.23±0.34	3.19±0.35	NS
(Urea(mg/dl	33.70±2.14 <sup>b</sup>	32.41±6.70 <sup>b</sup>	<sup>a</sup> 37.09±5.22	37.18±3.50	**
(ALP (U/L	84.90±6.30	85.89±6.43	86.8±9.57	88.89±6.70	NS
(AST (U/L	22.87±097 <sup>b</sup>	23.00±2.71 <sup>b</sup>	24.80±1.80 <sup>ab</sup>	26.30±1.35	*
(ALT (U/L	16.13±2.88	16.89±2.89	16.32±2.74	16.07±2.62	NS
(Cholesterol(mg/dl	56.85±4.53	50.39±4.19	53.55±4.91	45.47±5.94	NS
(Triglycerides(mg/dl	37.33±4.84ª	29.07±1.42 <sup>b</sup>	19.30±3.60°	19.63±2.02°	**
Total Bilirubin mg/dl	0.68±0.14ª	0.64±0.08ª	0.45±0.03 <sup>b</sup>	0.40±0.05 <sup>b</sup>	**
DirectBilirubinmg/dl	0.19±0.02 <sup>b</sup>	0.20±0.07 <sup>ab</sup>	0.16±0.02 <sup>b</sup>	0.27±0.06 <sup>ab</sup>	*

ALP Alkaline phosphatase. AST aspartate aminotransferase. ALT alanine aminotransferase. a, b, c, means in the .(same row values have different superscripts are significantly different (p<0.05 \* Significant (P< 0.05) \*\* Highly Significant (P< 0.01) NS not significant

### 3.7:- Goats kids feedlot performance:-

#### 3.7.1: Feed Ingredients and chemical composition:-

The ingredients and chemical composition of the diets used in the goat feeding experiment are shown in (Table 2.1) and (Table 2.2).

#### 3.7.2: Feed intake growth and feed conversion:-

The growth performance of goats kids fed diets with different levels of dried mesquite (*Prosopis juliflora*) pods for 7 weeks are presented in (Table 3.3). There were significant differences ( P<0.05 ) in DM, CP and ME intake. The highest intake was (756 g/day) on 20% Prosopis diet and the lowest (699 g/day) on the control diet. The CP and ME intake was highest on 20% prosopis (128g/day and 8.24 MJ/day) and lowest on the control (118 g/day and 7.41MJ/day). The final weight significantly differed with animals on  $T_2$  (prosopis 10%) being the heaviest and the lowest was recorded in animals on  $T_4$ . No significant difference on the daily body weight gain among treatments groups. The highest body weight gain (4.78Kg) was observed in treatment of 10% Prosodies pods and the lowest gain (3.58 Kg) was in 30% Prosopis treatment. FCR was best in  $T_1$ (control diet) and inferior in animals on diet  $T_4$  (30% Prosopis) but the difference was statistically non-significant (p<0.05). At higher levels of *Prosopis* pods 30%, the goats maintained weight with less gains.

Table (3.3)

Growth performance of Nubian goat kids fed graded level of Prosopis pods

oi Prosopis pous	Propoi	rtion o	f Proso	ppis poo	ds in the
	diet %				
Parameters	0	10	20	30	SEM
Initial weight (Kg)	13.03	13.10	13.12	12.98	0.27
Final weight(Kg)	17.75	17.88	17.58	16.57	0.29
Body weight gain (Kg)	4.72	4.78	4.47	3.58	0.27
DM intake (g/day)	699 <sup>b</sup>	756ª	745ª	743 <sup>ab</sup>	9.44
CP intake (g/day)	118 <sup>b</sup>	128 <sup>ab</sup>	124ª	123 <sup>ab</sup>	1.57
ME intake (MJ/Kg)	7.41 <sup>b</sup>	7.97ª	8.25ª	8.24ª	1.13
Feed conversion ratio(FCR)	7.6ª	8.1 <sup>b</sup>	9.7°	11.2 <sup>d</sup>	0.70

DM dry matter CP crude protein ME metabolzable energy SEM standard error of the means  $.(^{a, b, c})$  means within the same row followed by different superscripts are significantly different (p<0.05)

### 3.3. Rumen environment study:-

#### 3.3.1. Rumen pH

Rumen pH values of the animals fed the experimental diets are shown in (Table 3.4). No significant differences (p<0.05) were observed between the treatments group. The pH values were not affected by inclusion of prosopis (PJP) in the experimental diets. The pH values in all diets range was  $(6.13\pm0.4-6.8\pm0.1)$ . Animals on the control diet showed numerically higher values at all post feeding time than the other animals. The changes of pH exhibit cyclic fluctuations, and had maximum drop 2-4 h post feeding across all treatments. Nonetheless, values pH of all treatments groups were within the optimum for fibber digestion. By the end of day (at 8 h post feeding) the rumen pH stabilised again without significant differences between treatment diets (p<0.05).

#### 3.3.2 Rumen ammonia (NH<sub>3</sub>)

Table (3.5.) below presented the rumen ammonia (NH<sub>3</sub>) concentration of the goats kids fed the experimental diets at different times post feeding. Ammonia concentration at 2 hours post feeding were significantly (  $^{p<0.05}$  ) increased with increasing of prosopis pods in the diet. Animals in T<sub>4</sub> and T<sub>3</sub> fed high level of prosopis pods recorded the highest ammonia concentrations of (27.83 $\pm$ 2.92 mg/10<sup>2</sup>ml) and (26.60 $\pm$ 4.2 mg/10<sup>2</sup>ml) than animals in the other groups.

At eight h post feeding the concentration of ammonia nitrogen peaked again with values range  $(14.00\pm4.98\neg17.57\pm3.08 \text{ mg/}10^2\text{ml})$  for  $T_3$  and  $T_4$  respectively.

