CHAPTER ONE

INTRODUCTION

In Bangladesh, the peoples are facing a nutritional deficiency due to unavailability of a sufficient animal protein. Mutton (sheep meat) can play an important role to meet up the deficient amount of animal protein with comparatively lower prices. However, farmers who keep a significant number of sheep that are not ideally productive due to poor genetic merit (Rahman *et al.,* 2014), indiscriminate breeding, poor nutrition and management (Alam *et al.,* 2006), seasonal fluctuations of feed resources and diseases. On the other hand, feed unavailability is one of the most important constraints to the development of ruminant production in the developing countries in Asia, where the ruminant animals are usually raised on natural pastures, crop residues, agro-industrial by-products. These feeds are deficient in protein, energy, minerals and vitamins. Animals fed on these feeds fail to get adequate nutrients for their maintenance and production. These low quality roughages require supplementation with concentrates as sources of protein, energy and macro and micro minerals to support improved their performance. However, concentrates are expensive and may not be accessible to small holder farmers. There is a general shortage of concentrate feed in the Asian countries which is partially meet by importation. Imported feed ingredients lead to a higher production cost of livestock products. Therefore, strategies need to be developed through enhancing the production of indigenous feed resources and their efficient utilization for ruminants (Makkar, 2012).

A wide variety of multi-purpose tropical trees grown at the farmers' field can be used as nitrogen sources in supplementary feeds (Ondiek, Tuitoek, Abdulrazak, Bareeba & Fujihara, 2000). Tree forages specially *Azadirachta indica* not only provide a cheap source of nitrogen, energy and micro-nutrients but have also many other advantages like their wide spread on-farm availability and easy accessibility to farmers and their anthelminthic and laxative properties influence on the alimentary system which can replace commercial concentrates at a lower price (Ondiek *et al.*, 2000). Muhammad *et al.,* (2015) and Rahman *et al.,* (2015) are reported 25.01 and 23.51% CP content of neem leaves respectively. Gowda and Sastry (2000) also pointed out that, neem tree is a drought tolerant plant known to perform well in areas

with long dry seasons, even with rainfall as low as 130 mm per annum. In addition, neem tree is often available evergreen throughout the year when pastures and crop residue are depleted. Therefore, proper use of naturally available neem leaves as source of protein in critical periods of the year seems to be of particular benefit to poor small holder farmers. However, there are no any literatures that compare on supplementary value of sole and mixture of *Azadirachta indica* leaf with commercial concentrate. Therefore, this study was conducted to evaluate the feeding values of different proportions of neem tree leaves with/without concentrate mixture on the feed intake, digestibility, body weight gain, carcass parameters, as natural anthelmintic and meat quality of sheep.

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1.1 Objectives of the study:

The main objective of this study was to evaluate the effect of neem leaf meal as feed supplement on intake, growth and *in vitro* digestibility of indigenous Sheep. The specific objectives of this study are as follows:

1. To know the chemical composition of Neem leaves (*Azadirachta indica).*
2. To use them in ration of animals as protein supplement as well as Anthelminthic.
3. To evaluate the effect of neem leaf meal on growth performance, digestibility and meat quality of sheep.

CHAPTER TWO

LITERATURE REVIEW

2.1 Availability of Neem leaves

Neem (*Azadirachta indica*) is a tropical evergreen tree believed to be native to the Indian subcontinent (Girish and Shankara 2008) and also been introduced into several countries across the globe, including 46 African countries (Orwa *et al.,* 2009). According to Streets (1962) the plant was introduced in Ghana in 1915.

2.2 Neem as feed supplement

Neem biomass yield is estimated to be 0.35 tonnes per mature tree per annum (Panhwar 2005) and 5 to 50 tonnes/ha (Girish and Shankara 2008). Neem leaves can therefore be a potentially valuable alternative feed resource for small holder ruminant producers. However, there is a widely held perception that neem leaves are not accepted by ruminants (Nanang *et al.,*1997) because of their bitter taste. Some reports indicate a contrary view. Leaves of the neem tree are reported to be fed to ruminants in India and other parts of Asia during the dry season (Shukla and Desai 1988). The neem tree is listed among fodder trees in India (Singh 1982) due to its use in animal feeding. Neem leaves are reported to be palatable to sheep (Chandrawathani *et al.,* 2006) and goats (Seresinhe and Marapana 2011).

Neem leaves as supplement to basal diets of crop residues have been shown to improve feed utilization and animal performance in ruminants. In a study in which 30% of mustard straw was replaced with either neem or Albizzia lebbek leaves, both dry matter and crude protein intakes were increased to similar levels with a consequent increases in volatile fatty acid production (Raghuvansi *et al.,* 2007), indicating that neem leaves supplied critical nutrients needed to enhance ruminal microbial growth and fermentation of feed. Bais *et al.,* (2002) offered a sole diet of neem leaves to goats and observed a high voluntary intake of 3.12% of body weight. Other studies such as that of Paengkoum (2010) have shown that neem leaves can replace up to 50% of soya bean meal in ruminant diets with no negative effects on feed intake, dry matter and fibre digestibility as well as body weight gain.

2.3 Chemical composition of Neem leaves

The neem plant (*Azadirachta indica*) is a non-leguminous multi-purpose tree which belongs to the family Meliceae. It has been used in Ayurvedic medicine for more than 400 years due to its medicinal properties. Moreover, Neem leaves are high in crude protein. There are, however, wide variations in the reported values. Crude protein concentrations between 17.5% and 18.7% have been reported (Bais *et al.,* 2002; Bhowmik *et al.,* 2008). A few authors have reported values lower or higher than these. For instance, Ramana *et al.,* (2000) reported crude protein content of neem leaves as 9.7% whereas Ogbuewu *et al.,* (2011) reported a higher value of 20.9%. The variation in crude protein values may be due to varietal differences in the neem plant.

Available reports indicate neem leaves have low fibre content. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) levels of 38.0% and 27.0% respectively have been reported (Ramana *et al.,* 2000). Similarly, Bhowmik *et al.,* (2008) reported a crude fibre level of 11.3% for neem leaves. These are low compared with NDF and ADF ranges of 27.40 to 55.23 and 18.87 to 46.30 respectively for 15 tropical fodder trees (Kumar and Sharma 2003). Low fibre content of neem, coupled with the reported high nitrogen free extract (NFE) level of 53.9% (Bhowmik *et al., (*2008) may make it an important source of readily fermentable carbohydrates in ruminant feed.

There are few reports on the mineral content of neem leaves. Some available data on minerals reported in the literature are summarised in Table 2.1. The content of calcium, which ranges between 1.48 to 1.53 %, is similar to the value of 1.51% reported for sesbania leaves (Ngamsaeng *et al.,* 2006). Neem leaves are reported to be deficient in copper, manganese (Niranjan *et al.,* 2008), zinc and phosphorus (Rao *et al.,* 2011). Levels of minerals, especially trace minerals, are expected to vary widely due to differences in the mineral content of the soil in which the trees grow.

