DETOXIFICATION OF TANNINS FROM *ACACIA NILOTICA* PODS ON *IN VITRO* NUTRIENT DIGESTIBILITY AND MILK PRODUCTION IN LACTATING GOATS

THESIS SUBMITTED TO THE
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FOR THE AWARD OF THE DEGREE OF

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IN
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(ANIMAL NUTRITION)

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APPROVED BY

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This is to certify that the thesis entitled, "**DETOXIFICATION OF TANNINS FROM *ACACIA NILOTICA* PODS ON *IN VITRO* NUTRIENT DIGESTIBILITY AND MILK PRODUCTION IN LACTATING GOATS**" submitted by **MERGA BAYSSA** towards the partial fulfillment of the award of the degree of **MASTER OF SCIENCE (DAIRYING) in ANIMAL NUTRITION** of the **NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY),** Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

Dated: **June 13, 2006**

S.N. Rai
13.6.06

(S.N. RAI)
Major Advisor & Guide

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ABSTRACT

Acacia nilotica (babul) pods, having high tannins, inhibit the utilization of nutrients in the diet. To inactivate tannins these were treated with 3% calcium hydroxide (CH) and polyethylene glycol (PEG-4000) from 0.05 to 0.25 PEG: tannin ratio and evaluated for chemical composition, *in vitro* nutrients digestibility and partioning and degradation of tannins. Based on results of *in vitro* experiment, untreated and treated babul pods based total mixed ration (TMR) were fed to lactating goats to study their nutrients utilization and milk production performance.

Treatment of babul pods with CH as well as PEG alone improved IVOMD, and tannin degradation. However, combination of 3% CH and PEG at 0.10 levels was the best. Correlations between IVDMD and IVGP as well as tannin degradation and IVDMD were significantly positive ($P<0.05$), however, correlation between IVDMD and tannin content of babul pods was negative.

Control (I), untreated (II), 3% CH treated (III), and 3% CH+ PEG(0.10 PEG:Tannin ratio) treated (IV) babul pods containing TMR (R: C, 50:50) were fed to the lactating goats divided in to 4 groups of 6 each for a period of 112 days. DM intake (kg/d) was higher ($P<0.05$) in group I than in other groups. However, intake of CP, DCP, and TDN remained similar in all groups. Nutrients digestibility (CP, EE and HC) were higher ($P<0.05$) in group IV than in control. However, digestibility of CF and NDF were higher ($P<0.05$) in control than in other groups.

Balances of N, Ca and P were found positive in all the groups and N and Ca balances were higher ($P<0.05$) in group I than those in groups II and III. P balance remained similar in all groups. Milk yield and milk composition were similar in all groups during the experimental period of 112 days. Yields of milk fat, SNF and total solid were higher ($P<0.05$) in group IV than in other groups. Feed conversion efficiency and milk energy yield were significantly higher in group IV than in other groups.

It was concluded that 22% babul pods (tannins, 4%) can be effectively incorporated in the ration of lactating goats. In case of high tannins (tannins 6%) containing diets, these may however, be treated with 3% CH (cheaper than PEG) or PEG, to have better response of the diet.

सारांश

पशु आहार में बबूल (अकेशिया निलोटिका) दिम्बियां सम्मिलित करने से चयायचयन टेनिन स्तर उच्च के कारण दुष्प्रभावित होता है। टेनिन निराकरण हेतु इनका 3% चूना एंव पोलीइथाइलिन ग्लायकोल (पीईजी,400) 0.05 से 0.25 पीईजीःटेनिन अनुपात तथा उपचार करके रासायनिक सद्यंटन, अन्तपात्र शुष्क प्रदार्थ पाचकता (अशुप), टेनिन का निमतिकरण का ऑकलन किया गया। अन्तपात्र परीक्षण के परिणामों के आधार पर अश्पचारित व उपचरित बबूल दिम्बियां आधारित दूधारू बकरियों के सम्पूर्ण आहार में समावेश करने का उनके पोषक तत्वों की पाचकता व दूध उत्पादन पर प्रभाव का परीक्षण किया गया ।

केवल चूना अथवा पीईजी के उपचार से बबूल दिम्बियां का अशुप व टेनिन निमतिकरण बढ़ा किन्तु चूना व पीईजी (0.10) सम्मिलित उपचार सर्वाधिक था। अशुप व अन्तः पात्र गैस उत्पादन तथा टेनिन निमतिकरण व अशुप में परस्पर धनात्मक ($P<0.05$) सम्बध पाया गया जबकि अशुप व टेनिन स्तर ऋणात्मक सम्बध दर्शाया ।

नियन्त्रक (ए), अनुपचारित (II), 3% चूना उपचारित (III) व 3% चूना . पीईजी (IV) उनचारित बबूल दिम्बियों को सम्पूर्ण आहार (चाराःदाना 50:50) में समावेशित करके दूधारू बकरियों को चार वर्गो को 112 दिन तक खिलाया गया। वर्ग 1 में शुष्क प्रदार्थ अर्न्तग्रहण अन्य वर्गो की अपेक्षा अधिक ($P<0.05$) था, किन्तु क्रूड़ प्रोटिन, पचकीय क्रूड़ प्रोटिन व सकल पचकीय पोषकतत्वों को अर्न्तग्रहण सभी वर्गो में समान था। पोषकतत्वों (क्रूड़ प्रोटिन, वसा, हैमीसैल्यूलोस) की पाचकता वर्ग में नियन्त्रक वर्ग की अपेक्षा अधिक ($P<0.05$) थी। यद्यपि क्रूड़ रेशो व एनजिएक की पाचकता नियन्त्रक वर्ग में अन्य वर्गो की अपेक्षा अधिक थी।

सभी वर्गो में नाइट्रोजन, कैलशियम व फास्फोरस के शेष धनात्मक थे । नाइट्रोजन व कैलशियम शेष वर्ग I में वर्ग II व III की अपेक्षा अधिक ($P<0.05$) थें जबकि फास्फोरस शेष सभी वर्गो में समान थें। 112 दिन की परीक्षण अवधि में दूध वसा व दूध सधटंन सभी वर्गो में समान पाया गया, किन्तु दूध वसा एस.एन.एफ. व कुल ठोस प्रदार्थ वर्ग IV में अन्य वर्गो की अपेक्षा अधिक ($P<0.05$) था। वर्ग IV में योग्य दक्षता व दूध उर्च्व उत्पादन अन्य वर्गो की तुलना में सार्थक रूप में अधिक पायी गयी।

प्राप्त परिणामों से निष्कर्श निकाला गया कि दूधारू बकरियों के आहार में 22% बबूल दिम्बियां (टेनिन 4%) समावेशित की जा सकती है। टेनिन की अधिकता होन (4%) की स्थिति में इनका 3% चूना अथवा पीईजी द्वारा समान रूप से प्रभावी उपचार किया जाना चाहिए। पीईजी की तुलना में चूना उपचार की लागत अपेक्षाकृत निम्न पायी गयी ।

CONTENTS

ABBREVIATIONS

°C	---	Degree Centigrade
ADF	---	Acid Detergent Fibre
BP	---	Babul pods
CD	---	Crude Protein
CT	---	Condensed Tannin
DM	---	Dry Matter
EAA	---	Essential Amino Acids
EE	---	Ether Extract
Eg	---	Example
g	---	Gram
H_2SO_4	---	Sulfuric Acid
HC	---	Hemicellulose
HCI	---	Hydrochloric Acid
HT	---	Hydrolysable Tannin
IVCPD	---	*In vitro* crude protein digestibility
IVDMD	---	*In vitro* Dry Matter digestibility
IVGP	....	*In vitro* Gas production
IVOMD	---	*In vitro* organic matter digestibility
Kg	---	Kilogram
ME	---	Metabolisable Energy
µg	---	Micro gram
mg	---	Milli gram
N	---	Nitrogen
Na_2CO_3	---	Sodium Carbonate

NDF	---	Neutral detergent fibre
NH_3	---	Ammonia
O_2	---	Oxygen
OM	---	Organic Matter
PEG	---	Polyethylene glycol
PVP	---	Polyvenyl pyrrolidine
PVPP	---	Polyvenyl poly pyrrolidine
SE	---	Stand error
SE_m	---	Standard error of mean
TAsh	---	Total Ash
TD	---	Tannin Degradation
TP	---	Total Phenol
TT	---	Total Tannin

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1. INTRODUCTION

A high demand for food and restricted access of poor farmers to external inputs leaves the production of animal feed often at low priority in developing countries. Hence, feed shortage is the chronic problem in tropics and subtropics where the major feed supply is based on crop residues and poor quality native grasses which have low nitrogen and high fiber content. Many legume fodders trees and shrubs have high protein and energy contents in various edible parts and potentially promising supplements to overcome nutrient deficiencies of animals (Tolera *et al,* 2001).

The utilization of these feed resources by livestock brings economic benefits to the farmers as well as improvement in soil fertility and control of soil erosion. In general, neither multipurpose trees nor non-conventional agro industrial by-products can be considered as human food and hence may be diverted to animal use. However, the majority of these feed resources have one or the other antinutritional factors, which adversely affect the performance of animals when fed as such, particularly at higher levels in the diets for long periods (Ben Salem *et al, 2002;* Getachew *et al,* 2002; Barman and Rai, 2004).

Tannins are among these factors abundantly found in many fodders tree leaves, browse plants and agro industrial by-products. Tannins are water soluble natural occurring poly phenolic compound of various molecular weights and various complexity which differ from other natural phenolic compounds in their ability to precipitate proteins from solutions and to a lesser extent metallic ions, polysacharides and other compounds (Haslam,1981; Haggerman,2004).

Based on their structure and reactivity tannins are mainly grouped in to condensed and hydrolysable tannins. Condensed tannins are Oligomeric and polymeric flavonoid units which are less susceptible to acidic and enzymatic hydrolysis (Hagerman and Buttler, 1981). The precursor monomers are flavan-3-ols, where the position 3 on the ring is hyroxylated.

During heating and in the presence of acids, the interflavan bonds are cleaved by oxidation and anthocyandins are formed (Porter *et al,* 1986). Thus condensed tannins are called proanthocyandins.

Hydrolysable tannins are gallic acid and ellagic acid esters of sugar and catechin core molecules. They yield gallic acid or ellagic acid and sugar (usually glucose) by hydrolysis (Haslam, 1981; Muller-Harvey *et al,* 1992). Babul pods (*Acacia nilotica*) is one of tanniniferous unconventional feeds resources which is abundantly found in tropics and subtropics. It contains about 14% CP and 74% TDN.