#### 3.3.3. Volatile fatty acids (VFAs) production:-

Volatile fatty acids (VFAs) concentration of different levels of Prosopis in steer diets was shown in (Table 3.6). The rumen fluid volatile fatty acids (VFAs) concentration was not significantly ( p<0.05 ) different among the tested diets. However volatile fatty acids (VFAs) concentration increased with the time post feeding. Total VFAs concentration showed periodic fluctuation with higher values in all the groups during 2 h post-feeding. The lowest and the highest concentration of volatile fatty acid at 2 h were (10.22 $\pm$ 1.83 meq/100 ml) and (12.59 $\pm$ 0.57) meq/100ml for T<sub>2</sub> and T<sub>4</sub> respectively. Pre-feeding the highest total VFAs concentration was in animals on treatment T<sub>4</sub> and the lowest VFAs concentration was in animals in T<sub>2</sub>.

Table (3.4):

pH of rumen liquor at different sampling time (0-8hrs) of steers fed graded levels of Prosopis pods

	Prosopis pods inclusion level (%)				
Time hrs	0	10	20	30	Significan ce
0	6.80±0.10	6.57±0.35	6.77±0.64	6.37±0.78	NS
2	6.37±0.40	6.21±0.45	6.53±0.12	6.24±0.12	NS
4	6.30±0.38	6.22±0.61	6.43±0.57	6.13±0.40	NS
6	6.57±0.70	6.26±0.49	6.44±0.61	6.18±0.46	NS
8	6.73±0.67	±0.66 6.30	6.53±0.23	6.16±0.35	NS

Sign. Significance level NS: not significant

:(Table (3.5 Rumen ammonia (NH $_3$ ) (mg/10 $^2$ ml) concentration at different .time (0- 8hrs) of the steers fed graded level of Prosopis pods

	Prosopis pods inclusion level (%) Significan					
ce	30	20	10	0	Time hrs	
NS	20.83±1.42	16.07±0.64	19.05±1.75	15.43±4.20	0	
*	27.81±2.92ª	26.60±4.2ª	20.07±4.50 <sup>b</sup>	19.87±0.55 <sup>b</sup>	2	
	27.01±2.32	20.00±4.2	20.07 ±4.30	13.07 ±0.33	2	
NS	24.25±1.67	22.40±5.04	25.33±1.40	26.00±2.96	4	
NS	15.09±3.13	14.68±4.06	11.15±0.63	12.29±2.91	6	
NS	17.57±3.08	14.00±4.98	14.00±4.98	15.05±2.67	8	

Sign significance level

.(a,b,c,means in the same column values have different superscripts are significantly different(p<0.05 (Significant. At (P< 0.05 \* NS : Non significant

Table (3.6): Rumen volatile fatty acid concentration (VFAs) (meq/100ml) at different time (0-8hrs.) of steer fed graded levels of Prosopis pods.

	Prosopis pods inclusion level (%)					
Time hrs	0	10	20	30	Sig	
0	8.15±1.6	7.83±0.58	7.99±1.10	8.25±2.11	NS	
2	12.25±0.5	10.22±1.83	10.47±1.64	12.59±0.57	NS	
4	8.98±2.62	10.2±0.40	9.75±2.40	9.47±0.77	NS	
6	10.41±0.85	10.3±0.53	9.87±0.31	10.72±1.3	NS	
8	10.46±0.67	10.19±0.41	9.92±0.15	10.97±1.63	NS	

NS : not significant. Sign.L: Significance level

### 3.4. Rumen Microorganism:-

#### -: Protozoal count .3.4.1

The detailed values of rumen ciliated protozoal count is presented in (Table 3.7). The total protozoal count did not vary significantly (P< 0.05) among the dietary treatments. The values of protozoa numbers in this experiment range between  $3.73\pm0.70\times10^5 - 4.3\pm0.10\times10^5$ . Periodic fluctuation in protazoal count with time was observed, an insignificant (P< 0.05) increase in the number of protozoa was observed 2-4 h post feeding and decreased at 6 h in all treatments.

#### 3.4.2: Bacterial counts:-

Table (3.8) presented the various bacterial counts for rations with varying level of prosopis pods and at different time post-feeding. There was no significant difference (P< 0.05) among treatments. In this experiment bacterial count had shown fluctuations (decrease or increase) with time.

# In vitro gas production .3.5

#### .Chemical composition of Prosopis pods parts 3.5.1

The chemical composition of the different parts, the whole pod, sweet (pulp) and seeds is shown in (Table 3.9). There was significant (P<0.05) difference in the chemical composition of prosopis parts. The DM varied significantly (P<0.05) the highest was  $(97.05\pm0.09\%)$  for the seed and the lowest was  $(96.15\pm0.09\%)$  for whole pods. The CP was significantly (P<0.05) highest in the seed and lowest in the whole pods. The seed was significantly (P<0.05) the highest in CF followed by whole pods and the sweet (pulp). The NFE varied significantly (P<0.05) and the highest was in the sweet and the lowest was in the seed. The EE values which represented crude fat content was significantly different (P<0.05) among the

different parts the highest value was on the seed. The ash content of Prosopis part varied significantly (P<0.05) and the seed had the highest value.