Table2.1: Mineral profiles of Neem leaves

Macro minerals (% DM)			Micro minerals (ppm in DM)						References
Ca	P	Mg	Cu	Fe	Mn	Zn	Co	Cr	
1.48	0.11	1.26	5.24		30.4	47.7			Bhowmik *et al.,* (2008)
0.71	0.28	0.75	34.0	745	60	18.0	10.0	0.80	Ansari *et al.,* (2012)
1.47	0.12	0.40							Ngamsaeng *et al.*,(2006)
1.53	0.25		8.90	566	23.5				Niranjan *et al.,* (2008)

Neem leaves as supplement to basal diets of crop residues have been shown to improve feed utilization and animal performance in ruminants. In a study in which 30% of mustard straw was replaced with either neem or Albizzia lebbek leaves, both dry matter and crude protein intakes were increased to similar levels with a consequent increases in volatile fatty acid production (Raghuvansi *et al.,* 2007), indicating that neem leaves supplied critical nutrients needed to enhance ruminal microbial growth and fermentation of feed. Bais *et al.,* (2002) offered a sole diet of neem leaves to goats and observed a high voluntary intake of 3.12% of body weight. Other studies such as that of Paengkoum (2010) have shown that neem leaves can replace up to 50% of soya bean meal in ruminant diets with no negative effects on feed intake, dry matter and fibre digestibility as well as body weight gain.

Neem leaves have a relatively low nutritional value. In India, an estimated ME of 8.0 MJ/kg DM was reported (Ranjhan, 1980 cited by Puri, 1999). In Sudan, the in vitro OM digestibility of neem leaves was 51%, comparable to that of a local sorghum hay, but the estimated ME was much higher (10.0 vs. 7.8 MJ/kg DM) (Webb, 1988). In another in vitro study, the DM digestibility of neem leaves was 50% (Amanullah *et al.,* 2006).

In a trial in Thailand, neem foliage was included at 20% in the diet of growing goats as a partial substitute for soybean meal without affecting productive performance, rumen fermentation and N balance (Srisaikham, 2009). In India, Sheep fed on

multinutrient blocks that contained 30% neem leaves, as a supplement to a sorghum stover-based diet, increased intake and digestibility while blood parameters remained unchanged (Raghuvansi *et al.,* 2007a; Raghuvansi *et al.,* 2007b).

2.4 Anti-nutritional factors in neem leaves

The presence of some anti-nutritional factors such as tannins, phenolic compounds and oxalates have been identified in neem leaves. But tannin concentration in neem leaves is less than in *Leucaena leucocephala* and below the level that will depress feed intake (Niranjan *et al.,* 2008). The bitter taste in neem leaves is conferred by the presence of triterpenoids, particularly azadirachtin. However, ruminants, especially goats and sheep are known to tolerate bitter taste due to their ability to detoxify secondary plant compounds through allelochemical-type reactions that take place within them (Lu, 1988). Azadirachtin concentration in neem leaves varies with season and ecotypes (Dhaliwal *et al.,* 2004).

The concentrations of these compounds in neem leaves are similar to what have been reported for other ligneous fodder species. For instance, tannin concentration in neem leaves is less than in *Leucaena leucocephala* and below the level that will depress feed intake (Niranjan *et al.,* 2008). Lignin level in neem leaves fall within the range of 4.2 to 11.7 reported for Leucaena (Garcia *et al.,* 1996).

Table 2.2: Some anti-nutritional compounds identified in neem leaves

Anti-nutritional factors	Concentration (%)	Source
Condensed tannins	9.38	Ramana *et al., (*2000)
	11.4	Ngamsaeng *et al.,* (2006)
Crude saponins	2.80	Ngamsaeng *et al.,* (2006)
Oxalate	0.63	Niranjan *et al.,* (2008)
Lignin	10.2	Ramana *et al.,* (2000)
Azadirachtin	0.024	Radhakrishnan *et al.,* (2007)
	0.002	Ghimeray *et al.,* (2009)
Total phenolics	6.53	Ramana *et al.,* (2000)

There is a scarcity of information on the effects of anti-nutritional factors in neem leaves on ruminants. Bais *et al.*, (2002) offered a sole diet of neem leaves to goats and observed no adverse effects. Even in monogastrics, neem leaves feeding has not shown any deleterious effects. In poultry, inclusion of neem leaf meal up to 3g/kg did not affect the weights of the liver, spleen and heart but had an inconsistent effect on the bursa (Manwar *et al.,* 2007). Sonaiya (1993) fed a higher level of 10% neem leaves in poultry diet and did not observe any adverse effects.

2.5 Acceptability of Neem leaves by a livestock farmer

The production of neem biomass is about 0.35 tons per year per mature neem tree (Panhwar, 2005) & 5 to 50 tons /ha (Girish & Sankara, 2008). So neem leaves would be a source of potential feed for small ruminant holder producers.

However, there is a widely held belief that ruminants do not accept neem leaves because of its bitter taste. The presence of triterpenoids particularly azadirachtin in neem is responsible for bitter taste. However, ruminants can tolerate bitter taste as having ability to detoxify secondary plant compounds through alleolochemical-type reactions that take place within them (Adjorlolo *et al.,* 2016). But neem leaves are reported palatable to sheep (Chandrawathani *et al.,* 2006) and also to goats (Seresinhe and Marapana, 2011). In this study, it was observed that as the sheep were hungry and the neem leaves powder when mixed with the concentrate mixture then it was easily accepted by the sheep.

2.6 Effect of Neem leaves on growth and production of Animals

The proteins that are derived from the plant source are termed plant proteins. It is the cheapest and most abundant source of protein. Neem leaf is one of the sources of plant protein that can be used in animal feed. Feed costs are reduced and net return is increased when solitary neem leaf is added in place of commercial concentrate mix (Patil *et al.,* 2021). Neem leaf & Pigeon Pea mixture at different levels replaced the highly prized commercial concentrate feeds without impairing the growth and productive performance of goats (Dida *et al.,* 2019). In ruminant diets, neem leaves may replace 50% of soya bean meals without hampering the feed intake, dry matter and fiber digestibility as well as body weight gain (Paengkoum *et al.,* 2010). Neem leaf feeding improved ruminant performance may be due to the effects of the

bioactive chemicals in the leaves effects on intestinal worms. In another study, 30% of mustard straw was replaced by neem leaves as intakes of dry matter and crude protein were raised to comparable amounts, and synthesis of volatile fatty acids also increased at the same time. A sole diet of neem leaves is offered to goats and founded a high voluntary intake of 3.12% of body weight (Bais *et al.,* 2002).
So in conclusion, neem leaves may be fed as a supplement to animals to boost feed intake and diet quality.

2.7 Anthelmintic properties

The roundworm *Haemonchus contortus* is responsible for 80% of worm infections in small ruminants causing heavy economic losses especially to poor farmers in India. Presently, this infection is treated by giving synthetic chemical compounds (anthelmintics), which are costly, causing drug residues in food products. Studies on neem in animal production are mostly focused on its medicinal uses; mostly as an anti-helminthic agent (Chandrawathani *et al.,* 2006; Tiwary and Pandey, 2010).

Improved performance of ruminants fed neem leaves may be partly attributable to the effects of the bioactive compounds in the leaves on intestinal parasites. There is abundant literature on the effect of neem leaves and extracts on intestinal worms (Chandrawathani *et al.,* 2006; Tiwary and Pandey, 2010). According to Chandrawathani *et al.,* (2006) *Haemonchus contortu*s appears particularly sensitive to the intake of fresh neem leaves by the animal. Improved performance on neem leaves has also been reported for poultry (Sonaiya, 1993).

Thus they indirectly affect the health of the human beings. Further, over dependence on these anthelmintics has resulted in drug resistance in animals. To overcome these situations, several plants have been investigated for their anthelmintic properties. Significant decrease in faecal egg counts of *Haemonchus contortus* was recorded in Boer goats fed with *Acacia karoo* diets (Kahiya *et al.,* 2003).