Tannins have both beneficial and adverse effects (Min *et al*, 2005; Barman and Rai, 2004; Makkar, 2003a). The presence of tannins in diets at less than 4% (on DM basis) is advantageous to protect dietary proteins from excessive rumen degradation and enhance amino acid absorption and utilization by the ruminants (Waghorn *et al,* 1987; Barman and Rai, 2004), reduce occurrence of pasture bloat (Jones *et al*, 1976; Barry and McNabb,1999; Puchala *et al,*2005),reduce methane production in goats (Puchala *et al* ,2005) and have antiheliminthic property in goat (Molan *et al,*2000; Nguyen *et al,*2005).

However, tannins at greater than 4% in diet may have deleterious effect on feed utilization by reducing palatability and intake (Waghorn *et al*, 1994) nutrient digestibility (Rai and Shukla, 1977; Priolo *et al,* 2002; Barman and Rai, 2004) and animal production (Kumar and Vaithiyanthan, 1990; Reed, 1995; Rubanza *et al,* 2002; Makkar, 2003). Tannins reduce

the digestibility of nutrient either by binding the nutrients directly or by inhibiting the action of digestive enzymes of both microbial and animal origins.

Negative nitrogen balance was observed when Sal seed meal was incorporated in to concentrate mixture of lactating cattle (Rai and Shukla, 1977). Feeding quabracho tannin at the level of 50gm/kg basal diets in sheep reduced ruminal ammonia nitrogen, total volatile fatty acids, cellulase and xylanase enzyme activity (Salawu *et al,*1999).

Tannins reduce not only the digestion of proteins but also the digestion of carbohydrates, availability of minerals (Rhadikrishnan and Sivaprasad, 1980), vitamins (Rung ranangsak *et al,* 1977) and blood level of hormones (Barry and Manley, 1986). Higher level of tannins may also cause toxic damages to internal organs of animal system (Reed, 1995; Dawnson *et al*, 1999; Brooker *et al,* 2000).

In addition tannins are toxic to several rumen microbial species such as S*tereptococcus bovis, Butyrivibirio fibrisolvens, Fibrobacter succinogens, provetella rumenocolla, Ruminobacter amylophilus* (Mcsweeny *et al*,2001).

But a number of tannin tolerant rumen microbial species have been isolated from wild and domestic ruminants feeding on tanniniferous feeds (Brooker *et al,* 1994; Nelson *et al,* 1995; Nemato *et al,* 1995; Wriyanwan *et al,* 2001). This ability of gastro intestinal microbes to tolerate and detoxify tannins may explain why some animals can tolerate much higher diet levels of tannins than others. Barman and Rai (2004) found that goat is more resistant to tanninferous feed than cow and buffaloes.

Efforts have been made to improve the utilization of agro industrial by-products and unconventional feed resources for ruminants by reducing the tannin levels through physical treatments (Bensalem *et al,*2005; Vitti

et al,2005), chemical treatments (Getachew et al,2001; Makkar,2003; Berman and Rai,2004; Alem *et al,*2005; Bensalem *et al,*2005; Aemiro and Rai,2006; Dubey and Rai,2006), and biological treatments (Brooker *et al,*2000; Wriyanwan *et al*,2001; Makkar,2003; Aemiro and Rai,2004).

The most widely studied technique is the addition of PEG which can bind tannins and reduce its negative effects (Barry and McNabb, 1999; Priolo *et al*, 2000; viallaba *et al*, 2002). Although incorporation of PEG is effective, success of its adoption by farmers and feed industries depends on cost-benefit ratio. The use of calcium hydroxide as tannin antidote is relatively cheaper and effective in improving the utilization of various tanniniferous feed materials. The chemical is also easily available in local market (Aemiro and Rai, 2002).

Limited information is available on utilization of tanniniferous feeds by lactating goats with reference to efficiency of nutrient utilization, the response of chemical treatments of tanniniferous feeds on ruminant performance, the threshold level of tannins in lactating goats and the performance of goats on tanniniferous feeds in terms of milk production and composition. Such information is valuable in formulation of feeding strategies for dairy goats. Hence, the present study was designed to investigate:

1. The effect of chemical treatments on tannin inactivation and *in vitro* nutrient digestibility of *Acacia nilotica* pods.

2. The influence of treatment of Acacia *nilotica* pods on feed utilization, milk production and balances of Nitrogen, Calcium and Phosphorus balances in lactating goats.

2. REVIEW OF LITREATURE

Animal production system in the tropical and subtropical countries utilize a wide range of feed stuffs. This includes crop residues, industrial by-products, natural grasses, legume trees and shrubs. These trees and shrubs are of great importance in livestock production system because they can provide substantial protein and energy, especially during dry season of the year. But majority of these feed resources contains variable amounts of incriminating factors, which includes tannins.

The effect of tannins on animals ranges from beneficial to toxicity and even death depending on the type of animals, age of animals, type and level of tannins in the feeds, the biological activity of tannins, level of tannin intake, quality of basal diets etc. (Reed, 1995; Makkar, 2003).

Therefore, a better understanding of tannins properties, metabolism and its influence on efficiency of nutrients utilization in ruminant ration is of utmost importance. Hence, proper feeding management practices may be important to utilize these feed resources for strategic supplementation.

In this study attempt has been made to review the literature pertaining to plant tannins under the following headings:

2.1 Tannin contents of feed and fodders

2.2 Adaptation to feed tannins

2.3 Role of tannins in ruminant nutrition

2.3.1 Beneficial effects of tannins

2.3.1.1Effect on nitrogenous metabolism in ruminants

2.3.1.2 Effect on milk yield and composition

2.3.1.3 Tannins and immune systems of animals

2.3.1.4 Effect on reproduction and growth

2.3.1.5 Effects on body weight changes

2.3.1.6 Effects on bloats and parasitic infestations

2.3.2 Adverse effects of feed tannins

2.3.2.1 Effect on intake and nutrient digestibility

2.3.2.2 Effect on rumen microbial system

2.3.2.3 Mechanisms of tannins protein interaction

2.3.2.4 Effect on animal performances

2.4 Inactivation of tannins in feeds

2.5 Biodegradations of tannins in animal systems

Babul pods (*Acacia nilotica*)

The plant has many common names like Babul, Indian gum, Arabic tree, Thorn mimosa etc *Acacia* is a large genus comprising more than 1000 species belonging to the family Leguminosae, subfamily Mimosoideae. They are distributed in the warm and drier regions of the world mainly in the tropics and subtropics and are more prevalent in Australia and Africa. It is perennial shrub or tree, 2.5-20m tall, branches spreading, forming a dense flat or round crown with dark to black coloured stems and straight sharp spikes.

It can grow in dry regions and adapted to annual rain fall of 300-2,200mm, tolerates a wide range of soils types, thriving in alluvial and heavy clay soils with pH 5.0-9.0.

The plant has hard seed coats, leaves and pods generally well accepted by small ruminants. But cattle require prior experience or extended period of adaptation. Dried pods are relished by herbivores in the native range lands of Africa.

Leaves contains 2.2-2.6%N,16.9-20.0% NDF,13.3-14.1 ADF 7.2-8.7MJ/kg DM energy,10-21%CF and 6-12% condensed tannins.

Pods and seeds contain 1.6-2.2%N,10MJ/kg DM energy, 25% NDF, 17%ADF, 12-18% CF and 4-7%condensed tannins. Seeds contain crude protein 18.6%, ether extract 4.4%, fiber 10.1%, nitrogen-free extract 61.2%, ash 5.7%, silica 0.44%, phosphorus 0.29% and calcium 0.90% of DM. When bullocks were given the seeds and bran (2:1) with dry pasture grass daily DM intakes were 1.82, 0.91, and 5.35 kg respectively. Total DM intake/100 kg bodyweight was 1.40 kg. The animals retained 20.8 g N and 7.4 g Ca daily but the P balance was slightly negative (Pande et al, 1981). Walker (1980) reported CP 12.9%, and crude fiber 15.2% in Babul pods. The plant is abundantly found in India, Ethiopia and else where in the world. In addition of its feeding value for ruminants Babul pods and barks contain large quantity of gums for commercial purposes.

The annual availability of Babul pods in India is approximately 600,000 metric tons per year (Punji, 1988). The use of processed Babul seeds to 45% of the concentrate mixture did not affect the nutrient utilization in Kankrej bullocks (Pandey *et al.* 1981) but the use of Babul pods at 20 and 40% of TMR DMI the milk yield and fat composition were reduced but protein composition was increased in crossbred cow (Barman, 2004).

Acacia pods have traditional medicinal importance in different parts of Africa, for instance, Zulu tribe take bark for cough, Chipi tribe use root for tuberculosis, Masai are intoxicated by the bark and root decoction, said to impart courage, even aphrodisia, and the root is said to cure impotence. Astringent bark used for diarrhea, dysentery, and leprosy. Bruised leaves used to cure ulcers. According to Hartwell, the gum or bark is used for cancers and/or tumors (of ear, eye, or testicles) and indurations of liver and spleen, condylomas, and excess flesh. It can also be used for cancer, colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhea, ophthalmia, sclerosis, smallpox, and tuberculosis. Bark, gum, leaves, and pods used medicinally in West Africa. Sap or bark, leaves, and young pods are strongly astringent due to tannin, and are chewed in Senegal as antiscorbutic; in Ethiopia as lactogogue. Bark decoction drunk for intestinal pains and diarrhea. Other preparations used for coughs, gargle, toothache, ophthalmia, and syphilitic ulcers. In Tonga, the root is used to treat tuberculosis.

In Lebanon, the resin is mixed with orange-flower infusion for typhoid convalescence. Masai use the bark decoction as a nerve stimulant. In Italian Africa, the wood is used to treat smallpox. Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods (Duke, 1983a). Extracts are inhibitory to at least four species of pathogenic fungi (Umalkar et al, 1976).

Babul has been reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, acid, N-acetyldjenkolic acid, sulphoxides pentosan, saponin, tannin.

2.1 Tannin contents of feeds and fodders

Secondary plant compounds are thought to be produced as a defense mechanism against tissue invasion by microorganisms and

destruction by herbivore (insects, birds, and animals). Tannins are present in the leaves, stems, flowers, and seeds of plants (Douglas *et al.,* 1995; Aganga and Bessa, 1999)**.** Tannin content of feed are affected by many factors like species, genotype and stage of growth, parts of plant, season of growth and environmental factors such as temperature, rain fall, draught, cutting and defoliation by herbivores (Biswas *et al*., 1995; Bensalem *et al*; 2002; Singh, 2002). Further variation is also caused by different analytical techniques for tannin estimation and the method of tissue preparation for analysis. For example drying *calliandra* leaf by heat decreased both extractable and total condensed tannins by 27 and 21% respectively, but increased proportion of tannin bound to proteins and cell wall by 2.4 and 10.6 % (Perez-Maldano and Norton, 1996a). Tannin content of browses, tree leaves and pods etc of agro industrial by products has been presented in Table.....