:(Table (3.7 Total rumen protozoal count (10<sup>5</sup>/ml) at different time (0-8 . hrs.) for steer fed graded levels of Prosopis pods

Prosopis pods inclusion level (%)					
Significance	30	20	10	0	Time hrs
NS	0.70 3.73±	0.40 7± 3.9	0.57 4.13±	63. 3.83±0	0
NS	0.90 3.53±	0.36 4.10±	0.21 4.01±	0.66 4.10±	2
NS	0.10 4.30±	0.26 4.20±	0.57 3.97±	0.12 4.06±	4
NS	0.40 4.20±	0.45 4.17±	0.17 4.10±	0.40 3.90±	6
NS	0.36 4.00±	0.31 3.87±	0.36 4.00±	0.21 4.03±	8

Sign significance level .NS: not significant

:(Table (3.8 Total Rumen bacterial count (108/ml) in different time (0-8 hrs.)
.for steer fed graded level of Prosopis pods

Prosopis pods					
Significance	30	20	10	0	Time hrs
NS	1.55 ± 4.93	1.67 6.60±	1.60 4.13±	1.60 5.80±	0
NS	6.93 ± 5.20	6.42 5.13±	7.02 5.13±	1.17 5.47±	2
NS	$6.92 \pm 3.80$	6.42 5.07±	7.02 5.13±	1.17 5.07±	4
NS	6.02 4.43±	3.06 ± 5.33	8.50 4.83±	9.17 6.20±	6
NS	7.00 ± 5.20	5.77 5.53±	3.46 5.20±	6.92 5.60±	8

Sign significance level .NS : not significant

:(Table (3.9 Proximate composition (%) of Prosopis whole pods, Pulp .(Sweet) and seeds used in *in vitro* gas production

Prosopis sampl	es			
Siginficance	Seeds	(Sweet (pulp	Whole pod	Nutrient
*	97.05±0.091 <sup>b</sup>	<sup>a</sup> 96.85±0.06	<sup>b</sup> 96.15±0.09	%DM
*	a 23.36±0.24	<sup>b</sup> 7.74±0.072	c 12.64±0.099	%CP
*	$21.02 \pm 0.07^{b}$	c 21.71±0.58	21.71±0.051 <sup>a</sup>	%CF
*	025°.3.44±	$1.20^{c}\pm.015^{b}$	<sup>b</sup> 1.21±0.034	%EE
*	46.42±0.021°	64.11±0.025 <sup>a</sup>	57.07±0.025 <sup>b</sup>	%NFE
*	<sup>a</sup> 2.96±0.049	2.44±0.035°	2.62±0.03 <sup>b</sup>	%ASH

sig significance level

.(a, b, c, means in the same raw with different superscripts are significantly different (p<0.05

# 3.5.2 In vitro gas production of Prosopis pods, pulp (sweet)and seed:-

Data of in vitro gas production of Prosopis pods, seeds and sweet(plup) during the incubation period are presented in (Table 3.10) and figure (3.1). The gas production of the Prosopis components (whole pod, pulp and seeds) varied significantly (p < 0.05)from 3 h to 96 h incubation period. The cumulative volume of gas production increased with incubation time. Significant (p < 0.05)were observed between feed samples. At 3 differences and 96h the gas production on the pulp (sweet) was the highest  $(11.17\pm076 - 56.83\pm1.75 \text{ mg OM})$  and the lowest was  $(4.5\pm0.50-$ 51.67±0.5 mg/ OM) on the seed. The gas volume from the pulp was the highest than that produced from other feractions throughout the incubation periods. At 48h and 96 h incubation the highest gas production volume was  $(39.167 \pm 0.7 \text{ml/} 200 \text{g})$ and (51.67±0.577ml/200g) on the plup (SW) respectively. Gas production for the different parts of *Prosopis* at different incubation periods is shown in the Figure (1).

#### 3.5.3: In vitro gas production fermentation characteristics:-

The data of fermentation characteristics of *Prosopis*, pods, pulp and seed are presented in table (3.11). The *Prosopis* pulp was significantly ( p < 0.05 ) the highest in soulable fraction a and potentially degradable fraction a+b. The seed had the highest value for the insoluble fraction b and for the gas production rate c. Organic matter (OMD) digestibility and metabolozable energy (ME) were significantly(p< 0.0 5) higher (49.86 $\pm$ 0.02% and 7.29 $\pm$ 0.03Mj/Kg) on the pulp and lower (48.56 $\pm$ .085% and 7.20 $\pm$ .14 Mj/Kg) on the whole pod.

:(Table (3.10 In vitro gas production volume (ml/200mg) of Prosopis, pods, (pulp and seed incubated in buffered rumen fluid (0-96 hrs

Gas productio	n vol/ml			
	Prosopis	Prosopis	Prosopis	
Significance	seed	pulp	pods	Time hrs
**	$3.46\pm0.50^{\circ}$	10.19±0.87 <sup>a</sup>	$8.50\pm0.44^{b}$	0

3	$9.50\pm0.50^{b}$	$11.17\pm0.76^{a}$	$4.50\pm0.50^{\circ}$	**
6	14.83±0.76 <sup>a</sup>	16.50±1.32ª	9.50±0.50°	**
12	22.83±0.76 <sup>b</sup>	25.16±0.76ª	17.33±0.58°	**
24	30.83±0.79 <sup>b</sup>	32.67±0.57ª	27.47±0.50°	**
48	37.00±1.00 <sup>b</sup>	39.167±0.76ª	37.17±0.76 <sup>b</sup>	**
72	17 <sup>ab</sup> .50.90±	52.83±0.24ª	46.50±5.22 <sup>b</sup>	**
96	51.68±0.58ª	56.83±1.75ª	51.67±0.57 <sup>b</sup>	**

Sig significance level .( $^{a,\,b,\,c}$  means within the same row followed by different superscripts are significantly different (p<0.05

p<0.01 .Highly Significant \*\*

Figure.3.1 In vitro cumulative gas production (ml/200mg) of Prosopis whole pod, pulp (sweet) and seed

:(Table (3.11

In vitro Gas production degradation characteristics of Prosopis whole pods, pulp
and seed incubated with rumen buffered liquor