Anthelmintic efficacy of some medicinal plants, especially neem (*Azadirachta indica*) seeds and leaves has been evaluated against gastro-intestinal nematodes of ruminants in Bangladesh (Ahmed *et al.*, 1994, Mostafa *et al.*, 1995, Rob *et al.*, 2004; Khalid *et al.*, 2005). Rob *et al.*, (2004) observed that water extracts of neem was 53.72% effective against hemonchosis in sheep. Brelin (2002) found that fresh neem leaves significantly reduced *H. contortus* in the abomasum of the treated sheep. Arunachal *et al.*, (2002) noted that neem leaves, seeds and bark were 53%, 49% and 38% infective against gastrointestinal helminths in sheep respectively. Rahman (2002) found the effects of water extract of neem leaves was 62% in goat.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Location of the study

The experiment has conducted at Chattogram Veterinary and Animal Sciences University. The research station was located at 23˚42’0”N, 90˚22’30”E and at an altitude of 4 meters above sea level.

3.2 Neem leaf Collection

Azadirachta indica were collected from different locations of Chattogram. This selection point was based on the activities being carried out within and around the sampling point. Collected samples were wrapped in a black polythene bag and properly labeled before transporting to the laboratory for further analysis.

Neem tree Neem foliage Neem leaves

Image 3.1: Different parts of neem

3.3 Neem leaf preparation

Hybrid neem leaves has harvested mostly from 1to 3 years old trees. After collection of leaf the fresh samples were dried at sunlight and then grounded into powder using a laboratory grinder to reduce particle size and then kept in plastic bags for Laboratory analysis and further use as Animal feed in experimental diet.

Neem leaves Ground leaves Ground leaves foliage

Image 3.2: Neem leaf preparation

3.4. Chemical analysis of Neem leaves

The proximate analysis of the samples (neem Leaves) for moisture, total ash, crude fibre, crude protein were carried out in triplicate using methods described by AOAC (AOAC, 2005).

3.5. Experimental animals

A total twelve male indigenous sheep with initial body weight Approximate 12 ± 1.0 kg (means ± standard deviation) were selected from a flock of 30 animals of the CVASU animal farm and these animal were used in the experiment. The animals were quarantined for seven days to get them used to their new environment and to observe their health condition. At the end of the quarantine period, the sheep was randomly allocated into four dietary treatments. All sheeps were treated with anthelmintics (Endex, Novertis, India limited) prior to feeding experimental diets. The selected sheeps were gradually adjusted to feeding with neem leaves and paddy straw before the start of feeding the experimental diet.

3.6. Experimental diet and management

The collected *Azadirachta indica* consisted of leaves, petioles and soft rachis. The hard woody rachis was removed from the foliage. The whole foliage was chopped and sun dried on thick plastic sheets for three to five days, then grind and stored in bag until further use. The concentrate ingredients such as broken maize, rice polish, wheat bran, soybean meal were purchased from the local market chattogram and the mixed dietary concentrate were prepared according to Table 3.1 as weekly basis for feeding the sheep. The concentrate mixture (supplement feed) were offered at 300 g DM/ (animal/d) and this concentrate mixture was replaced gradually using dry neem foliage according to Table 3.2. All animals were free access to water. Before the experiment starts, samples of supplement ingredients were analyzed for chemical composition of DM content. The total concentrate mixture and dry neem foliage for each treatment were weighed once a day. They are divided in to two parts. One part was offered at 08:00, another part was given at 14:00. The feeder and water buckets were cleaned daily before the fresh feed and water were offered. The feed offered and refusals were recorded on a daily basis throughout the experimental period to estimate voluntary dry matter intake. The feeding experimental trial was lasted for 60 days. All animals were weighed before morning feeding at the start of the experiment and each week interval. The average daily live weight gain was calculated by regressing cumulative live weight on the days of feeding during the experimental period. The feed conversion efficiency was calculated as a proportion of live weight gain to feed intake for the whole experimental period.

Table 3.1. Composition of basal diet (concentrate Mixture)

Name of ingredients	**Amount of ingredients (kg)**
Maize	52
Rice police	18
Wheat bran	15
Soybean meal	12
Di-calcium phosphate	1.5
DL methionine	0.5
Vitamin Mineral premix	0.5
Salt	0.5
Total	**100**

3.6.1 Experimental design

The selected sheep were distributed into four equal groups each containing three animals, and the following four diets were randomly allocated to four dietary groups of animals in a Completely Randomized Design (CRD) with three replications. Concentrate mixture was replaced with neem foliage at 0, 50, 100 and 150g among remaining 300g of diet and four experimental treatment were as follows according the Table 3.2.

T1 = 300g concentrate mixture

T2 = 250 g concentrate mixture + 50g Dry neem foliage

T3 = 200 g concentrate mixture + 100g Dry neem foliage

T4= 150g concentrate mixture + 150g Dry neem foliage

Table 3.2 : Treatment arrangement

Treatments	Rhodes grass and Straw	Feed offered, g/d		
		Concentrate Mixture (Wheat bran, Rice polish, Soybean meal or till oil cake)	Neem Leaf Meal	Total Supplement
T1	Ad libitum	300	-	300
T2	Ad libitum	250	50	300
T3	Ad libitum	200	100	300
T4	Ad libitum	150	150	300

3.7 In-vitro dry matter and organic matter digestibility

In-vitro dry matter and organic matter digestibility of the neem leaf meal and other four diet samples will be measured according to the modified technique where the feed samples in the serum bottles were incubated using a rotary incubator. When a feed is incubated in vitro with buffered rumen fluid, the amount of gas produced reflects the production of VFA, which are a major source of energy for ruminants.

3.7.1 Rumen fluid collection

Ruminal contents were obtained from the 8 month aged slaughtered sheep. The rumen fluid was collected early in the morning. The collected ruminal fluid was squeezed and the extracted fluids was strained through cheese cloth that had been folded four times and was placed in a glass bottle with cap. The bottles were subsequently capped and immediately transported to the laboratory while maintaining the temperature at 39˚C in a water bath in the laboratory.

Collection of rumen fluid | Measuring pH after 24h *In-vitro* digestion | Gas production estimation

Image 3.3 : Different stages of estimation of gas production

3.7.2 Preparation of buffered solution

The medium was prepared following the method described by Asanuma *et al., (*1999) with the following composition in mg/L: Dipotassium phosphate(K_2HPO_4), 450; Monopottasium phosphate(KH_2PO_4), 450; Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), 190; Calcium chloride dehydrate ($CaCl_2.2H_2O$), 120; Sodium chloride (NaCl), 900; Cysteine hydrochloride ($C_3H_7NO_2S.HCl$), 600; Ammonium sulfate ($(NH_4)_2SO_4$), 900; Trypticase peptone (BBL; Becton Dickinson, Cockeysville MD), 1000 and Yeast extract, 1000. The chemicals were poured in distilled water of one liter. Firstly, all the chemicals were poured and a very small amount of distilled water was put for the solution to mix evenly. Yeast extract and trypticase peptone were dissolved by hands since they clump immediately when these come in contact

with air. Thereby, immediate mixture of these chemicals were needed. In this process, a certain p^H is required for the efficient function of *in vitro* test which is 6.9.The p^H was balanced by adding one to two drops of Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl). Afterwards, the buffer was dispensed with 100% Nitrogen (N_2) gas for creating anaerobic condition. Lastly, the buffer was autoclaved at 121˚C for 15 minutes. Finally, the buffer was collected collected after almost one hour when the buffer was cooled after autoclaving and preserved till the next day for mixing with freshly slaughtered rumen fluid.