Table: Tannin content of some browses, tree leaves and agro industrial by-products

Species of plant	**%Tannin (DM)**	**References**
Terminalia baleirica (L)	6.41	Lohan *et al.,*1980
Prosopis juliflora pods	0.76	Barman and Rai,2004
Salseed Meal (Shorea robusta)	8.39	Barman ,2004
Grewia retina (L)	8.7-12.70	Aganga *et al,*1999
Mangifera indica (s)	7.98	Barman ,2004
Acacia nilotica pods	18.71	Barman and Rai,2004
Acacia tortilis	12.10	Rubeniza *et al.,* 2003
Acacia polyacantha	9.50	Rubeniza *et al.,* 2003
Acacia albida	11.00	Getachew *et al,* 2001
Dismodium intortum	7.70	Getachew *et al,* 2000
Dismodium unicinatum	6.42	Getachew *et al,* 2000
Calliandra calothrysus	13.90	Getachew *et al*., 2002

2.2 Adaptation to tanniniferous feeds

Higher levels of tannins intake by animals can produce toxicity and death (Garg *et al,* 1992). Animals normally consuming tannin rich feeds appear to develop defensive mechanism against tannins. In some animals the salivary proline rich protein (PRP) are considered to be the first defense line against dietary tannins. In these animals, less protein is required to bind all the tannins resulting qualitative saving of nitrogen.

In addition, since PRP being rich in non essential than essential amino acids which are excreted and results in quantitative saving of nitrogen (Makkar, 2003). But no evidence was reported about the presence of PRP in the saliva of cattle, sheep and goats. However, other small proteins were known to present in the saliva of these animals consuming tannin rich feed and have high affinity to complex tannin. However, the primary role of these salivary proteins did not appear to neutralize dietary tannins (Makkar *et al*, 1997). Micro organisms resistant to high level of condensed tannin have been reported to be present in ruminants consuming high levels of tannin containing feeds (Nelson *et al,* 1999; Brooker *et al,* 2000).These microbes might, however, have some adaptive mechanisms which enable them to degrade hydrolysable tannins faster (Odenyo *et al*, 1999) or decrease the activity of hydrolysable tannin through methylation of the phenolic hydroxyl groups.

The adaptive mechanisms of rumen microbes to high levels of tannin in the feed might give protection of key membrane by strategic deployment of lipids (Pell et al, 1999), secretion of extra cellular polysaccharides (Brooker and Odenovo, 2000; Denis *et al*, 2005) which have affinity to bind tannins and or through formation of thick high tannin affinity glycoprotein (Mcsweeny *et al*, 2001; Krause *et al,* 2005) and thus prevent tannin to cause adverse effects on rumen microbes.

Feral goats and camels fed on acacia spp and *calliandra callothyrsus*, which have high level of tannins, were capable of tolerating tannin in the diets due to presence of high populations of tannin resistant bacteria like *Streptococcus caprinus* and S*elenomonas ruminantum* K2 (Tjakrandidjaja, 1999).

In addition, this higher ability of goats to tolerate condensed tannin to extent might be due to their higher urea recycling capacity per day and higher salivary secretion capacity (Coccimano and Leng, 1963; Brooker *et al*, 1994)

2.3 Role tannins in ruminant nutrition

2.3.1 Beneficial effect of tannins

2.3.1.1 Effects on dietary proteins in the rumen

The major benefits of tannins in feed are protection of dietary protein from excessive degradation in the rumen making it available for digestion and utilization in the lower digestive tracts (Waghorn, 1990; Norton, 1999).

Barry and McNabb (1999) reported that the ideal concentration of condensed tannins in forage legumes to protect the proteins from rumen microbial attack was 20-40g/kgDM.

Norton and Ahn (1997) have demonstrated that tannins in Calliandra calothrysus (2.5 to 3.7%CT) protected protein degradation in the rumen and increase flow of nitrogen to small intestine with out net gain in nitrogen retention. The efficiency of absorption from small intestine decreased from 58 to 50% when PEG was added to the diet, suggesting that tannins in some way enhanced absorption and utilization of nitrogen in small intestine. Harves et al (1999) reported that quabracho tannins at a rate of 15g/100g of soybean meal (SBM) can be used as chemical

additive to decrease rumen degradation of SBM in sheep with out affecting intestinal digestibility. The presence of CTs in legumes can reduce protein degradation in the rumen due to stable CT: protein complex formation at pH 4.0 to 7.0 in rumen that subsequently released protein in the acidic condition of the abomasum. This enables proteins to by-pass degradation in the rumen and undergoes enzymatic hydrolysis in the abomasum and absorbed from small intestine (McNabb et al, 1998; Min et al, 2000, 2002, 2003). Further more tannins form complexes with surfaces of bacterial cell wall and with bacterial enzymes which alter the bacterial growth and reduce proteolytic enzymes activities (Jones et al, 1993).

Min et al (2005) reported that CT tannins from Lotus pendiculatus at 200, 400 and 600mg /ml reduced the growth rate and optical density of most proteolytic bacterial strains compared to controls, and concluded that CTs from lotus the rate of proteolysis inhibited the growth of rumen microbes.

2.3.1.2 Effect on milk yield and composition

Rai and Shukla (1979) noticed that non significant increase in milk production in lactating cows fed on 10 percent salseed meal (11% tannic acid) in concentrate mixture, which reduced the feed cost to the extent of Rs70.4/tons as compared to control diet. Errante et al (1998) found that increased milk yield in cows was noticed due to feeding of farmantan @ 120g/day. Similarily, Barry and McNabb (1999) reported CT in L.carniculatus at 30-40g/kgDM increased EAA absorption from small intestine and there by increased milk production, wool growth and reproductive rate without affecting voluntary feed intake.

Wood ward et al (1999) reported dietary forges containing (20 to40g CT/kgDM) resulted in increase milk protein by 40% in dairy cow and sheep. Barman and Rai (2004) noticed increase in milk protein by 6% at

20% and 7% at 40% inclusion of Babul pods (18.01%tannins) in crossbred cows but milk yield and milk fat contents were reduced.

2.3.1.3 Effect on reproduction and wool growth

Min et al (1999) reported that ewes grazing on L.carniculatus containing 17gCT per kg DM without PEG supplementation increased the plasma concentration of branched chain amino acids by 57% and essential amino acids by 52% as well as resulted in increased production of wool and increased reproductive efficiency.

Ramirez-restrepo et al (2005) reported that mating sheep on L.carniculatus (18-29 g CT /kg DM) increased lambing percentage and weaning percentage of lambs per ewe by 16% and 32% respectively due to more multiple and less single births and reduced lamb mortality .They noticed increasing days of grazing on the L.carniculatus before ovulation (0,10,21 and 42 days) linearly increased ovulation rate ,number of lambs weaned ,wool growth and fiber length. They speculated an increase in efficiency of reproduction is because of decreased degradation of soluble proteins of L.corniculatus by CT, thereby reduced the plasma ammonia and urea concentrations which also reduced uterine toxic ammonia and urea concentration and improved uterine micro environment at the time of conception, implantation and early fetal growth.

2.3.1.4 Effect on body weight gain

Farmantan, a natural extract from Chestnuts tannins improved body weight gain and feed conversion efficiency in lactating cow (Dumanovski and sotoseki, 1998). Similarly, Errante et al (1999) reported farmantan at 120 g/day increased daily weight gain and feed conversion efficiency by 33% and 7%, respectively in dairy cow.

Feeding low level of *Acacia albida* pods (100 g/day) to sheep on oil seed cake enhanced weight gain (Nsahlai et al, 1999). Similarly,

Bensalem et al (2002b) reported that supplementation of lambs feeding on spineless cactus pods with 100 g of air-dried Acacia leaves and SBM increased growth rate to 102 g/d from 75 g/d of control diet.

Wriyanwan et al (2001) found increased *in vitro* DM digestibility of Calliandra when inoculated with different levels of tannin degrading bacterial inoculums and found higher IVDMD with 10^8cfu/ml-inoculum dose. Inoculation of these bacteria to unadapted animals fed on 100% Calliandra @ $3x10^{11}$cfu/ml resulted in reduction of body weight in the first 20 days of inoculation and there after body weight increased at a higher rate than control. However, inoculated goats adapted Calliandra feeding faster than non-inoculated goats.

2.3.1.5 Effect on bloat and parasitic infestation

Barry and McNabb (1999) reported that feeding diets containing 5 g CT per kg DM reduced the frothy bloat in cattle and intestinal parasitic infestation in sheep. At 15-20 mg Quabracho CT /g DM of wheat pasture, the rate of ruminal gas production and methane gas production were reduced by 33% and 23% respectively in crossbred steer (Min et al, 2005).

Ten mg CT /gm DM avoid pasture bloat in sheep (Waghorn, 1990; McAllister et al, 2002). Puchala et al (2005) reported CT in *Sericea lespedza* at 17.7% reduced bloat incidence in Angora goats as compared to tall fuscue at 0.5% CT .The methane production per se by the goats were lower for *Sericea lespeza* (7.4 g/d) than for tall fescue (10.6 g/d) and methane production relative to DMI was 6.9 g/d for sericea as compared to tall fascue16.2 g/d.

Molan *et al (*2000) demonstrated that condensed tannin extracts from *Lotus* spp reduced the rate of larval growth of parasites by 91%,

number of eggs hatching by 34% and decreased the mobility of L3 larvae by 30%.

Kahn and Diaz-Hernandez (2000) reported CT extracted from *Lucaenea* spp at 6 to 12 mg/ml was most effective in reducing migrating L_3 larvae in sheep and goats. They also noticed that the development of nematodes to L3 larvae reduced by 40 to 70% in the presence of *A.anuera* and *A.saligna* in sheep and goats. Sokerya and Rogeriquerz (2001) and sokerya and Preston (2003) showed that EPG counts in goats feed on cassava declined from 4000-5000 to 1500 EPG in the first 30 days of the experiment. Nguyen *et al (*2003) reported feeding jackfruits, cassava leaves and *Lucaenea* leaves to goats have reduced the infection rate of intestinal parasites and increase live weight gain in goats.

2.3.2 Adverse effect of tannins

Tannins play a significant role in the nutrition of animals, causing either beneficial or adverse effects on nutrient utilization, health and production .The beneficial and negative effects of tannins are based on the level in the feed and duration of feeding.

2.3.2.1 Effect on intake and nutrient digestibility

Tannins may reduce intake by decreasing palatability and by negatively affecting digestion. Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoprotein. Astringency may increase salivation and decrease palatability (Reed et al, 1995). Therefore low palatability depresses feed intake and, thus, animal productivity.

Tannins in *Lotus pendiculatus* (8%) reduced VFI in sheep by 27 **%** (Barry and Duncan, 1984). Waghorn *et al* (1994a) found that decrease in ruminal turnover and rate of digestion was the main cause of decrease in intake of sheep feeding on *L.pendiculatus*.