	Sample of Prosopis parts					
Fitted values	Whole pod	(Sweet (pulp	Seed	Significance		
a	8.26±0.24 <sup>b</sup>	10.89±0.02 5°	1.91±0.025°	*		
b	48.28±0.11°	51.30±0.05 5 <sup>b</sup>	035°.53.88±	*		
a+b	56.31±0.02b	62.22±0.1°	042°.55.79±	*		
С	001 <sup>b</sup> .0.025±	042°.0.021±	002°.0.026±	*		
%OMD	085°.48.56±	<sup>a</sup> 02.49.86±	041 <sup>b</sup> .49.15±	*		
MEMj/Kg	14 <sup>b</sup> .7.20±	03 <sup>b</sup> .7.29±	a 02.7.473±	*		

a= the gas production from immediately soluble fraction (ml). b= the gas production from .(insoluble fraction (ml

a+b= gas production from potentially degradable fraction.c= the gas production rate constant from insoluble fraction (b). MEMj/Kg = metabolisable energy OMD= organic matter .digestibility

Means within the same column with different superscript are significantly different\* significant (level at (p < 0.05)

## -: Rumen degradability study :3.6

#### -: Chemical composition of Prosopis samples :3.6.1

The mean values of proximate analysis on dry matter basis of the Prosopis pods and seed are shown in (Table 3.12). The seed was significantly (p < 0.01) highest in CP and CF (21.16±0.26 and 23.02±0.2%) than the pods (12.64±0.21 and18.89±0.30 %). The pods were significantly (p < 0.01) highest in the NFE. No difference in ash content and tannins of feed samples.

#### 3.6.2 Dry matter disapaerence for Prosopis pods and seeds.

Dry Matter disappearance for Prosopis pods and seeds are presented in (Table 3.13) and figure (3.2). The degradation of DM in test sample differed significantly (p< 0.0 5) in their disappearance rate in the different incubation time. The disappearance of the DM contents the Prosopis samples by the end of 48 h of incubation had shown the highest value for pods and the lowest value for the seeds. Over 50% of DM of in all the samples had been degraded. All of the tested feed samples were over 70% degraded at 96 h of incubation.

# 3.6.3: Dry matter (DM) degradation characteristics of Prosopis pod and seed:-

The degradation characteristics for DM in *Prosopis* pods and seeds incubated in the rumen of the steers are presented in (Table 3.14). The DM of the test samples differed significantly (p < 0.05) in degradation characteristics except the (c) and pd values. The value

of soluble fraction (a) was significantly ( p < 0.01 ) highest in the pods and the seed was the highest in insoluble fraction (b). The degradation constant (c) did not significantly different in the feed sample and was highest in the pods and lowest in the seed. Effective degradability for DM decreased as the rumen outflow rate increased.

The Ed at 2% flow rate was significantly ( p < 0.05 i highest in the pods and lowest in the seed at 8% outflow rate.

:(Table (3.12)

Percent chemical composition of Prosopis pods and seeds (White Nile State) used for *in situ* experiment

Sample of Prosopis				
Significance	Seeds	Pods	Nutrient	
NS	94.90±0.20	0.09.95.17±	DM	
**	21.16±0.26ª	12.64±0.21 <sup>b</sup>	СР	
**	23.02±0.21ª	19.76±0.30b	CF	
NS	1.35±0.06	1.21±0.08	EE	
**	46.09±0.73 <sup>b</sup>	57.78±0.31ª	NFE	
NS	3.26±0.10	3.87±1.19	ASH	
NS	030.+2.19	06.+2.13	нт	
NS	020.0.56±	01.0.53±	СТ	

HT = hydrosouble tannins. CT = condensed tannins

p<0.01 €

NS non significant \*\* highly significant

) Sign Significance level

:(Table (3.13

In situ dry matter disappearance (%) of Prosopis seeds and
.pods at (0- 96hrs) incubation periods in the rumen

Significance	seeds	pods	Incubation time
**	7.72±0.622 <sup>b</sup>	41.05±1.66 <sup>a</sup>	0
**	21.08±1.62 <sup>b</sup>	48.94±2.13 <sup>a</sup>	3
**	23.97±2.60 <sup>b</sup>	54.87±2.67ª	6
*	45.57±3.06 <sup>b</sup>	63.66±0.99ª	12
**	54.39±3.71 <sup>b</sup>	65.57±0.96ª	24
*	59.29±2.94 <sup>b</sup>	67.43±0.43ª	48
*	72.28±1.61 <sup>a</sup>	69.67±1.24 <sup>b</sup>	72
*	75.09±1.26ª	70.61±1.44 <sup>b</sup>	96

(p<0.05) significant at\* p<0.01 highly significant \*\*

Figure 3.2. Dry matter disappearance of Prosopis pods and seeds.

Table (3.14):

In situ dry matter degradation characteristics and effective degradability of Prosopis pods and seeds.

		Feed samples		
Degradation		Prosopis pods	Prosopis seeds	significance
parameters				
	a	28.13±2.32ª	9.22±0.20 <sup>b</sup>	**
	b	40.89±2.07 <sup>b</sup>	62.29±2.86ª	**
	C	0.079±0.020	0.067±0.01	NS
	Pd	69.02±0.38	71.5±2.99	NS

(Ed(0.02	64.94±0.30 <sup>a</sup>	55.19±0.61 <sup>b</sup>	**
(Ed(0.05	60.64±0.72ª	42.25±1.47 <sup>b</sup>	**
(Ed(0.08	58.16±0.62ª	35.04±1.64 <sup>b</sup>	**

a = soluble fraction b= insoluble fraction Pd= potential degradable. c= degradation rate Ed = effective degradability .(a, b, means in the same column values have different superscripts are significantly different (p<0.01

# 3.6.4: Crude protein (CP) disappaerence for Prosopis pods and seeds:-

The disappearance of CP content of Prosopis pods and seeds is presented in (Table 3.15) and figure (3.3). The rate of disappearance the CP increased with increased rumen incubation time of feed samples. Throughout the period of incubation the disappearance of CP of seed was significantly(  $^{p<0.01}$  ) highest than the pods. At 24 hrs to the end of the incubation period CP disappearance of the pod was more than 70% and more than 80% on the seed.