3.7.3 Preparation of buffered rumen fluid

The rumen fluid was mixed with the buffer the next day collection of freshly slaughtered cow and rumen fluid. The upper residue of the rumen fluid was removed fluid was removed while the middle portion was collected and used in the experiment. The pooled and particle free rumen fluid was transferred to a buffer medium bearing p^H 6.9 (Hino *et al.,* 1992) in a 1:3 rumen fluid : buffer ratio. 4000 ml of total liquid was required, but excessive 500 ml was prepared in order to prohibit shortage of liquid in case liquid is lost while pouring in serum bottles.

3.7.4 Preparation of serum bottles

50 ml of buffered rumen fluid was anaerobically transferred under a constant flow of N_2 gas atmosphere in order to make it oxygen free as per suggested by Asanuma *et al.*,(1999) to 100 ml serum bottles containing the 0.5 g concentrate feed. Finally, the prepared rumen fluid buffer was poured in different serum bottles for the ultimate *in vitro* experiment. Sealing with rubber septum stopper and aluminium cap (Asanuma *et al.*,1999) of the bottles containg the feed samples and buffered rumen fluid will follow which will then be incubated subsequently at 39˚C for 6, 12, 24 and 48 h in a shaking incubator with 120 rpm (Hattori and Matsui,2008).

The final bottle set up was made according to the following treatments : treatment 1(T1),treatment 2 (T2), treatment 3 (T3), treatment 4 (T4) and keeping five replication of each treatment. Thereby, the incubation times were 6 hours, 12 hours, 24 hours and 48 hours. There were 5 bottles fixed for every 6, 12 and 24 h at each treatment group. Finally, all the bottles of both control and treatments group were put

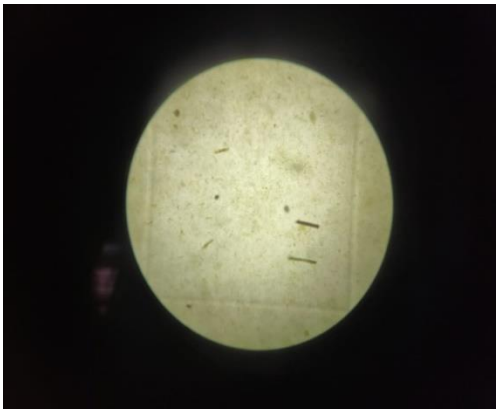
into shaking incubator at 39˚C temperature for in vitro gas production with 120 rpm (Hattori and Matsui, 2008).

3.7.5 *In-vitro* dry matter (IVDM) and organic matter (IVOM) digestibility

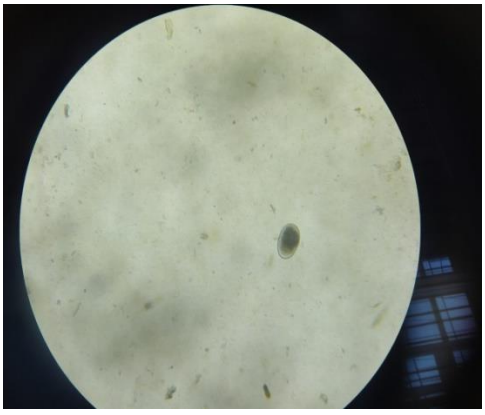
Neem leaves dry matter (DM) and organic matter (OM) were determined by drying it at 105°c for 20-24 hours and ashing it at 550°c for 6 hours, respectively. The obtained DM% and OM% were used to calculate the substrate's initial DM (DMI) and initial OM (OMI) in grams. After the designated incubation period, fermenta samples from each serum bottle were drained into dried, pre-weighed nylon bags and knotted using nylon thread, then splashed with flowing water for 15 minutes or until the turbidity of the water caused by washing dissipated. After that, the approach of Van soest and Robertson (1985) for determining actual digestibility was used, which involved refluxing the incubated residues for 1 hour and then filtered in pre-tarred crucibles to recover the based on incubated samples minus oven dried samples and the ivom was estimated using incubated samples minus the residue of ash.

3.8. Laboratory Analysis for Faecal egg counting

Daily faecal samples (2 days / week) were collected from each animal in the morning and this was continued for 6 weeks. These samples were subjected to the McMaster faecal egg counting technique, using 3 g individual faecal samples (Christopher et al., 1992).



Examining fecal egg using Mcmaster technique



Worm’s Egg in fecal sample

Image 3.4 : Fecal egg examination

3.9. Slaughter procedure and carcass sampling

At the end of the feeding trial, two sheep were randomly selected from each treatment for slaughtering. All Eight selected animals were fasted for twenty four hours and slaughtered according to the 'Halal' method. The live weight of fasted animals was recorded before slaughtering, and immediately after evisceration each hot carcass weights were recorded. Non-carcass components (skin, head, feet, lung, heart, liver, spleen, kidneys, kidney fat, and gastro-intestinal tract fat) were removed and weighed individually. The stomach (rumen, reticulum, omasum and abomasum) and post-ruminal tract (small intestine, large intestine and caecum) were removed and weighed separately. Dressing percentage was calculated as hot carcass weight relative to fasted body weight. The carcasses were divided into equal halves along the midline using a carcass saw. The left half was used for the determination of meat quality and chemical composition, while the right half was assigned for determining carcass composition (lean, bone and fat) and carcass cut. Approximately 150 g of both *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles were sampled from the left side of the carcass and store at -20ºC for further analysis of chemical composition and fatty acid and amino acid analysis.

Image 3.5 : Skinning and evisceration of the carcass

3.10 Meat quality

3.10.1 Measurement of pH

The pH of sheep muscles at 24 h post slaughter was measured using a transportable pH meter (Mettler Toledo, AG 8603, Switzerland) following the procedure of Bendall (1975). The pH meter was standardized at 4.0 and 7.0 buffers before the use. Immediately after the animals were dressed, about 10 g of meat was taken from the longissimus dorsi muscle. Then 1 g of meat sample was placed into a blender beaker and 10 ml deionized water was added. The sample was allowed to blend for 10 sec at maximum speed. During analysis, the solution was continuously stirred on a stirrer plate to ensure that accurate pH values were obtained. All samples were measured in duplicate.

3.10.2 Measurement of drip loss and Cook loss

The fresh sub-samples of Longissimus dorsi (LD) were weighed (approximately 20 g) and recorded as initial weights (W1). The samples were then kept in polyethylene plastic bags and sealed it, vacuumed, and then put on a tray in a chiller at 4 °C. After specific time (3, 5, 7 days) of storage, the samples were taken from the chiller and immediately removed from the bags, gently blotted to dry, weighed and weights recorded as W2. The percentage of drip loss was calculated and expressed as the percentage of differences of sample initial weight and sample weight after specific days storage divided by the sample initial weight (Honikel, 1998).

$$\text{Drip loss (\%)} = [(W1 - W2) \div W1] \times 100$$

Where,

W1 (g) = sample initial weight

W2 (g) = sample weight after 3, 5 and 7 days storage

3.10.3 Measurement of cooking loss

The frozen sub-samples of muscles (LD) were removed from a -80°C freezer into a 4°C chiller overnight to thaw. The thawed samples were weighed individually and recorded as initial weight (W1), and placed in water-impermeable and sealed plastic bags. The muscles were then cooked in a water bath set at 80°C for 30 minutes.