Tannins more than 4% reduce palatability, intake of DM, OMD and NDFD in ruminants (Bensalem *et al.,* 2002; Singh, 2002; Barman and Rai, 2004). Tannins at higher levels have negative linear relationship with nutrient digestibility (Thadi *et. al*., 2001; Getachew *et al* 2002; Rubanza *et al* 2003). Proanthocyandins are not absorbed through the digestive tract. Instead, free tannins and complexes forms remain in the rumen, decreasing protein and plant cell wall digestibility.

Several studies have shown that tannins decrease organic matter and fiber digestion. The lower digestibility is the result of the interaction of tannins with cellulase enzymes and rumen bacteria (Barry and manely, 1984; Barry, 1986; Reed, 1995). In some cases, lower fiber digestibility can be the result of a shortage of ruminal-fermented nitrogen due to tannin-protein complexes.

Field drying and treatments with PEG are able to limit these negative effects. Barry and McNabb (1999) stated that the high tannin concentration in *Lotus pendiculatus* (95-105g/kgDM) reduced rumen digestion of readily fermentable carbohydrates (sugar, pectin) and hemicelluloses.

In *in vivo* studies, protein digestibility is greatly reduced when tanniniferous feeds are part of the diet. Plants high in proanthocyandins often have proteins linked tightly to the plant cell wall (neutral-detergent insoluble nitrogen, NDIN) and lignin (acid detergent lignin, ADL) components, and, thus, may show negative digestion coefficients when ingested (Reed *et al,*1990;Wiengard *et al*,1995). After ingestion, PAs may also form detergent insoluble tannin-protein complexes with proteins they encounter. These two factors may cause the amount of NDIN and ADL excreted in the feces to exceed the amount ingested. When dietary content of tannins increases, faecal nitrogen excretion increases due to lower digestibility of nitrogen fractions and formation of tannin-protein complexes (Reed, 1995 Rubanza *et al,* 2003).

Feeding deoiled Sal seed meal containing about 11% tannins at a level of 20% in total ration of lactating cow reduced the digestibility of proteins and increased the ether extract digestion. Nitrogen retention was reduced and caused negative nitrogen balance (Rai and Shukla, 1977)

Singh (1979) also did not find effects of Sal seed meal on digestibility of nutrients at 30% level in lactating buffaloes. But he found that inclusion of treated (0.1% NaOH,3% tannins) and untreated Sal seed meal (5.75% tannin) at the level of 45 percent in the concentrate mixture of lactating buffaloes reduced dry matter intake and digestibility of DM,CP,CF,NFE than control. Ether extract was higher in both treated and control groups. He showed treatment of Sal seed meal with 0.1% NaOH improved the nitrogen balance.

Feeding *Quercus caliprious* leaves with PEG to goats increased the digestibility of CP intake (Silankove *et al,* 1994). Addition of PEG -6000 into 0.6 gm/40ml of rumen liquor containing 0.5 gm of tannin rich feed .Gas production was increased significantly.

Degen *et al* (1995) found negative nitrogen balance as well as digestibility of NDF, ADF and ADL when *Acacia saligina* leaves fed to sheep and goats. This is because tannin protein complexes bind NDF, ADF and ADL, there by increased their amount in faces than intake level.

Experimental dosing of merino sheep intrarumially with different dose levels of quebracho tannin extract (0,28,83, and 168 g/kg DM) containing 78 g condensed tannin per kg live weight for 21 days showed that animals taking 168g/kg DM tannin extract developed sign of toxicity on day ten of the experiment. This *in situ* data showed that these animals did not have any rumen fermentation activity at day 9. The experiment revealed daily administration of 28g/kg DM of the extract did not affect rumen fermentation parameters but 83g/kg DM had negative impact on parameters such as CPD, in situ DM, NDF, ADF disappreance from barley straw. IVDMD from all the three and cumulative gas production from

alfalfa hay and *Erica arborea* were negatively affected when three diets were offered to all the groups (Hervas *et al,* 2005). They found that the highest dose of quebracho tannin extract was toxic to rumen microbial population.

Increasing level of condensed tannin in the diet (0-2.3%) decreased nitrogen digestibility but had no significant effect on feed intake, organic matter or neutral detergent fiber digestibility (Perz-Maldodo and Norton, 1996).

Digestion coefficient of nutrients significantly reduced ($p<0.01$) with increasing level of Acacia nilotica pods (0, 5, 10 and 15 %) in the diets of goats (Sotohy *et al,* 1997). This is because the condensed tannin content the pods in this experiment was too high (320g/kg DM).

Tannin reduced transportation of amino acids across the brush border of membrane of intestine by binding proteins. Tannic acid concentration of 0, 0.5, 0.1, 0.25, 0.5, 1.0 and 2.5 per cent (w/v) and at PH 7.4 reduced ($p<0.05$) the transport of L-thereonine across the brush border membrane of duck intestine by 77 per cent compared to control. Under the sodium condition, the maximal inhibition of thereonine transport was 45.155 ($p<0.05$) (King *et al,* 2000).

Feed consumption and nutrient digestibility of tree leaves in goats were not affected by the presence of tannins at 3.4 and 5.5 per cent level. However, phosphorus balance was found to be negative while that of calcium and nitrogen was positive (Ahy and Kunji kutty, 2003). But in another study the ruminal ammonia concentration was reduced by 21 per cent when 0.25% hydrolysable tannin was used in Rusitech (Sliwinki *et al,* 2003).However, despite the decrease in apparent nitrogen digestibility, nitrogen retention does not always decrease with increasing tannin concentration in the diet. In many cases, nitrogen retention increases as a result of decreased urinary excretion.

If protein digestibility is not affected

by tannins, proteins behave as a uniform fraction, with a regression coefficient (true digestibility) equal to or larger than 0.88, with a negative intercept estimate of metabolic endogenous nitrogen, usually about 0.5% of dry matter intake or smaller) and with a low standard error (Thaedi et al, 2001). Hence**,** if tannins affect protein digestibility, the proteins will behave as a non-uniform fraction, with a regression coefficient (true digestibility) smaller than 0.88, and with a larger negative intercept and a higher standard error (Thaedi et al, 2001; Reed, 1995).

Tannin solubility plays a role in determining tannin's efficiency in binding proteins and/or fiber. If the ratio of soluble to insoluble tannins is high, then protein digestibility is affected more than fiber digestibility and if the same ratio is low, fiber digestibility is the most affected. Miller and Ehlke (1994) reported that CT concentration from birds' foot trefoil in the range of 27-85 g/kg DM can reduce rumen protein degradation.

Tannin reduced the bioavailability of divalent metallic ions (Bage palli *et al*, 1982). Inclusion of 45 % treated with 0.1% NaOH and untreated Sal seed meal containing 3.0 and 5.75 percent tannin in concentrate mixture of she buffaloes did not affect calcium and phosphorous balances but untreated sal seed meal significantly lower the calcium and phosphorous balance than control and treated groups (Singh, 1979). Rai and Shukla (1977) also did not find any significant effect on Ca and P balances when Sal seed meal fed to lactating cows at 10 and 20 per cent levels in the concentrate mixture.

Supplementation of *Acacia sieberina* pods @ 0,15,30 45 and 60 per cent in the diet of lambs increased the daily body weight up to a level 45 per cent incorporation (111,120, 149,165g/d). Beyond 45 per cent level the body weight gain was observed to decline. Feed intake was also increased up to 45% levels in the diet (Kibon and Mina, 1993).

2.3.2.2 Effect on rumen microbial system

Tannin toxicity to rumen microorganisms has been described for several bacterial species such as *Streptococcus bovis, Butyvibrio fibrosolvens, Fibrobacter succinogens, Prevotella ruminicola, and Ruminobacter amylophilis* (Bae *et al.,* 1993 Mcsweeny *et al,* 2001). Catechin gallate are found to be toxic to rumen microbes (*Streptococcus bovis*) when grown in the pure culture (Muller_Harvey *et al.,* 1988). They have identified three mechanisms of toxicity; enzyme inhibition and substrate deprivation, action on membranes and Metal ion deprivation (Krause *et al*, 2005).

Tannins induce changes in morphology of several species of ruminal bacteria. Hydrolysable tannins are toxic to ruminants. Tannin toxicity from HTs may occur in animals fed oak (*Quercus* spp.) and several tropical tree legumes (e.g. *Terminalia oblongata* and *Clidema hirta*) .Microbial metabolism and gastric digestion convert HTs into absorbable low molecular weight metabolites. Some of these compounds are toxic.

The major lesions associated with HT poisoning are hemorrhagic gastroenteritis, necrosis of the liver, and kidney damage with proximal tuberal necrosis, high mortality and morbidity were observed in sheep and cattle fed oaks and other tree species with more than 20% HT. (Reed, 1995; Alldrege *et al.,* 1999).

Toxicity from PA is difficult to separate from their effects on the digestion of proteins and carbohydrates. Because PAs are not absorbed by the digestive tract, but they may damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients, and may reduce the absorption of essential amino acids. CT at higher concentration (5-8%) reduced the absorption of EAA by 15% in sheep (Makkar, 2003; Waghorn, 1990).

The most susceptible amino acids are methionine and lysine. Decreased methionine availability could increase the toxicity of cyanogenic glycosides, because methionine is involved in the detoxification of cyanide via methylation to thiocyanate.

2.3.2.3 Mechanisms of tannin-protein interactions

Tannins react with proteins and form tannin-protein complex. Four types bonds have been suggested to participate in the formation of tannin-protein complexes.

These are:

1. Hydrogen bond formation between phenolic hydroxyl and carboxylic oxygen of proteins (Haslam, 1974).

2. Ionic bond formation between phenolate anion and cationic sites of proteins (Loomis, 1974),

3. Covalent link formation by oxidation to quinones and subsequent condensation with nucleophlic groups in proteins (Haslam, 1979) and

4. Hydrophobic interactions between aromatic regions of proteins and tannins (Hagerman and Buttler, 1980)

Tannin protein complex formation is affected by PH of the gut environment (4-7) and structure and size of both tannins and proteins. Tannin-protein interactions are specific and depend on the structure of both the protein and tannin. Protein characteristics that favor strong bonding are large molecular size, open and flexible structures, and rich in proline. Tannin characteristics that favour strong bonding are high molecular weight and high conformational mobility.

2.3.2.4 Effect on animal performance

Tannin can cause reduction in growth rate and milk production from animal when fed at higher level in the diets. Barman and Rai (2004) reported that inclusion of Babul pods (18.01% tannin) in concentrate mixture of crossbred cows at 20 and 40% level reduced milk yield by 13 and 10 percent respectively as compared to control ($P<0.01$). He also noticed that milk fat per cent was reduced.

Panda *et al* (1983) found that grazing of goats on E .jambolana (6.5% tannin) resulted in the loss of body weight (22.3 g/kg body weight) and the body condition of the animals also reduced progressively. Feeding of P. cinereria (5-6.2 tannin) to sheep reduced 50.62 g/ kg body weight (Upadhyay, 1985). Frutos *et al* (2004) observed feeding of chest nut skin (13% hydrolysable tannin) to sheep showed progressive reduction of body condition and body weight loss (50 g/kg body weight).