#### 3.6.5:- Crude protein degradation characteristics of Prosopis pods and seeds:-

The results of the in situ protein degradation characteristics of Prosopis pods and seeds are presented in (Table 3.16). The all of the degradation parameters were significantly(p < 0.01) varied except for (c). The soluble fraction (a) of the pods was significantly (p < 0.01) higher than that of the seeds ( $45.10\pm5.08a$  Vs  $21.83\pm0.58b$ ). The insoluble fraction (b) of the pods was significantly lower than that of the seeds ( $30.21\pm0.91b$  Vs  $61.96\pm0.30$ ). At all the known flow rates (0.02, 0.05 and 0.08) the pods registered the highest value for effective degradability

:(Table (3.15 In situ crude protein disappearance (%) of Prosopis seeds and pods, at different incubation periods in the rumen of .steers

	Feed samples			
Incubation time	Prosopis pods	Prosopis seeds	significance	
0	40.18±0.73 <sup>b</sup>	62.14±0.00°	**	
3	60.96±1.03b	65.83±0.64°	**	
6	62.22±1.03b	70.84±0.84°	**	
12	63.33±1.20 <sup>b</sup>	76.84±4.09°	**	
24	70.19±5.66 <sup>b</sup>	79.08±4.60°	*	
48	71.45±3.31 <sup>b</sup>	83.17±1.75ª	**	
72	75.44±3.33 <sup>b</sup>	83.67±1.59ª	**	
96	77.02±3.31 <sup>b</sup>	84.67±0.54°	*	

a, b, means in the same column values have different superscripts are significantly ...different