The cooked samples were removed from the water bath, allowed to adjust at the room temperature and removed from the bag, blotted to dry without squeezing, and re-weighed (W2). The cooking loss percentages were calculated using the following equation (Honikel, 1998).

$$\text{Cooking loss (\%)} = [(W1 - W2) \div W1] \times 100$$

Where,

W1 (g) = initial sample weight

W2 (g) = cooked sample weight

3.12 Statistical analysis

The analysis of variance will be done on the data on feed intake, growth performance, proximate analysis, IVOMD, fatty acid profile meat quality of sheep using the one way ANOVA in the SAS 9.2 software (2007). The differences in the means will be compared by Duncan's multiple range tests at 5% level ($P<0.05$).

CHAPTER FOUR
RESULTS

4.1 Chemical composition different parts of *Azadirachta indica* (Neem)

The Chemical composition different parts of Azadirachta indica (Neem) leaf are presented in Table 4.1. In our study DM% of neem foliage, leaf, stem are 34.79%, 32.42%, 37.87% respectively. It is observed that neem stem contents more dry matter than leaf and foliage. Moisture contents are respectively 65.21%, 67.58%, 62.13% which shows neem leaf has more moisture content than foliage and stem. Ash content of neem is 8.815% in foliage, 9.82% in leaf, 6.33% in stem which means leaf content more ash. CP% of foliage is 12.75%, leaf is 14.07%, stem is 5.28%. Leaf content more protein than other part of plant. CF% of foliage is 19.33%, leaf is 12.50%, stem is 42.95%. Here stem content more fiber.

4.2 Chemical Composition of Supplements Used in Growth and Digestibility Study

The proximate composition of the different experimental diet that used in the growth trial are shown in Table 4.2. Treatments 1 had DM, Ash, CP, CF values of 90.62%, 30.50%, 15.31%, and 6.0%. The DM content of dietary group did not differ. The crude protein content of dietary supplement decreased slightly with increased of dry neem leaves in to the diet however there were no significant difference among the dietary group. However , CF content of supplement increased significantly ($P<0.05$) with increased of dry neem leaf into the diet where the lowest value was in treatment T1(6.0%) and highest values was in T4(8.25%) whereas, there were no differences among the treatment T1,T2 and T3. The Ash content decreased with increased of neem leaf in diet.

Table 4.1: Chemical composition of different parts of *Azadirachta indica* (Neem) (Mean ±SE; n = 3)

Variables	Neem tree			P value	Level of significance
	Foliage	Leaf	Stem		
DM (%)	34.79[b]	32.42[b]	37.87[a]	0.01	*
Moisture (%)	65.21[a]	67.58[a]	62.13[b]	0.01	*
Ash (%)	8.81[b]	9.82[a]	6.33c	0.001	**
CP (%)	12.75[b]	14.07[a]	5.28[c]	0.001	**
CF (%)	19.33[b]	12.50[c]	42.95[a]	0.001	**
OM (%)	91.19[b]	90.17[c]	93.67[a]	0.001	**

[a,b,c] Means in the same row and in each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

Table 4.2: Chemical Composition of the Experimental Supplement

Nutrient (%)	Treatments				SEM	P value
	T1	T2	T3	T4		
Dry matter	90.62	91.51	90.00	91.29	0.27	0.08
Crude protein	15.31	13.65	13.65	14.05	0.33	0.24
Crude fiber	6.0[b]	6.12[b]	6.45[b]	8.25[a]	0.41	0.03
Ash	30.50[a]	26.83[b]	21.17[c]	20.67[c]	1.48	0.003

[a,b,c] Means in the same row and in each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

*T1 = 300g concentrate mixture,T2 = 250 g concentrate mixture+ 50g Dry neem foliage,T3 = 200 g concentrate mixture+ 100g Dry neem foliage, T4= 150g concentrate mixture+ 150g Dry neem foliage.

4.3 Effect of dietary Neem foliage on growth performances of sheep

The weight gain and feed conversion efficiency of the Sheep are represented in Table 4.3. The initial weights of the sheep were ranged from 12.10 to 12.50 kg. The average daily weight gain of sheep increased significantly with when animal fed increased amount of dry neem leaf and significantly higher live weight were found sheep fed T3 and T2 diet. The feed conversion efficiency of sheep on the basal diet (Treatment 1) and Treatment 4 were similar ($P>0.05$) but significantly ($P<0.05$) higher than sheep on Treatments 2 and 3 diets. Sheep on Treatments 2 and 3 were however similar ($P>0.05$).

Table 4.3: Effect of dietary Neem foliage on growth performances of sheep

*Variables	Treatments				P value	Level of significance
	T1	T2	T3	T4		
Initial BW (kg)	12.10	12.17	12.50	12.33	0.75	NS
Final BW (kg)	16.30	16.73	17.17	16.80	0.28	NS
Average daily live weight gain (g d^{-1})	70.00^{b}	76.11^{a}	77.78^{a}	74.44^{ab}	0.01	*
FCR	8.57^{a}	7.89^{b}	7.72^{b}	8.06^{ab}	0.008	*

^{a,b,c} Means in the same row and in each treatment with different superscripts differ significantly among cutting interval at $P<0.05$ level; n= observation numbers.

FCR= Feed conversion efficiency. *T1 = 300g concentrate mixture,T2 = 250 g concentrate mixture+ 50g Dry neem foliage,T3 = 200 g concentrate mixture + 100g Dry neem foliage,T4= 150g concentrate mixture + 150g Dry neem foliage.

4.4 Faecal egg Counts (FEC) and FAMACHA estimations

The mean FEC of the Neem treated diet and control groups was higher amount of EPG and almost the same all of the group until day 14 of the experiment ranging from 30000 e.p.g to 34000 e.p.g. However, after 14 days the EPG decreased rapidly in Neem treated group and had lower mean e.p.g in sheep those fed neem treated diet

compared to those fed control diet. Week 4th onwards the e.p.g in neem treated group decreased gradually and lowest value was obtained in week 8 which was ranges 500 to 600 (Figure 4.1).

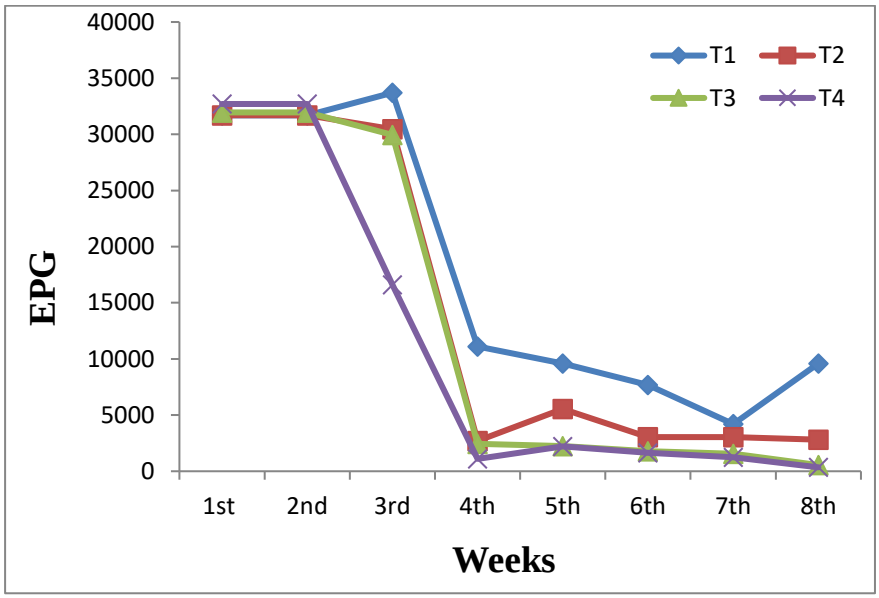


Figure 4.1 Mean nematode faecal egg counts (e.p.g.) of the Control and Neem fed (Treated) group.