Tannin in *Acacia anuera* was reported to cause increased loss of endogenous structural and secretary proteins(Brooker et al, 1994; Komolong et al., 2001) and induce histological changes in tissue morphometry and reduce activity of intestinal brush border enzymes (Robins and Brooker.2005). Monogastric animals fed diets with a level of tannins fewer than 5% experiences symptoms like depressed growth rates, low protein utilization, damage to the mucosa lining of the digestive tract, alteration in the excretion of certain cations, and increased excretion of proteins and essential amino acids.

Inactivation of tannins in feeds and fodders

Tannins present in feeds and fodders at higher concentration are well known to affect animal performance negatively. So, it is desirable to deactivate these tannins or to reduce to the harmless level to the animals. In principle, deactivation of tannin methods can be applied to any feed

resource rich in tannins. The common methods of tannin inactivation in feed and fodders are:

1. Chemical treatment
2. Biological treatment.

Chemical treatment

Chemical detoxification of tannins includes use of alkalis, organic solvents, oxidizing agents, wood ash and metallic ions (Makkar and Singh, 1992; Aemiro and Rai, 2002)

Alkali treatment promotes oxidation of phenolics at higher PH and decrease polymerisation of CT (Deshpande *et al,* 1986). Various alkalis like sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium carbonate, sodium bi-carbonate and limewater have been tried to remove tannins. Out of them sodium hydroxide, potassium hydroxide, calcium hydroxide and limewater have been proved to be most efficient in different feeds. The reduction in tannins in oak leaves using alkalis ranged between 70 and 90%. The reduction in tannins due to alkali treatment is due to oxidation of phenolics by oxygen (present in air) at higher pH values.

Treatment of Sal seed meal by sodium hydroxide (0.05M) was reported to reduce 68-74 per cent tannins (Wah et al, 1977). Aemiro (2002) reported treatment of Babul pods and mango seed kernel by 3 per cent calcium hydroxide resulted in 81 and 85 per cent reduction in tannin respectively. The treatment resulted in improvement in *in vitro* organic matter digestibility of Babul pods and mango seed kernel by 17.8 and 22.6 per cent.

Dubey and Rai (2006) also found that treatment of Babul pods with 3 % calcium hydroxide at 20% moisture for day reaction period resulted in 15.64% increase in IVOMD.

Cumulative gas production in response to alkali and PEG treatments at 24 and 48 hours post inoculation was less pronounced in *Acacia nilotica* as compared to *Dicrostachys cinerea* fruits. In *Acacia nilotica* sodium hydroxide solution (0.6%) was most effective in reducing the detrimental effect of tannins on *in vitro* fermentation. Gas production was increased by 29% as a result of tannin inactivation (PEG) while treatment with 20% wood ash solution caused a 26% increase. For *D.cinerea* ,the treatment of 20% wood ash solution was most effective in inactivating tannins causing a 23% increase in gas production(Mlambo *et al,*2005).They concluded that alkali treatment alone did not cause significant improvement in degradability of A.nilotica fruits at 24 hours post inoculation .

However, PEG+sodium hydroxide treatment improved IVDMD. In *D.cinerea* fruits both sodium hydroxide and wood ash solution were improved the IVOMD.

In vivo studies of treatment of tree leaves and fruits with calcium hydroxide were reported to show variable results ranging from no effect to positive effect on the nutritive values of browses. Alkali treatment reduced the concentration of extractable tannin by as much as 92%.

Alam *et al* (2005) observed that feeding *Albezi*a foliage (300g/kg of diet) to growing goats on a basal diet of hay and wheat bran either un treated or treated with either calcium hydroxide (20g/100ml), PEG (2:1) or calcium hydroxide +PEG resulted in increased nitrogen digestibility (0.72, 0.70 versus 0.60,0.61), nitrogen retention (0.43,0.48 versus 0.26,0.27 mg N/mg N intake), and microbial nitrogen supply (23.7,21.4 versus 14.2,12.4 g/day) respectively when both PEG and calcium hyroxide+PEG groups compared to control and calcium hydroxide alone treated groups. Mean dry matter intake and mean dry matter digestibility were similar across all the treatments. They concluded that calcium hydroxide alone did not improve the feeding value of *Albzia procera* (6.64% tannin).

Soaking *Acacia villosa* leaves in calcium hydroxide solution (20g/l) removed about 75% tannins. Feeding of the soaked leaves to goats on sugar cane tops basal diet and 100 g of cassava flour did not improved intake (56.5 versus 59.7 g/kg Lwt 0.75) and digestibility (0.60 versus 0.56) of *accia* leaves (Wina et al,2005). They concluded that the relatively higher intake of unsoaked leaves by goats might be due to the ability of goats to withstand higher astringency of tannin rich feeds.

Treatment of *Albezia* leaves with alkali (calcium hydroxide or potassium dichromate at 20g/kg leaf dry matter) reduced the content of extractable total tannin by 93%. This reduction was improved marginally to 97% when the leaves where subjected to drying in sun for 3 days after treatment (Alam *et al,* 2005). Despite the dramatic reduction in extractable tannin contents achieved following treatment of alkali, in *vivo* assessment showed that tannins were still present and active in *Albezia procera* treated with calcium hydroxide as similar to untreated leaves which resulted in no significant difference in growth rates and nitrogen utilization of goats supplemented with treated or untreated leaves.

Urea treatment of tree leaves at 20g/kg DM reduces the tannin content by 66-70% (Vitti *et al*., 2005; Bensalem *et al.,* 2005). Addition of urea and then storage (urea-ammoniation) increased the extent of tannin inactivation. Urea treatment was utilized to improve the nutritive value of some feed stuffs (Makkar, 2003). This is because they are highly alkaline which destabilizes tannin protein complexes and oxidizes tannin to phenols. It was reported to be effective in tannin deactivation and also resulted in increased crude protein content of feeds (Bensalem et al, 2005). But urea treated materials resulted in significant increases in the level of NDF,ADF and ADL which might be due increase in lignin ,decrease in cellulose content or the presence of polymerized acid detergent insoluble products as the consequence of treatment (Bensalem

*et al,*2005;Makkar and Singh,1993). IVDMD, IVOMD and IVGP were not improved by the treatment.

Extraction with aqueous organic solvents (30% acetone, 50% methanol, 40% ethanol) removed about 70% tannins from oak leaves. (Makkar and Becker, 1996). The advantage of using these organic solvents is that these can be recovered for reuse and the tannins can be used for the tanning of leather.

3. Extraction with aqueous organic solvents (30% acetone, 50% methanol, and 40% ethanol) removed about 70% tannins from oak leaves. (Makkar and Becker, 1996). The advantage of using these organic solvents is that these can be recovered for reuse and the tannins can be used for the tanning of leather. Ferrous sulphate (0.015 M), a tannin-complexing agent reduced tannins by 85%.These treatments affected the extractable condensed tannins to a larger extent than the bound condensed tannins. Ferrous sulphate is known to form complex with tannins (Deshpande *et al.,* 1986) and increase in the degree of polymerization in the treated material could be due to binding of phenolics through ferrous ions. Specific activity of tannins (protein binding capacity per unit phenols) in the treated oak leaves was lower than that of the untreated leaves.Treatment of Babul pods with different levels of ferrous sulphate (0 to 3 per cent) did not improve *in vitro* dry matter digestibility, organic matter digestibility and gas production. Average IVDMD and IVOMD were suddenly decreased ($P<0.05$) by about 10% when moisture level was maintained at about 30 and 40% along with increased level of ferrous sulphate from 0 to 0.5%. IVGP (ml/g) was also reduced from 124.3 to 123.4 but did not differ significantly (Barman and Rai, 2004). They also found that this treatment did not significantly affect average tannin degradation in Babul pods. Wood ash, available easily and at no cost in villages, is a good source of alkali. A 10% solution of oak wood ash decreased the content of total phenols, condensed tannins and protein precipitation capacity by 66, 80 and 75% in oak leaves, whereas these values for pine wood ash were 69, 85 and 80%, respectively (Makkar and Singh, 1992c). Higher effectiveness of pine wood

ash was attributed to a higher level of alkalis in the pine wood ash (pH values of 10% ash solution of pin).

Bensalem *et al* (2005) found that treatment of acacia cynophylla with wood ash solution (120g/l) increased NDF and ADF contents, crude protein digestibility, and NDF digestibility in sheep. Nitrogen intake and nitrogen retention were also significantly increased ($P<0.05$). However dry matter intake showed non-significant increase while organic matter intake remained similar ($P<0.05$). Microbial crude protein synthesis was also not significant ($P<0.05$).

Adsorbents like polyethylene glycol (PEG), poly vinyl pyrrolidine(PVP) and poly vinyl poly pyrrolidine(PVPP) have been demonstrated to bind tannin even more strongly than proteins and might be utilized to reduce the formation of tannin protein complexes and thus alleviating the deleterious effect of tannin(Makkar *et al,*1995; Bensalem *et al*,1999; Rubenza *et al,*2003; Moujahed *et al*,2005).

Benefits from PEG as tannin neutralizing agent to improve the nutritive value of tannin rich feeds has been well documented (Slanikove *et al*, 2001; Makkar, 2003a;Dubey and Rai, 2006). PEG is a synthetic polymer which has ability to disrupt tannin protein complexes and increase nutrient availability for rumen microbes and the host animal (Bensalem *et al,* 2005).

Systematic investigations were conducted on the binding efficiency of PVP (molecular weights: 10,000, 40,000 and 360,000) and PEG (molecular weight: 2000–35,000) in order to identify the most effective tannin-complexing agents (Makkar et al., 1995c). The affinity of PVPs for tannins was lower than of PEGs. Furthermore, binding of insoluble PVP (PVPP) to tannins was lowest at pH = 7 and the binding with PEG 6000 was the same from pH = 4.7–7, except for quebracho tannins for which the binding increased as the pH approached 7. The binding with PEG 2000

decreased to a greater extent, as the pH reached near neutral and for PEG 4000 this decrease was slightly lower.

The PEGs were the most effective followed by PVPs and PVPP. The PEG 35,000 was the least effective amongst PEGs. The efficiency of other PEGs was similar. The PEG 6000 may be preferred for inactivation of tannins in feedstuffs as its binding to tannins was highest at near neutral pH values. Although, amongst various tannin-complexing agents investigated, PEG of molecular weight 6000 was the most effective in binding to tannins at near neutral pH values, PEG of molecular weight 4000 or 6000 had similar effect on increase in the gas production from tannin-rich feeds when incubated *in vitro* in presence of rumen microbes. The efficiency of PVPs for binding to tannins and increase in the gas production in presence of PEG was much lower compared to PEGs (Makkar *et al.,* 1995c).