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( p < 0.05 ) significant at* p < 0.01 .( ; highly significant **
```

Figure 3.3. Crude protein disappearance (%) for Prosopis pods and seeds

:(Table (3.16 In situ crude protein degradation characteristics and .effective degradability of Prosopis pods and seeds

Dogradation			Feed	samples
Degradation				significan
parameters		Prosopis pods	Prosopis seeds	ce
	a	45.10±5.08ª	21.83±0.58 <sup>b</sup>	**
	b	30.21±0.91 <sup>b</sup>	61.96±0.30ª	**
	С	00.14±0.08	00.08±0.01	NS
	Pd	75.30±4.25 <sup>b</sup>	83.79±0.48ª	**
	(Ed(0.02	64.94±0.30 <sup>a</sup>	55.19±0.60 <sup>b</sup>	**
	(Ed(0.05	60.64±0.72ª	42.25±1.474 <sup>b</sup>	**
	(Ed(0.08	58.16±0.62ª	35.04±1.64 <sup>b</sup>	**

 $a = soluble \ fraction \ b = insoluble \ fraction \ Pd = potential \ degradable. \ c = degradation \ rate \ Ed = effective \ degradability$ 

(a, b, means in the same column values have different superscripts are significantly different (p<0.01

# CHAPTER FOUR DISCUSSION

Haematological indices are index and reflection of the diet effect on the animals in terms of quality of feed ingested and nutrient available to the animal to meet physiological requirements. In the present study the goat kids were in excellent condition throughout the experimental period without signs of ill health. Feeding *Prosopis* pods to the goats for 50 days did not affect their health or growth. Mahgoub *et al.*, (2007) also reported that there was no effect on health observed with feeding *Prosopis* pods to goats. The health problems were encountered in goats fed *Prosopis* in a study by (Tabosa *et al.*, 2000).

The PCV is used as index of anemia, but its normal value in this experiment indicated the absence of normocytic anaemia which reportedly is characterize by normal MCV and MCH and only detected by the decreased number of RBC or PCV. The normal PCV observed in the experiment was higher than that observed by Mahgoub *et al.*, (2008) for sheep fed non-conventional diet. A severely depressed PCV were observed by Olafadehan (2011a) for goats fed tannin rich *Pterocarpus erinaceus* fodder.

The Hb an iron containing conjugated protein involved in transport of oxygen and carbon dioxide, did not differ significantly between dietary treatments and were within the reference range for goats. The results indicated that the animals fed *Prosopis* diet did not suffer from respiratory or circulatory problems. The normal Hb concentration was indicative to absence of microcytic hypochromic anaemia caused by iron deficiency. Hb level lower than the levels of this study were reported in kids and lambs fed *Prosopis cineraria* leaves (Bhatta *et al.*, 2007).

The blood indices (MCV, MCH and MCHC) which are important in diagnosis of anaemias were not significantly affected by feeding *Prosopis* pods. The indices were within the normal range and comparable to those reported by Girma, *et al.* (2012) in broiler chicken fed on Prosopis pods diet. The RBCs count and indices values in all of the test groups were within the normal range and further confirms the absence of anaemia particularly hypochromic microcytic type. These finding were compabale to those reported by Mahgoub, *et al.* (2005a) in sheep.

The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body. The values of ESR reported from the present work were within the range reported for normal goats (Meck Vet Manual, 2012).

Total leukocyte count (TLC) were not affected by inclusion of Prosopis pods in the diet and were within the normal physiological range of clinically healthy goats (Kaneko, *et al.*, 2008). The TLC reported in this study were on line with the values of Adamu, *et al.*, (2011) on rabbits fed diet of Prosopis pulp. Higher white blood cells (WBCs) count than in the present study were reported in sheep and goats fed tanniniferous diets (Olafadehan 2011a; Mahgoub *et al.*, 2008).

Concentration of blood glucose have been used to monitor nutritional status in animals. Blood glucose concentrations in this study were not significantly varied among the treatments. The current glucose concentrations were higher than those of Shaker et al., (2014) in Shami doe goats fed salty browse mixture. Very low level of glucose in animals result in gross reduction in weight gain (Mahgoub, et al. 2005a). Since protein parameters are influenced by total protein intake, the values from this study suggested nutritional adequacy of the dietary protein (Zubcic, 2001). The serum total protein and albumin were not affected by inclusion of Prosopis in goats kids diet. The concentration of total protein observed in the current study was comparable to the that reported by Al-soquer and Fahy (2014) for poultry fed Prosopis pods. The serum protein profile was not affected by feeding Mesquite and remained within the limits of normal goats (Kaneko, et al., 2008). Abnormal serum albumin usually indicates an alteration of normal systemic protein utilization and blood protein are reflection of quality and quantity of dietary protein. The inclusion of *Prosopis* in the diet did not affect goat protein serum levels. The concentration of serum albumin was affected by feeding *Prosopis* pods to poultry (Al-soquer and Fahy 2014).

Blood urea concentration is a reflection of the rumen NH-N level. In the present rumen ammonia increased along with blood urea concentration. The effect of feeding Prosopis pods on serum urea concentrations were highly significant. The blood urea levels in the present work were within the values of (10-60 mg/dl) reported by Nasr *et al.* (2011) on castrated and un-castrated Sudan Nubian goat and in Shami goats (Shaker *et al.*, 2014). The high blood urea concentrations values could be due to high CP of the experimental diets. A close relationship between protein intake, rumen ammonia level and BUN levels was observed by (Anantasook *et al.*, 2013).

In the present study none of the enzymes, except enzyme AST were significantly affected by inclusion of *Prosopis* pods in the diet. The level of enzyme AST was significantly increased with increasing proportion of *Prosopis* pods in the diet, but the enzyme values were within the normal range. The enzyme AST activity is used as measurement for evaluation of heptocellular injury (Temizel *et al.*, 2009). The enzyme did not increase above the normal values suggesting that no damage to the liver occurred. The increase on the enzyme AST activity in the present study were in agreement with that reported by (Silanikove *et al.*, 1996; Olafadehan, 2011) but contradicted the observation by Tabosa *et al.*, (2000) that antinutritional factor in the *Prosopis juliflora* pods caused tissue damage in goats.

The level of cholesterol is directly related with food available to the animals. The level of serum cholesterol was not significantly different among the dietary treatments. The cholesterol level in this study is comparable to Sahel goats (Waziri *et al.*, 2010) and are lower than the physiological range reported for goats (Zubcic, 2001). The low cholesterol level may be due to liver damage Silankove, *et al.* (1996) or to the presence of anti-nutritional factors affecting lipid metabolism (Matsuura, 2001). The cholesterol level in this study was comparable to that reported by Omidi, *et al.* (2012) on ostrich fed *Prosopis farcta*. Low cholesterol levels were encountered by Shaker *et al.*, (2014) in goats fed salt tolerant shrub like *Prosopis*.

Concentration of serum triglyceride was significantly different among treatments. The concentration of serum triglyceride in the experimental treatments were within the normal range reported for goats (Kaneko, *et al.*, 2008 ). Significant decrease in concentration of serum triglyceride with inclusion of *Prosopis* pods in this study were comparable to those reported in goats (Ikhimioya and Imasuen, 2007) and in sheep (Astuti *et al.*, 2008).