*T1 = 300g concentrate mixture, T2 = 250 g concentrate mixture + 50g Dry neem foliage, T3 = 200g concentrate mixture + 100g Dry neem foliage, T4 = 150g concentrate mixture + 150g Dry neem foliage.

4.5 Carcass characteristics, internal and lymphoid organs of Sheep fed different dietary treatments

The effects of dietary supplementation different level of neem foliage on carcass characteristics, internal and lymphoid organs of sheep are presented in Table 4.4. The sheep fed dietary supplements using 50 to 100g dry neem foliage had significantly ($P<0.05$) higher hot carcass percentage and numerically higher breast yield compare to those fed other diets. The head stomach, loin and heart percentage differ significantly among the treatments. The internal organs (liver, kidney, spleen) percentage did not differ ($P>0.05$) among the dietary treatments.

Table 4.4: Effect of neem leaf on carcass characteristics of sheep

Parameter	Supplements				SEM	P value
	T1	T2	T3	T4		
Slaughter weight	15.45[b]	18.73[a]	17.53	15.90[b]	0.53	0.03
Hot carcass (%)	95.95[ab]	96.95[a]	98.75[a]	92.76[b]	0.90	0.05
Dress percentage	48.87	51.66	52.02	46.90	0.95	0.15
Skin (%)	10.20	12.28	10.96	8.8	1.50	0.72
Head(%)	8.05[b]	8.28[ab]	8.7[ab]	9.11[a]	0.18	0.01
Hind limb (%)	2.57	2.36	2.37	2.20	0.07	0.30
Loin (%)	5.21[ab]	5.79[a]	4.50[bc]	4.35[c]	0.06	0.02
Heart (%)	0.700	0.580	0.800	0.937	0.06	0.01
Liver (%)	4.26[ab]	4.19[ab]	4.16[b]	4.53[a]	0.23	0.12
Kidney (%)	1.47[ab]	1.35[b]	1.65[aba]	1.95	0.05	0.14
Spleen (%)	0.49	0.443	0.466	0.512	0.06	0.17
Viscera (%)	18.99	16.56	18.83	20.60	0.03	0.34

[a,b,c] Means in the same row and in each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

*T1 = 300g concentrate mixture, T2 = 250 g concentrate mixture + 50g Dry neem foliage, T3 = 200g concentrate mixture + 100g Dry neem foliage, T4= 150g concentrate mixture + 150g Dry neem foliage.

4.6 Drip loss and pH of meat at different postmortem aging periods of sheep fed dietary treatments

The muscle pH and drip loss values of breast muscle in broiler chickens fed different dietary treatments is shown in Table 4.5. Dietary treatments had no effect (P> 0.05) on the pH value of muscle in sheep at 1 and 3 days of postmortem aging. At 7 days pH of muscle increased in treatment group T3 however, no differences observed among the other treatment group. Regardless dietary treatments, pH at 7days significantly increased in treat T3 whereas, pH did not differ (P> 0.05) among other treatments.

At day 1 and 7 postmortem, drip loss of sheep muscle was influenced (P> 0.05) by dietary treatments and drip loss decreased (P< 0.05) in treatment groups sheep fed dry neem leaves group. The drip loss of muscle in sheep fed T2, T3 and T4 was lower (P< 0.05) compared with those fed control diet (T1). Irrespective of the dietary treatments, drip loss of meat increased with increase of postmortem ageing period, whereas no further changes of drip loss observed from 5 to 7 postmortem storage days dietary treatments supplied 100 to 150 g neem leaves (T3 to T4).

Table 4.5: Drip loss and pH of meat at different postmortem aging periods of sheep fed dietary treatments

Parameter	Post-mortem days	Treatments				P value
		T1	T2	T3	T4	
Drip loss (%)	**1**	0.75^{az}±0.02	0.66^{bz}±0.04	0.63^{by}±0.013	0.62^{by} ±0.02	0.01
	3	2.08^{ay} ± .02	1.78^{ay}±0.02	1.80^{ax}±0.06	$1.81^{a\,x}$±0.016	0.18
	7	4.55^{ax} ± .02	3.18^{bx}±0.2	2.59^{bx}± 0.027	3.35^{bx}±0.118	0.004
P value		0.001	0.001	0.01	0.003	
pH of meat	1	5.25±0.155	5.63^{y}±0.015	5.65±0.09	5.03±0.09	0.76
	5	5.35±0.024	5.79^{xy}±0.08	5.81±0.015	5.87±0.055	0.13
	7	5.85^{b}±0.05	5.91^{abx}±0.1	5.93^{a}±0.05	5.91^{ab}±0.02	0.04
P value	**0.237**	0.237	0.05	00.07	0.49	

^{a,b,c} Means in the same row and in each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

x,y,z Means in the same column and each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

*T1 = 300g concentrate mixture,T2 = 250g concentrate mixture + 50g Dry neem foliage, T3 = 200g concentrate mixture + 100g Dry neem foliage, T4= 150g concentrate mixture + 150g Dry neem foliage.

4.7 Cook loss of meat sheep fed different level of neem leaves

The cooking of muscle of sheep fed different dietary treatments are presented in Table 4.2. At day 1 postmortem the cook loss of meat increased with increase of dry neem foliage in the basal diet and significantly (P<0.05) higher cook loss was obtained sheep fed 100 to 150g dry neem foliage used in dietary treatments that is T3 and T4. However no significant differences (P> 0.05) were observed in the cooking loss of meat among the dietary treatments among the treatment T2, T3 and T4.

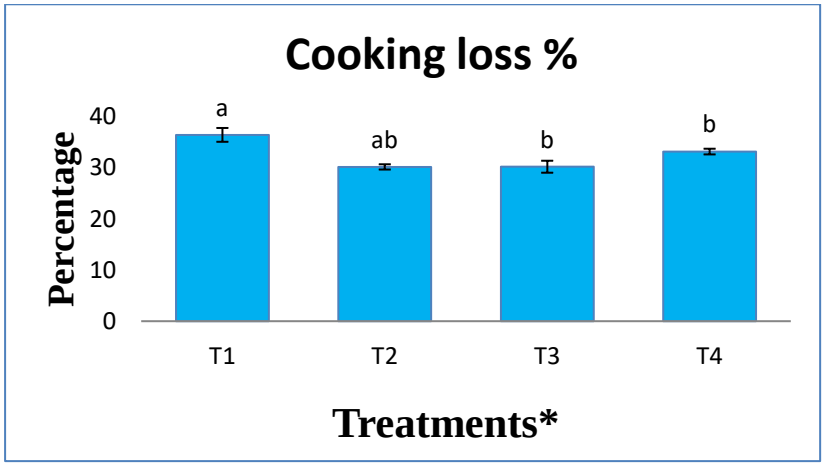


Figure. 4.2 Cooking loss of meat sheep fed different level of neem leaves

*T1 = 300g concentrate mixture,T2 = 250 g concentrate mixture+ 50g Dry neem foliage, T3 = 200 g concentrate mixture + 100g Dry neem foliage, T4= 150g concentrate mixture + 150g Dry neem foliage.