In most in vivo studies on ruminants, PEG of molecular weight 3500 or 4000 has been widely used and were found to have both beneficial and adverse effects in ruminant nutrition depending on the concentration and nature of tannin in the feed and the level of protein in the diet (Barry et al,2001;Waghorn and Shelton ,1997;Makkar and Becker ,1996).

Rubanza *et al* (2003) reported *in vitro* gas production and *in vitro* organic matter digestibility increased by 46.6 and 23.4% when *Acacica nilotica* leaves were treated by 1 g of PEG-4000 per 500 g feed sample. They concluded that increased in IVOMD and IVGP due to addition of PEG were an indication of the inhibition effect of the potential nutritive value of browses by tannins.

Effect of PEG-4000 treatment of Babul pods on nutrient digestion and gas production was demonstrated (Dubey and Rai, 2006). The result indicated PEG to tannin ratio of 0.5:1.0 was the best treatment and improved IVDMD (73.73 versus 54.55%), IVOMD (74.74 versus 59.16 %) IVCPD (38.94 versus 17.43%)

and IVGP (253.7 versus 144.3) respectively, for treated and untreated Babul pods.

Feeding fresh *Acacia cynophlla* leaves (43g CT/kg DM) to sheep and goats with 40 g PEG -4000 administered at different frequencies (once, twice, and three times per day) showed that irrespective of the dosing frequencies of PEG, DMI, apparent digestibility of OM, and CF were not significantly affected ($p<0.05$) but CP digestibility and nitrogen retention were improved for groups receiving PEG daily or twice a day (Moujahed *et al,* 2004).

Supplementation of PEG-6000 @ 5 g per animal per day to kids browsing on Prosopis cineraria improved dry matter intake ,(871 versus 691 g/d, $p<0.05$),CP digestibility(50.4 versus 43.6%,$p<0.05$) ,intake of digestible CP(72 versus 52 g/d),hemoglobin level(15.52 versus 11.82 mg %,$P<0.05$) compared to control(Bhatta *et al* ,2003)

PEG supplementation at the rate of 0.67 per cent in concentrate along with *Acacia* foliage (6.6% total tannin & 6.0% condense tannin on DM) fed to lamb resulted in increased DM intake, CP digestibility, nitrogen retention allantoin voided in urine, microbial nitrogen supply and lamb growth (Bensalem *et al,* 2002).

Activated charcoal binds with tannins and reduces toxic effects. This binding agent is widely used in human and veterinary medicine to remove toxic compounds from gastro intestine before absorption to the blood system. Bensalem et al (2005) reported that treatment of A.cyanophylla and Q. coccifera with activated charcoal at 100g/l reduced the tannin by 60 and 65 percent respectively..Biological treatment of tanniniferous feed stuffs were known to reduce tannin in the plants and improve the nutritive values of the feed materials. Aemiro and Rai (2003) reported that *Pleurotus ostreatus* requires 55 and 60 % moisture for their optimal growth on mango seed

kernel and Babul pods respectively. He also noticed that the crude protein content of the kernels increased from 10.5 to 12.3 per cent when incubation period was increased from 6 to 9 days with tannin degradation increased from 10.55 to 18.95%. They also noticed higher tannin degradation (75% in Babul pods) when moisture content was kept at 60% with incubation periods of 9 days. They concluded tannin degradation of Babul pods by *Pleurotus* was 83% while it was only 32% for mango seed kernel

Tannin degradation in animal system

Simple plant phenolics undergo varying degrees of transformation and degradation by micro organisms during passage through the gastro intestinal tract of ruminants(Martin,1982).Phenolic glycosides such as rutin and naringin and flavonoides (quercitrin) are metabolized in the rumen by hydrolysis of glycosides and cleavage of heterocyclic ring (Mcsweeny and mackie,1997;Lowry and Kenedy,1996).The product of flavonoid degradation in the rumen includes acetate, butyrate, di and mono hydroxyphenolics and phloroglucinol(Chang et al,1969;Simpson et al,1969).But there is no evidence of cleavage of heterocyclic ring system of flavan-3-ol (catechin and epicatechin) that are monomers of condensed tannin(Lowry and Kenedy,1996).

Hydrolysable tannin are complex esters consisting of a poly alcohol core in which two or more hydroxyl are esterified with gallic acid (gallotannin) or the dimmer hexahyrdoxy diphenic acid (ellagitannin).

Some rumen microbes (*Selenomonas ruminantium* and S*treptococcus* spp) produce the esterase tannin acyl hydrolase (Brooker et al,199) to form tannin metabolities by diffirent path ways .Positive relationship between phenolic degradation and methanogenic bacteria was noticed (Field and Letinga ,1992) because in the absence of the microbes ,the cleavage of aromatic ring is unfavorable.

Nutrient balance studies on sheep and goats fed on tannin rich *Acacia saligna* leaves showed virtual absence of extractable tannins in faeces (Perz-Maldonado and Norton, 1996; Degen *et al,* 1995; Terrill *et al,* 1994). In this balance studies little free condensed tannin have been observed in faeces. The condensed tannin did not get degraded but the free condensed tannin in the feed get bound to fibre fraction and proteins in the gastrointestinal tract and were present in faeces as unextractable form.

Therefore, the out put of condensed tannin and protein in the acid detergent fiber and acid detergent lignin fractions was substantially higher (Degen *et al*, 1995). Hence, the negative digestibility of both these fractions have been observed (Makkar, 2003a; Rubanza *et al,* 2003).

On the other hand, Rai and Barman (2006) demonstrated the extent of tannin degradation from Babul pods and its balance and types of metabolites in different biological samples. They found that total tannin intake (g/d) was 215.72+13.34 and 327.57+28.61 at 20 and 40 percent Babul pods supplemented groups. Degradation of total tannin, hydrolysable tannin and condensed tannin were 94.89, 94.74, 96.21 % at 20 percent and 97.94, 98.05, 96.86 % respectively at 40 per cent level of Babul pods in concentrate mixture. The urinary excretion (g/d) of the degraded product was higher at 40% while faecal and milk excretion were reduced ($P<0.05$) as compared to 20 percent level of Babul pods. The tannin metabolites identified and quantified in biological samples were phloroglucinol, gallic acid, (+) catechin, (-) epicatechin and catechin gallates in faecal, serum and urine samples .In addition ,resorcinol was identified in urine samples. Milk samples were also reported to contain phloroglucinol, gallic acid, (+) catechin, and (-) epicatechin.

3. Materials and methods

Phase 1: Influence of chemical treatment of babul pods on chemical composition

Babul pods (*Acacia nilotica*) containing seeds and pulps were selected largely due to presence of high level of tannins and abundant availability in tropics and subtropics. Babul pods were purchased from the market and dried at room temperature and then ground to pass through 1mm sieve size and stored in polythene bags until further analysis.

3.1 Chemical treatment of babul pods

3.1.1 Polyethylene glycol (PEG) treatment

Hundred grammes ground samples of babul pods were treated with PEG-4000 MWt. @ 0.05:1, 0.1:1, 0.15:1, 0.20:1and 0.25:1 ratio of PEG: tannin. Measured amount of ground babul pods was taken on clean dry aluminum tray in duplicates and to this 0.9 1.8,2.7,3.6 and 4.5 g of PEG were mixed thoroughly on each separate tray and kept for 24 hrs for reaction to take place in polythene bags at room temperature. The bags were then opened and the samples were transferred to trays and kept in oven for over night at 65 ^{o}c to stop the reaction. The samples were then kept back in the polythene bags in dry place for further analysis.

3.1.2 Calcium hydroxide (Ca (OH) $_2$) treatment

3 g of Ca (OH) $_2$ was taken a beaker and 16.1 ml of water was added and completely dissolved by mixing and this was mixed with 100 g of ground babul pods which were in duplicates and kept over night in air tight polythene bags at room temperature. The same procedure above followed after the reaction was completed.

3.1.3 Combined treatment of calcium hydroxide and Polyethylene glycol

The above levels of PEG-4000 were treated in combination of 3 per cent calcium hydroxide treated babul pods.3 % Calcium hydroxide treated samples were transferred to clean dry tray and mixed with the above levels of PEG in duplicates and kept for another 24 hrs at room temperature for the reaction to

complete. After completion of their specific reaction time, samples were opened and dried in hot air oven at 65 OC. The samples were stored in a plastic container until further analysis for their chemical composition (OM, CP, NDF, ADF, HC, total as),*in vitro* nutrient digestibility, tannin portioning was also done as total phenols, condensed tannins , total tannins and hydrolysable tannins.

3.2 Estimation of proximate principles

Ground samples (1 mm sieve size) of treated and untreated babul pods were analyzed for proximate principles as per standard procedures (AOAC, 1990) and fibre fractions were determined as per the standard procedure of Vansoest *et al,* 1991.

3.2.1 Dry matter (DM)

A measured quantity of sample was taken in pre-weighed crucibles and then kept in hot air oven at 100 ±2 0C for 24 hours. The dry matter content was estimated as percentage of the sample taken.

% DM =weight of sample after drying/ weight of sample taken

3.2.2 Organic matter(OM)

The percent organic matter in the sample was determined by subtracting percent ash contents of treated and untreated samples from 100.

% OM= 100-% Total ash

3.2.3 Crude protein

Crude protein (Nx6.25) was determined by kjeldhal technique. A known quantity of dry ground sample was digested with concentrated sulfuric acid and a digestion mixture (potassium sulphate and copper sulphate in the ratio of 9:1). After digestion, the contents were made to 100 ml in a volumetric flask. A suitable volume of digested sample was transferred in to the kjeldhal distillation apparatus and an approximate quantity of 40% sodium hydroxide solution was added to make the content alkaline. A 100 ml of distillate was collected in a conical flask containing 2% boric acid solution with mixed indicators (0.1% methyl red and 0.1% bromocresol green in the ratio of 2:1 in absolute alcohol). The distillate was then titrated against standard sulfuric acid.

3.2.4 Crude Fibre (CF)

A measured amount of moisture and fat free samples were successively refluxed with weak sulphuric (2.04N) acid and sodium hydroxide (2.05N) in a spoutless beaker on a hot plate for 30 minutes, each followed by filtration and repeated hot water washings. The remaining residue was quantitatively transferred to a silica basin and then oven dried and ashed in a muffle furnace at 550 ^{o}c after the weight was recorded. The loss in weight by ashing of the residue was calculated as crude fibre when divided by sample weight on DM basis.

The calculation was as follows:

(a-b) x100/w

Where,

a= weight (g) of silica basin plus oven dried sample

b= weight (g) of silica basin plus ash

3.2.5 Ether Extract (EE)

A known amount of moisture free samples with petroleum ether (60-80 ^{o}c) was extracted in a soxhlet apparatus on a hot plate for 6-8 hours. The collected extract in the flask was dried to a constant weight at 100 ^{o}c in hot air oven to a constant weight and expressed as percentage on dry matter basis.