Bilirubin concentration in the blood is considered a measurement for the true function of the liver, as it reflects the ability of the liver to secrete bile. Serum total bilirubin in this study was significantly affected by dietary treatment. The low values were on the *Prosopis* fed groups but were within the range of 0.00- 0.9 mg/100/ml reported for healthy goats (Singh, 2004). The serum concentrations of the unconjugated (direct) bilirubin were high in *Prosopis* fed animals. This implies that livers of *Prosopis* fed animals has less capacity to detoxify toxic substances. Bilirubin concetrations above normal ranges were observed in cattle consuming tannin rich immature oak leaves (Garg *et al.*, 1992).

The experimental diets were isocaloric and isonitrogenous, contained 17% CP which are adequate for growing goats kids. The values of other dietary ingredients of experimental ration indicated that the feed has good nutritional potential to support maintenance and growth. The CF content were within the values for effective rumination and normal rumen function. *Prosopis* pods is high in fibre, which is more than twice as much as soft wheat bran (Batista, *et al.*, 2002). The CP content of *Prosopis* in the this experiment is similar to that used by Mahgoub *et al.* (2005b) and Obeidat *et al.*, (2008) for fattening lambs. Feed that contain 20% or more CP are classified as protein supplements (Kellems and Church, 2002).

Animal performance is the product of nutrient concentration, intake, digestibility and metabolic efficiency of absorbed nutrients. In the present study the highest DM, CP, and ME intakes were observed in group fed 10% *Prosopis* in consistent with Mahgoub *et al.* (2005). Indicating that such feed treatment provide better nutrients balance compare to other treatments. In the contrary to finding of Abdullah and Abddel hafes (2004) the inclusion of *Prosopis* more than 10% in the current experiment did not depress feed intake. This is may be due to associate effect of other dietary ingredients making the ration more palatable and/or due to less length of feeding period in this study. As feeding Prosopis leaves for longer periods result in depression of food intake (Bhatta *et al.*, 2007). The results showed that goats can tolerate a diet up to 30% *Prosopis* and this was in accordance to earlier studies (Maghoub *et al.*, 2004).

Generally there is an inverse relationship between the concentration of tannin in the diet and voluntary feed intake in ruminants (Bhatta, *et al.*, 2004). It was reported that tannin content of 3% or above in browse diet, not only acts as a feeding deterrent, but also reduces ruminal degradability of that feedstuff and of the whole diet (Silanikove *et al.*, 1996). Increased in dry matter and protein intake in the on Prosopis diets in the present study were also reported by (Koech, *et al.*, 2010). The highest CP intake was in the group fed 10% *Prosopis* (T<sub>2</sub>) is comparable to that reported by (Mahgoub *et al.*, 2005). The highest body weight gain was in 10% *Prosopis juliflora* pods (PJP) fed group compared to other groups. A higer body gain than in this study was reported on Awassi lambs fed Prosopis pod (Obeidat *et al.*, 2008). The change in body weight in the current study is comparable to that obtained by Shaker *et al.* (2014) for Shami goats fed salt tolerant shrub mixture. Mahgoub *et al.* (2004) reported a reduction in ADG with higher level of *Prosopis cineraria*, at levels of 300 and 450 g/kg of the diet.

Goats kids fed 10% had the highest gain than goats on the other treatments, and they maintain body gain even at 30% Prosopis level. On the contaray Mahgoub et al. (2004b) repored that goats fed 30% Meskit had lost weight by the end of experiment which last for 50 days. The group fed 30% Prosopis had lowest body weight gain similar to the observations from pervious studies (Abdullah and Abdel Hafes, 2004; Mahgoub et al., 2005). The low FCR are considered to be more economical in animal production and should be positive. FCR showed significant difference among the experimental groups. In this study the goats kids fed 0 and 10% Prosopis pods had the best FCR compared to the groups fed on 20 and 30 %. Among Prosopis fed groups the best FCR value was reported for group fed 10% pods. A (FCR) value for group fed 10% was similar to that reported by Abdullah et al., (2011) for goat kids. FCR for Prosopis fed lamb and goats better than in this study were repored by Obeidat et al., (2008) and Koech et al., (2011) repectively. The growth performance of goats kids was not affect by inclusion of Prosopis pods in the diet up to 30%, similar observations were reported by (Koech et al., 2011; Talpada et al., 2002).

Rumen pH is the most direct and important indictor reflecting rumen fermentation activity. It varies in a regular manner depending on the nature of the diet and on the time it is measured after feeding and reflects changes of organic acid quantities in the ingesta. In this study the diets were isonitrogenous, isocaloric and the feed intake was almost the same among calves. There was no variation on the pH among steers fed *Prosopis* pods diets post feeding and were consistently within the normal range optimum for microbial digestion of fiber and protein in the rumen (Wanapat *et al.*, 2000). These observations were in consistence with Talpada and Shukla, (1987) in cows fed 30% Prosopis pods and Sharma, (1997) on sheep fed concentrate mixture of 75% Prosopis pods. The stabilization of ruminal pH at 8h post feeding in the four treatment groups observed

in this study may be due slow rate of degradation and increase buffering capacity within the rumen.

The ammonia-N in the rumen fluid is the principal intermediate in the microbial degradation and synthesis of protein. Concentration of ammonia produced from Prosopis pods fed to the steers was sufficient for optimum microbial growth and was comparable to that reported by Allam *et al.* (2012). Lower level of rumen ammonia than in this work was on kids fed complete feed block containing *Prosopis cineraria* leaves and (PEG)-600 (Bhatta *et al.*, 2005).

The observed increase in ammonia-N in all of the experimental diets reflect abundant rumen degradable nitrogen (RDN) supply for rumen fermentation and adequate rumen activity.

Rumen fermentation processs and TVFAs production were no affected by inclusion of Prosopis pods in the diets of steers. Similar findings were reported from lambs fed 30% Mesquite pods (Allam *et al.* (2012; McDonald *et al.*, 2002) ). Bhata *et al.* (2005) found higher concentration of VFAs than in the present work in kids fed diet contained *Prosopis* leaves. Increased TVFA level at 2 h post-feeding observed here in this work may be attributed to presence of fermentable carbohydrates in the diets. The increased in TVFAs 2h post feeding which was in consistence with findings of (Chaturvedi and Sahoo 2013).

Ciliated protozoa composition has been used as an index of the condition of the rumen. In the present work rumen protozoal population was not affected by feeding Prosopis pods and their numbers were on the normal range (Dehority, 2005). Feeding Prosopis pods in the present work did not show defaunating activity. Similar findings were reported by Raghuvansi *et al.* (2007).

The rumen bacterial population count was not affected by inclusion of *Prosopis* pods in the experimental diets and their numbers were within the normal range of 10<sup>9</sup> to 10<sup>10</sup> bacteria per mililiter (Singh, *et al.*, 2011). Higher ruminal bacterial population than in this work was reported in dairy cow fed rain tree pods (Anantasook *et al.*, 2013). In this study a higher population of bacteria, and lower population of protozoa in the rumen of steers was observed, a trend which was also reported previously in browse feeding studies. (Chanjula *et al.*, 2004; Anantasook *et al.*, 2013). In this study *Prosopis* pods were separated into three fractions, whole pod, pulp and seeds. Batista *et al.* (2002) separated Prosopis pods by hand into two fraction, seed and