4.8 Chemical composition of sheep meat

The chemical composition of meat sheep fed different level dry leaf with basal diet is represented in Table 4.6. The percentage of moisture, ash and CP content of meat were unaffected across the dietary treatments. The ether extract content of meat of sheep fed increased proportion of neem leaf in basal diet decreased numerically both CP and EE than those of control diet.

Table 4.6 Chemical Composition of the Experimental Meat

Nutrient (%)	Supplements				SEM	P value
	T1	T2	T3	T4		
Dry matter	24.10	23.6	23.9	23.9	0.16	0.27
Crude protein	23.8	22.93	22.84	21.26	0.59	0.82
Ether extract	1.05	1.00	1.00	0.96	0.03	0.83
Ash	1.14	1.21	1.08	1.07	0.03	0.59

4.9 *In vitro* gas production and pH

The total gas production increased with increased neem leaf leaves in the diet but no significant difference were observed among the treatment all the incubation period. However, the total gas production were increased significantly ($p<0.05$) with increased of incubation period and difference was observed 3, 6 and 24 h of incubation period. Total gas production was higher at 24h compared to 12h and 6h (Table 4.7).

The in-vitro rumen fluid pH at 24 hours incubation period is represented in Figure 4.3. From the figure it was observed that at 24 hours incubations there was significant difference in pH among different dietary supplements. At 24 h incubation period significantly ($p<0.05$) highest pH was observed in diet containing 150gm dry neem foliage (T4) and lower pH were obtained in diet containing 50 gm dry neem foliage (T2).

Table 4.7: Total gas production of different dietary supplements at different incubation period

Parameter	Hour of Incubation	Treatments				P value
		T1	T2	T3	T4	
Total gas production (ml)	**6h**	$26.40^{z} \pm 4.0$	$26^{z} \pm 0.004$	$30.50^{y} \pm 0.5$	$31.5^{z} \pm 1.5$	0.48
	12h	$39.5^{y} \pm 4.5$	$40^{y} \pm 3.0$	$39.5^{x} \pm 0.5$	$44.5^{y} \pm 0.5$	0.55
	24h	$56.0^{x} \pm 4.0$	$50.50^{x} \pm 4.5$	$52.50^{x} \pm 2.5$	$56.50^{x} \pm 1.5$	0.58
P value		0.03	0.04	0.04	0.02	

x,y,z Means in the same column and each treatment with different superscripts differ significantly among cutting interval at P<0.05 level; n= observation numbers.

*T1 = 300g concentrate mixture, T2 = 250 g concentrate mixture + 50g Dry neem foliage, T3 = 200g concentrate mixture + 100g Dry neem foliage, T4= 150g concentrate mixture + 150g Dry neem foliage.

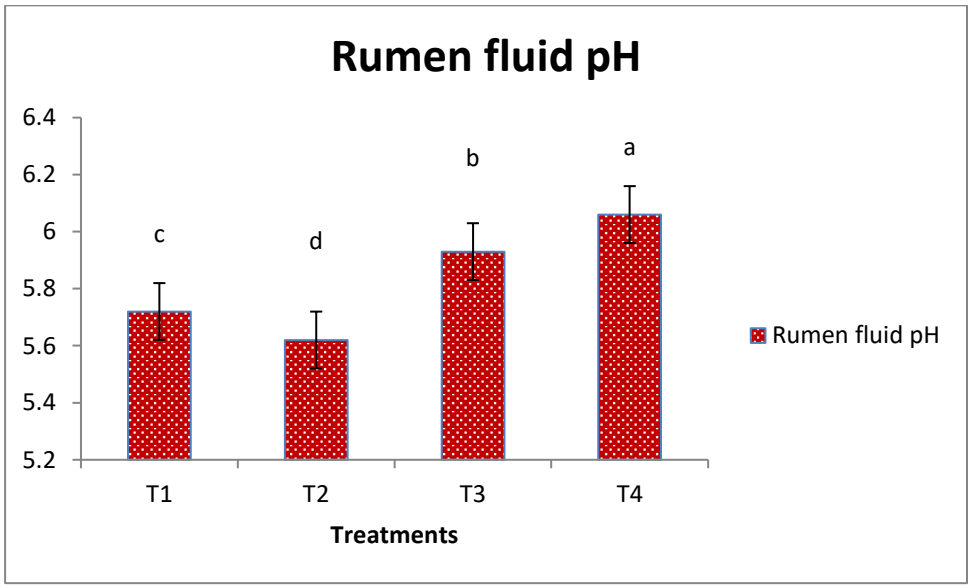


Figure 4.3 In-vitro rumen fluid pH at 24h incubation

*T1 = 300g concentrate mixture, T2 = 250 g concentrate mixture + 50g Dry neem foliage, T3 = 200g concentrate mixture + 100g Dry neem foliage, T4= 150g concentrate mixture + 150g Dry neem foliage.

4.10 Dry Matter and Organic Matter and ash digestibility

There was no significant difference on DM, OM and ash digestibility in among the different dietary supplements but tended to highest DM and OM digestibility was observed in using supplements used 50 to 100gm dry neem leaves. Similarly, the ash digestibility was higher 94.75 % than DM and OM digestibility for all treatments (Table 4.3).

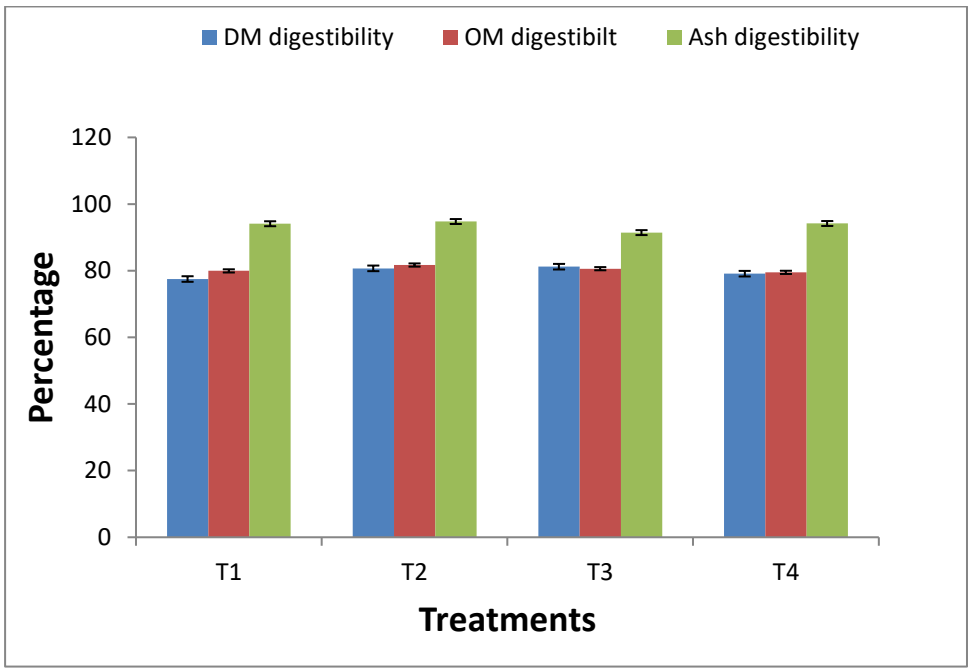


Figure 4.4 Invitro digetibility of dietary treatments using different level of neem leaves

*T1 = 300g concentrate mixture, T2 = 250 g concentrate mixture + 50g Dry neem foliage, T3 = 200 g concentrate mixture + 100g Dry neem foliage,T4= 150g concentrate mixture + 150g Dry neem foliage.