Ether extract (%) =(y-x)/w

Where,

Y= weight of oil flask after extraction,

X= weight of oil flask before extraction

W=weight of oven dried sample

3.3 Estimation of cell wall components

Treated and untreated samples of babul pods were analyzed for cell wall fractions by the method of Goering and Van Soest (1970) as described below:

3.3.1 Neutral Detergent Fibre (NDF)

Preparation of neutral detergent Fibre solution

Reagents: Sodium lauryl sulphate 30 g
Di sodium ethylene di amine tetra acetate (EDTA) 18.69 g
Sodium borate deca hydrate 6.51 g
Di sodium hydrogen phosphate 4.56 g
2-Ethoxy –ethanol 10 ml

EDTA and sodium borate deca hydrate were put together in a large beaker with some distilled water and the contents were heated until dissolved by heating. Sodium lauryl sulphate was dissolved separately. Both the above solutions were then put together in a volumetric flask and maintained the volume .

Procedures of Determination

Approximately 1.0 g of sample was taken in to a 1000ml spoutless beaker .To this 100ml neutral detergent solution,2ml decalin and 0.5 g sodium sulphite were added and the contents refluxed on a hot plate for one hour .After refluxing, the sample was filtered through pre-weighed sintered crucible grade using vacuum pump. The sample was dried in a hot air oven (100 ^{0}C) and weighed.

NDF (%) = (B-A) x100/weight of sample on DM basis

Where,

A= weight of empty crucible, and

B= weight of crucible and cell wall components.

3.4.6.2 Acid Detergent Fibre (ADF)

Acid detergent solution: 20 g of cetyl trimethyl ammonium bromide (CTAB) was added and dissolved in 1 liter of 1N sulfuric acid.

Procedure of determination

Approximately, 1 g sample was taken in a 1000 ml spoutless beaker. To this, 100 ml of acid detergent solution and 2ml of decalin were added and the contents boiled on hot plate for exactly 1 hour. After boiling the sample was filtered through pre- weighed sintered glass crucible grade 1 using vacuum pump. The sample in crucible was dried at weighed after 2-3 water washings. The ADF was calculated as follows

ADF (%): (Weight of crucible+ Fibre) – weight of crucible /weight of sample on DM basis.

3.2 *IN VITRO* NUTRIENT DIGESTIBILTY (IVDMD, IVOMD, IVCPD, IVGP) AND GAS PRODUCTION OF TREATED BABUL PODS.

3.2.1 Collection of rumen liquor

Rumen liquor was collected from lactating goats with the help of stomach tube fitted with plastic syringes of 60-ml size. The goats were under similar schedule of feeding (5.4 kg concentrate mixture containing maize grain -33 part,GNC-20 parts, deoiled rice bran-11 parts, mustard cake-12 parts, wheat bran-21 parts,

mineral mixture-2 parts, common salt -2 parts and maize green fodder -30 kg offered in group).The samples were filtered through double layered muslin cloth in to a previously carbon dioxide gas flushed thermos flask and the strained rumen liquor was then brought to laboratory for *in vitro* trials.

3.2.4 Estimation of *In vitro* nutrient digestibility (IVDMD, IVOMD, IVCPD)

The *In vitro* nutrient digestibility of treated babul pods was estimated according to Tilley and Terry (1963). The two stage nutrient digestibility consists of microbial fermentation of feed followed by enzymatic digestion.

In the first stage, 0.5 g of sample was incubated with 40 ml of McDougal's buffer which consisted of di sodium hydrogen carbonate-9.8 g, sodium hydrogen phosphate-9.3 g, sodium chloride-0.47 g, potassium chloride-0.57 g, magnesium chloride-0.06g per liter of distilled water and the PH range from 6.7 to 6.8) and 10 ml of strained rumen liquor for 48 hours at 39 ^{O}C after flushing the whole content with carbon dioxide. This was followed by addition of 2 ml of 10% sulfuric acid to

arrest microbial fermentation. The second stage was pepsin digestion in which 0.1 g of pepsin powder in 2ml of 1N HCl was injected in to the vial and incubated for 24 hours.

In vitro dry matter digestibility was determined in samples by measuring dry matter content of the sample before and after the in vitro digestion. Similarly ,in vitro organic matter digestibility was carried out by igniting the digested and undigested samples in a muffle furnace at 600 ^{0}C for 1 hour .*In vitro* crude protein digestibility was determined by filtration of whole content of bottles through grade 1 filter paper and digestion of the filtrate for protein estimation as per standard AOAC(1990) methods. Blank samples were subtracted from the observed reading of samples to get the actual reading of titration.

3.3 In vitro gas production

Total gas production was measured according to the techniques of Theodrou *et al* (1994). A detachable pressure transducer and the LED digital display read out voltmeter (Bailey and Mackey Ltd, Bringham, UK) were used to measure the gas pressure of the fermenting bottles. The transducer was connected to the inlet of disposable lever lock three way stop cock; the first out let connected to hypodermic needles and the second outlet was connected to 60 cc disposable plastic syringe. The gas pressure was measured by fitting the syringe in to the needle and the transducer; where by the needle is injected in to the fermenting bottle through rubber stopper. The volume of the gas is measured on withdrawal of the plunger of the syringe until the pressure of the gas returned in to zero reading on display unit.

3.4 Determination of tannin in the samples

The dried treated and untreated sample was ground to pass through a sieve of 1 mm size diameter. Four hundred mg of samples was taken in a conical flask and 40 ml of diethyl ether containing 1% acetic acid (v/v) was mixed to remove pigments and fats materials, After 5 minutes, the supernatant was carefully discarded and 20 ml of 70 % aqueous acetone was added. The flask was sealed with cotton plug covered with aluminum foil and kept in an electrical shaker for 2 hours for extraction. Then it was filtered though whatsman filter paper No 1 and the samples were kept in a refrigerator at 4 ^{O}c until analysis.

3.4.1 Estimation of total phenols and tannins

It was estimated according to the standard procedures of Makkar et al (1993). Fifty micro liter of tannins extract was taken in a test tube for each samples and the volume was made to 1.0 ml with distilled water.

Then 0.5 ml of folin ciocalteu reagent was added and mixed properly. Then 2.5 ml of 20% sodium carbonate solution was added and mixed and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in spectro photo meter and concentration was estimated from standard curve prepared from tannic acid solution. Total tannin was estimated as tannic acid equivalents and expressed on DM basis.

Non tannin phenol was estimated by precipitating tannins with poly vinyl poly pyrrolidine (PVPP), which binds tannin. 200 mg PVPP was taken in test tube and then 2.0 ml of distilled water and 2 ml of tannin extract were added. Then it was vortexed and kept in refrigerator for 15 minutes at 4 ^{o}c. Then the mixture was vortexed and filtered through filter paper No 1 Filtrate was taken for estimation of non-tannin phenols. 150 micro liter of filtrate was taken in test tube and volume was made to 1 ml with distilled water and then processed like that of

total phenol estimation. Concentration of non tannin phenol was calculated from standard curve and expressed on DM basis.

Total tannin was calculated by subtracting non tannin phenol from total phenols. The standard was prepared from the stock solution of tannic acid (0.5 mg/ ml) From this 0,10,20,30,40 and 50 micro liter were taken in test tubes and volume was made to 1 ml to obtain a tannic acid concentration of 0,5,10,15,20 and 25 micro gram respectively. Then 0.5 ml of folin ceocaliteau reagent and 2.5 ml of 20% sodium carbonate was added. The whole content was mixed properly and after 40 minutes reading was taken at 725nm using spectro photometer.

3.4.2 Estimation of condensed tannin (proanthocyandins)

Condensed tannin was estimated according to Porter *et al* (1986).

0.5 ml of tannin extract was taken in test tube in triplicate and 3.0 ml of butanol HCl and 0.1 ml of ferric reagent was added. Tubes were vortex to insure proper mixing .The mouth of the tube was covered with glass marble and then boiled for 60 minutes. Similarly, blank was prepared for each sample with out heating the reagent. The tube was cooled to room temperature and reading was taken at 550nm using spectro photometer. Condensed tannin was expressed as leucocyanidin equivalent and was calculated as:

% condensed tannin= (A_{550} nm x 78.26xdilution factor)/ (% DM)

3.4.3 Determination of hydrolysable tannin

Hydrolysable tannin was determined by subtraction of condensed tannin from total tannin phenol.

3.4 Statistical analysis

The data were analyzed by standard procedures of snedecor and Cochran (1985). Correlation analysis was done to find out the relation between *in vitro* dry matter digestibility (IVDMD), organic matter digestibility (IVOMD), crude protein digestibility (IVCPD) and in vitro gas production (IVGP). Student T-test was done to compare different treatments and levels to find out weather or not other factors like CP, NDF, ADF and NFE involved in variation of IVDMD, IVOMD and IVGP.

PHASE II: LACTATION STUDIES

3.6 Effect of dietary treatments on voluntary intake, nutrient utilization and balances of nitrogen and minerals

3.6.1 Selection and distribution of animals

Twenty-four lactating goats were divided in to 4 groups of 6 animals each in a complete randomized block design (CRD) based on their milk yield, parity, stage of lactation and body weights

Table 3.1: Initial body weights, average milk yield and parity of experimental animals

Treatment	Animal NO	Initial body weight (Kg)	Initial average milk yield(kg/day)	Parity	Days in lactation
T1	496	36.5	1.9	2	27
	575	45	2.1	3	25
	610	39	1.4	2	26
	696	43	1.3	2	29
	735	34.5	0.7	2	29
	45	34	1.2	3	30
	average	38.6667	1.433333		
T2	647	37	1.8	3	33
	662	35.5	1.8	2	32
	674	46	1.9	2	25
	697	46	0.9	2	29
	717	32.5	1.0	2	25
	52	38.5	0.9	3	
	average	39.25	*1.383333*		
T3	678	41	2.1	2	27
	727	35	0.6	3	28
	745	39	0.8	3	29
	26	37	1.9	2	29
	37	38	1.0	2	34
	48	41.5	1.9		33
	average	38.5833	1.3833		
T4	679	37	1.8	3	29
	740	36.5	1.1	2	30
	44	42.5	0.9	2	25
	46	39	1.2	3	29
	51	44.5	1.6	3	24
	998	35	1.4	2	31
	average	39.08333	1.333		

3.6.2 Feeding and housing management of experimental animals

Lactating goats were kept on isocaloric and isonitrogenous diet according to NRC (1981) feeding standard. The animals were kept in the shade in a group feeding pens. The experimental diets were offered to the goats as total mixed ration (TMR) of concentrate and roughage of 50:50 ratio. The feed was given to the animals two times a day; in the morning (8:00PM) and noon (2:00AM).

Goats were handed milked twice a day; early in the morning (5:00 PM) and noon (3:00AM).