pericarp. Dry matter of *Prosopis* pod, pulp and seed in this study was higher than that reported by (Koech, *et al.*, 2010) for *P. glandulosa* and Ansari nik *et al.* (2013) for *Prosopis farcta* fruit. The crude protein of the seeds reported in the study was lower than that reported by (Obeidat, *et al.*, 2008). The result of the chemical analysis of Prosopis pods and seeds in this study was close to that reported by (Salah and Yagi, 2011). The CP of Prosopis pods and seed in this work was above the range of 70-80 g/kg DM suggested by Van Soest (1994) to the critical limit below which intake by ruminants and rumen microbial activity would be adversely affected.

Tannin content in this study is comparable with those reported by Pena-Avelino *et al.* (2014) for red and white Mesquite (*P. laevigata*) and lower than the tannic acid reported by Gonzalez-Galan *et al.* (2008).

Tannin level in the Mesquite pods may not always be a limiting factor in its dietary inclusion level, as suggested by (Mahgoub *et al.* 2004). The results of gas production of *Prosopis* pods, pulp and seeds were within the range reported for browse trees (Abdulrazak *et al.*, 2001). The variation in gas production between the whole pods, pulp (mesocap) and seed can be attributed to compositional differences of these fractions of *Prosopis*, especially on degradable non-structural carbohydrates, CP, CF and nature and concentration of the anti-nutritional component. The relatively high gas production observed in the present study for all of the of *Prosopis* samples incubated were expected since the fractions were high in crude protein. Hillman *et al.* (1993) reported that gas production is positively related to microbial protein synthesis. The pods of browse tress contain protein, vitamins and minerals necessary for the growth of rumen microbe that degrade the feedstuff in the rumen.

Results of in vitro gas production from this study were similar to gas produced from African bread fruit (*Treculia africana*) (Arigbede *et al.*, 2008) and were higher than those reported for vegetative stage or flowering stage of *Prosopis cineraria* leaves (Elahi *et al.*, 2014). Njidda *et al.* (2013) reported higher values of gas production from *Prosopis Africana* than in this study. The fermentation parameters, gas production at 24 and 48 h and metabolizable energy and dry matter digestibility for *Prosopis chilensis* (Juarez *et al.*, 2013), were higher than in this study. The *in vitro* degradation parameters for the whole Prosopis pods in this study were comparable with values reported by Pena-Avelino *et al.* (2014) for whole red Prosopis pods. The high *in vitro* degradation parameters in the pulp in this experiment could be related to lesser proportion of CF and high NFE. The digestibility coefficient for whole pods

were higher than those suggested by Batista *et al.* (2002) but were comparable with conventional cereal grain such as wheat and corn (Aye-Saldar *et al.*, 2012).

The disappearance of DM from the *Prosopis* pod and seeds in the study was observed to be well above 50% of their reported potential degradability values after 48 hrs incubation. According to (Ehargava and Orskov, 1987) high digestibility values after 48 hrs of incubation imply high digestibility since degradability at this time are regarded as being equivalent to digestibility.

The high values of degradability of pods and seeds obtained in this study is suggestive for their potential value as feed for animals. Over 70% disappearance of dry mater by 96 hrs of incubation for both pods and seeds was reported in this study which was higher than that reported by Ansari nik *et al.* (2013) for *Prosopis farcta.*.

The DM degradation kinetics estimates of *Prosopis* pods and seeds in this study were significantly different. However their overall values were within the range of various browse trees evaluated by (Labri *et al.*, 1998). The difference could be due to the variations in structure and solubility characteristics of the seed and pods. The rapidly degradable fraction" a" DM was observed to be generally low with least value being in seeds and the pods had relatively high values. The increase in DM of soluble fraction in the pods may have resulted from accumulation of soluble carbohydrate in the pods. If " a" is positive, then there is a component which is degraded rapidly or compound which soluble or fine enough to escape from the bag simply by soaking and washing. Whether " a" represents rapid degradation or simply washing losses can be determined with control bags which are simply soaked in water and then washed and dried in the normal way.

When "a" has negative value this means that there is lag time for degradation to start. The "b" fraction differ between investigated seed and pod but mostly high. The seed were rich in the "b" fraction due to cell wall content.

The low rate of degradation of the seeds was in consistence with observation of (Getachew, et al., 2000) that the dry matter

degradation characteristics may be due to cell wall configuration of their polysaccharides and their effect on microbial attachment and colonization of digested particles. The effective degradability of DM in the seed and pods were calculated using out flow rate of 0.02, 0.05 and 0.08. The (ED) of seed and pods varied significantly. According to (Mupangwa *et al.*, 1997) the variation in the effective degradability of DM in forage closely corresponds with the proportion of potentially degradable and level of NDF. The degradation rate of potential degradability for pods and seeds are higher than those reported by (Ansari nik *et al.*, 2013) for *Prosopis farcta* fruit.

## CHAPTER FIVE

## CONCLUSIONS

The study demonstrated that, the chemical composition of *Prosopis* pods and seeds justifies their use as good alternative source of protein supplement for ruminant animals.

The pods can be classified as medium quality protein feed since they contain CP of 12.76 on DM.

Goats kids fed Prosopis pods maintained body weight and good health.

The blood composition of goats were not affected by inclusion of 30% *Prosopis* pods in the diet.

The rumen environment parameter such as pH, ammonia and total volatile acids were not affected by feeding *Prosopis* pods.

The ruminal bacteria and protozoa numbers were not affected by inclusion of *Prosopis* pods in the diet.

*In vitro* gas production of *Prosopis* showed that the pods, seed and sweet part have potential fermentation efficiency as a source of digestible protein and energy to be incorporated in goatsl rations.

The rumen degradation characteristics data indicated that the *Prosopis* pods have relatively good nutritive value and the data could be used for adjusting their inclusion in the ruminants diets.

It is concluded that inclusion of *Prosopis juliflora* pods up to 30% in goat diet had not any adverse effect on nutrient intake and utilization as well as blood or ruminal attributes. This implies that there is a great opportunity for utilizing *Prosopis* as livestock supplement and in contributing to the management of its control.

## Recommendations

Development of indigenous knowledge for the mangement of the Prosopis trees for their effective utilization.

Direction of efforts towards better utilization of the tree and conservation rather than elimination.

Development of effective methods for prepration of the pods before mixing in compound feed and storage facilities for the crop.

Further investigation on the high level of inclusion of the pods in the diet for longer periods than the present study.

More research should be done to investigate the presence of antinutritional factors and practical methods to deactivate them.

An economical appraisal of the use Prosopis pods as animal feed is recommended.

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