CHAPTR FIVE

DISCUSSIONS

5. 1. Chemical composition of Neem foliage, leaf and stems

In other study Moisture, Ash, CP, CF of neem plant shows result 14.30 ± 0.02%, 4.03 ± 0.10%, 1.22 ± 0.22%, 10.86 ± 0.11% (A.Otache.*et al.*,2017) which are much lower than the value we found. These values are also lower than the values of 13.42% protein, 11.93% ash and 5.7% fiber recorded in other study (Atangwho *et al*., 2009). The dry matter composition of the neem leaves was lower than the value 93.50% reported by Paengkoum (2010). Raghuvansi *et al.,* (2007) reported a dry matter content of 95.16% for neem leaves which were higher than the current study. The difference in dry matter content can be attributed to method of drying the leaves and stage of harvesting the leaves. The dry matter content of neem leaves in the present study compares favourably with other tree leaves and browses. The high dry matter content of neem leaves makes it a good source of feed for animals in the dry season. The high dry matter of the diet will help the animal to take in more of the diet which will lead to nutrient intake. This will also lead to increase in productivity.

The CP content of the Neem leaves was higher than the 9.7% stated by Ramana *et al*., (2000) but lower to the values of 20.9% found by Ogbuewu *et al*., (2011). The variation in CP values can be attributed to changes in the variety of the neem plant. High percentage of CP in the Neem foliage diet means there will be additional protein made available to the rumen microbes which should lead to increase in digestibility of the feed resulting in increase in productivity especially when fed to sheep in the dry season. The Ash content of neem leaves was 8.81% to 9.82 % which were almost comparable to the value of 7.1% reported by Ogbuewu *et al*., (2011). High ash content is an indication of high concentration of minerals (Kwabiah *et al*., 2003). Therefore, the variation of ash content might be due to the composition of soil in which the plants grew. The higher level of ash in the neem foliage means higher amount minerals will be accessible to the sheep which will help improve productivity of the sheep.

5.2 Growth performance and Feed conversion Efficiency of Sheep

The initial and final body weight of the sheep was almost similar. Sheep fed on T2 and T3 (50g and 100 g Neem foliage) diet had higher daily weight gains than those on control and T4 (150g) diet. The higher daily body weight gain among the treatments T2 and T3 might be due to the differences in daily DM, and CP intake as well as DM and CP digestibility between treatment groups. Despite neem leaves superior in nutritional quality, weight gain trends do not follow the increasing proportion of neem leaves in supplementary feed (T4) and this might be associated with due to higher amount of fibre content and lower amount CP content in T4 which might be not apt for the activity of rumen microorganisms.

Significant difference of FCR for Treatments 2 and 3 followed by Treatments 1 and 4 can be attributed to the numerically higher digestibility of neem foliage (Table 4.2) due to the lower amount of parasites in rumen for sheep on that treatment. Also the neem foliage could have improved the rumen environment which helped in quicker digestion of diet for sheep on Treatment 2 and Treatment 3 which helped them make better use of their diet.

5.3 Faecal egg Counts (FEC) and FAMACHA estimations

The mean FEC of the Neem treated diet almost the same all of the group until day 14, after that the EPG decreased rapidly in Neem treated group compared to those fed control diet. Week 4th on wards the e.p.g in neem treated group decreased gradually lowest value was obtained in week 8 which was ranges 500 to 600. A study conducted by Khadijah *et al.,* (2005) and Wong *et al.,* (2005) on the use of fresh Neem, and pelleted Neem, observed no significant difference in faecal egg counts, compared with control sheep, although the control sheep had higher mean faecal egg counts.

5.4 Carcass characteristics, internal and lymphoid organs of Sheep fed different dietary treatments

In the present study the sheep fed dietary supplements using 50 to 100mg dry neem foliage had significantly ($P<0.05$) higher hot carcass percentage and numerically higher breast yield compare to those fed other diets. This observation is comparable to Faji Dida *et al.,* (2019) observed that supplementation of sole neem leaves had higher dressing percentage than sole pigeon pea and mixture of neem leaves and pigeon pea.

The internal organs (liver, kidney, spleen) percentage did not differ (P>0.05) among the dietary treatments of the present study is similar to many researcher findings (Ahamefule *et al.*, 2006; Assefa., Kijora, Kehaliew, Bediye & Peters,2008 and Freweini, 2014) who observed liver was not affected by neem leaves, which could be an indicative of low level of anti-nutritional compounds in the leaves used in the current study that could otherwise have demanded the liver to grow to undertake detoxification.

5.5 Effect of neem leaves on meat quality

Water-holding capacity (WHC) of meat is the important tool for evaluating meat quality for both the industry and purchaser (Modzelewska-Kapituła *et al*., 2015). The heme pigment and soluble flavour component of meat could be loss during loss of moisture from meat (Savage *et al*., 1990; Luciano *et al*., 2009). In the current study, dietary supplementation of neem leaves decreased drip loss and cook loss compared to control this result might be due to the phenolic compound presence in neem leaves. Demiray et al. (2009) stated that the neem is medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents usually associated to wide range of polyphenolic compounds.

5.6 Influence of dietary inclusion of neem leaf meal on in-vitro digestibility

The sheep fed 50 to 100g neem foliage tended to highest DM and OM digestibility compared to control and higher dose (150mg) neem leaves. The high dry matter digestibility of the diets fed to sheep in the current study could be attributed to the ability of the neem supplements to have had an influence on the rumen microbes to increase digestion. Other studies have indicated significant increase in dry matter digestibility between goats fed graded levels of cassava leaf meal with corn bran based diet (Yousuf *et al*., 2007).

CHAPTER SIX

CONCLUSION & RECOMMENDATION

6.1 Conclusion

From the aforesaid results and discussion, body weight gain and carcass parameters values in the present finding outlined that, neem leaf as sole supplement is comparable to the supplementary value of concentrate mixture to improve sheep performance. The addition of neem leaf on diet of sheep found positive effect on growth performance and increased digestibility and dress percentage when dry neem foliage was used 50g to 100g. In addition to, it was observed that neem leaf also has positive effect on reducing parasitic load of sheep. The average daily weight gain was higher in neem fed group (T1&T2) compared to control group. FCR value also indicated positive impact on neem fed group. Moreover, dietary supplementation of neem leaves at a level 50gm to 100gm decreased drip loss and cook loss compared to control this result might be due to the phenolic compound presence in neem leaves. Supplements containing up to 30% (100gm) Neem leaf meal can be fed to sheep without deleteriously affecting their dry matter intake, digestibility and meat quality and to reduce feed cost and increase of net return. However further study is recommended with large number of sheep to make final conclusion on different cultivars and times of harvesting of Neem leaves to determine whether the results reported in this investigation are consistent.

6.2 Recommendation

1. It can be recommend that supplement containing up to 30% (100g) neem leaf meal can be used to feed sheep in the dry season.
2. Further research should be conducted to determine the effect of neem leaf meal on rumen microbial population and intestinal specific helminthic.
3. Further research should also be conducted to determine the effect of neem leaf meal on detail of meat quality.
4. In addition, the determination of the effect of neem leaf meal on the biochemical parameters of ruminants is being suggested.

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