Table 2: Ingredient compositions of the concentrate mixture used for feeding experimental animals

Ingredients	Parts in concentrate mixture			
	Group I	Group II	Group III*	Group IV*
Babul pods	0	42.2	63.3	63.3
Maize	38.4	14.0	5.0	5.0
GNC	18.3	22.0	24	24
Deoiled rice bran	10.1	4.8	1.7	1.7
Mustard cake	11.0	5.0	1.8	1.8
Wheat bran	19.2	9.2	3.2	3.2
Mineral mix	2.0	2.0	2	2
Common salt	1.0	1.0	1.0	1.0
Total	100	100.2	100.3	100.3

*Babul pods in concentrate mix III and IV was treated by calcium hydroxide 3% and 1.8 % PEG-4000.

The experimental diets were offered to the goats as total mixed ration (TMR) consisted of concentrate and roughage (chopped green maize) in the 50:50 ratio. The feeding schedule was as follows:

TMR1: Control diet, concentrate mix I and maize fodder

TMR2: Babul pods equivalent to 4% tannin, Concentrate mix II and maize fodder

TMR3: 3% Calcium hydroxide treated babul pods equivalent to 6% tannins concentrate mix III and maize fodder

TMR IV: Combined 3% calcium hydroxide and PEG treated babul pods equivalent to 6% tannins, concentrate mix IV and maize fodder

3.6.3 Observations recorded during lactation trial

A **Feed intake**: Dry matter intake was recorded for five consecutive days in a fortnight by weighing feed offered and residue left over with 24 hrs during the entire experimental period.

B. **Milk yield and composition**

Daily milk yield of individual goats was measured using spring balance and milk composition of each animal was determined at each fortnight interval. The samples from morning and evening milking were pooled proportionately together to represent milk samples of each animal.

The pooled samples of each animal were subjected to fat, protein, and solid not fat and total solid analysis as per standard methods of AOAC (1996).

C. **Fortnight body weight**: Body weight of the animals was recorded at fortnightly interval for two consecutive days before offering feed and water.

D. **Metabolic trial**: It was conducted at the middle of lactation trial for 7 days periods during which proper records of feed and water offered ,and residue left over were maintained. The animals were weighed for two consecutive days before and after the metabolic trial.

E .**Collection of samples**

Representative samples of feed, residue and milk were collected at each fortnight for dry matter, organic matter and proximate composition during lactation trial from representative samples feed offered, residue left over, faeces, urine and milk samples were collected and pooled together. Feed, residue, and faeces were oven dried every day .the pooled samples were ground to pass through 1mm sieve size and used for proximate and fibre analysis.

Total amount of faeces voided and urine excreted by each animals per day were recorded and a known amount (1/20th part) faecal aliquot and (1/40th part) of urine aliquot were taken in a pre-weighed bottles each day for each animals.
For nitrogen, the faeces and urine samples were preserved by 25% suphuric acid and for minerals in a known quantity of toluene. At the end of the trial, the bottles were weighed and representative samples were used for analysis.

F **Milk composition**

Milk composition was analyzed as per AOAC (1996). Total nitrogen in the milk was determined by kjeldhal method and crude protein was calculated by multiplying the nitrogen content by 6.38. Milk fat was estimated by automatic fat analyzer. Solid not fat was calculated from corrected lactometer reading by the standard formula of Indian Standard Institute (1984).

% SNF = CLR/4 + 0.2 F% + 0.14

Where, SNF =solid not fat

CLR= corrected lactometer reading

F= fat content of milk

Total solid content of milk was determined by the following procedure: Ten gram of pre warmed milk sample was taken in to a pre weighed silica crucible and kept in a steam bath for 10-15 minutes and then kept in hot air oven at 98-100 ^{o}c for over night. After complete during, the sample was cooled in a desiccators and weighed.

Calculations

a. Fat corrected milk (4%)

This was calculated by the following equation given by Tyrell and Reid (1965)

FCM (kg) 0.4 M+ (15xFxM)/100

Where,

M= milk yield

F= fat percent

b. Gross protein efficiency for milk production was calculated by using formula of Jumah *et al* (1965).

Gross protein efficiency= ((milk yield protein %) /100) XDCP intake) x 100.

C Milk energy: It was calculated (Tyrell and Reid, 1945) as follows:

Milk energy (Mcal) =SCMX0.75

SCM= Solid corrected milk.

SCM=12.3(F) +6.56(SNF) +0.0752(M)

F= kg of milk fat

SNF= kg of solid not fat

M= kg of milk yield

d. Gross energetic efficiency of milk production was calculated according to the procedure of Brody (1945) as follows

Gross energetic efficiency (%) = (4% FCMX750)/ (TDN consumed (kg) x 4000) x100

3.7 Parasitic egg counts in faeces

In both experimental and control groups faeces were collected directly from the anal ampulae of animals at every 30 days interval. The samples were preserved in 10% formalin or in refrigerator at 0 ^{o}c for further analysis. Faecal egg counts of individual animals were carried out using modified Mc Master Technique (What lock, 1948). 3 g of fecal sample was grounded using the pistil and mortar and homogenous solution was made in 42 ml of concentrated salt solution. 0.15 ml of

this sample was then transferred to the counting chamber. The prepared sample was then observed under 10x microscopic power for parasitic fecal eggs and oocysts. The faecal egg counts/ gram were log transformed for analysis and back transformed.

3.8 Statistical analysis

Generalized linear model of SAS (1990) programme was used for analysis of the data from feeding and lactation trial for student t test was used for mean comparison of treatments. The same programme was used for parasitic egg counts

4.0 RESULTS AND DISCUSSION

4.1 INFLUENCE OF CHEMICAL TREATMENTS ON PROXIMATE COMPOSITION, *IN VITRO* DIGESTIBILITY AND PARTITIONING OF TANNINS IN BABUL(*ACACIA NILOTICA*) PODS

4.1.1 Effect of polyethylene glycol (PEG) treatment on chemical composition of babul pods

Chemical composition of treated Babul pods (*Acacia nilotica*) with different levels of polyethylene glycol (PEG) is presented in Table 4.1. There was significant ($P<0.05$) difference in OM content of treated Babul pods and the values decreased from 92.80 percent to 88.60 percent on DM basis with increased level of PEG treatment from 0.05 to 0.25 (PEG :tannin ratio) as compared to control. But organic matter contents of treated Babul pods were non-significantly different ($P<0.05$) between treatment groups. Unlike OM, the total ash content of treated samples were increased significantly ($P<0.05$) in treated groups. However, variations between levels in each treatment were non significant. Crude protein contents of treated Babul pods ranged from 15.01 percent in control to 13.86 percent in 0.25 level of PEG: tannins treatment. NDF content decreased significantly ($P<0.05$) as the level of PEG increased. Similar trends were observed in ADF contents of treated Babul pods.

4.1.2 Effect of calcium hydroxide and PEG treatment on chemical composition of babul pods

Chemical composition of treated Babul pods (*Acacia nilotica*) with calcium hydroxide followed by different levels of polyethylene glycol (PEG) is presented in Table 4.2.

Table 4.1: Effect of PEG treatment on chemical composition (% DM) of babul pods

Particulars	Levels	OM	CP	NDF	ADF	Total ash
Control	Untreated	92.81±0.33	16.01±0.38	44.26 ±1.95	29.43 ±0.92	7.19 ±0.33
PEG: Tannin	0.05	90.36±0.33	14.53±0.38	32.47±1.94	19.76±0.79	9.63 ±0.33
	0.1	89.34±0.33	14.32±0.38	32.75±1.86	20.08 ± 0.86	10.66±0.33
	0.15	89.59±0.33	14.71±0.38	33.40±1.86	22.35 ±1.17	10.40±0.33
	0.2	88.61±0.33	14.50±0.38	32.27±1.39	21.25 ±0.88	11.40±0.33
	0.25	91.37±0.33	14.36±0.38	33.72±1.86	20.69±0.86	8.63 ±0.33
	SEm	0.25	0.33	1.54	1.18	0.25
	CD	0.78**	1.02*	4.76**		0.77**

Table 4.2: Effect of Calcium hydroxide and PEG treatment on chemical composition (% DM) of babul pods

Particulars	Levels	OM	CP	NDF	ADF	Total ash
$Ca(OH)_2$	3%	90.36±0.33	14.68±0.38	36.66 ±0.95	24.13 ±0.92	9.63 ±0.33
$Ca(OH)_2$ +PEG (PEG: Tannin)	0.05	91.03±0.33	15.27±0.38	36.66±0.95	23.28 ±0.98	8.96±0.33
	0.1	90.54±0.33	14.42±0.38	32.67 ±1.71	22.32 ±1.01	9.45 ±0.33
	0.15	89.78±0.33	14.87±0.38	34.50 ±1.71	21.65 ±0.57	11.21±0.33
	0.2	90.5±0.33	14.22±0.38	32.77 ±2.11	20.93 ±0.99	9.40 ±0.33
	0.25	88.83±0.33	13.86±0.38	33.21±1.710	21.45 ±0.98	11.17± 0.33
	SEm	0.39	0.43	0.99	0.52	0.39
	CD	1.21*	NS	3.78NS	1.60**	1.21*

* Significant at P<0.05; * *Significant at P<0.01

The OM content of treated babul pods with calcium hydroxide and PEG ranged from 88.83 percent in level 0.25 to 91.03 percent in level 0.05 and it was similar in all the treatment levels. CP content of treated babul pods were 14.68, 14.47, 14.42, 14.87, 14.22 and 13 86 per cent for 3% calcium hydroxide and 0.05, 0.10,0.15,0.20 and 0.25 levels of combined treatment. The CP content was not affected by the level of treatments. Similarly, NDF, ADF and Ash contents were not affected by the levels of treatments.

In present study, OM, CP, NDF, ADF reduced significantly ($P<0.05$) with increasing level of PEG, from 0.05 to 0.25 PEG tannin ratio. This may be due to dilution effect of chemical treatment. Ca $(OH)_2$ treated Babul pods also reduced significantly ($P<0.05$) in chemical composition as compared to control.

Aemiro and Rai (2002) reported reduction in chemical composition of treated Babul pods when level of Ca $(OH)_2$ increased from 3 to 5 percent. Combined treatment of 3 percent $Ca(OH)_2$ and PEG resulted in significant ($P<0.05$) reduction in OM, CP, NDF, ADF as compared to native Babul pods but increase in total ash content. This might be due to solubilization of nutrients by alkali treatment and increase in ash content was due to increase in inorganic Ca salt in the treated Babul pods.

4.1.3 Effect of polyethylene glycol treatment on *in vitro* nutrients digestibility of treated Babul pods

In vitro digestibility of nutrients in treated Babul pods are presented in Table 4.3. The IVDMD increased significantly ($P<0.05$) from 50% in control to 64.73% in 0.25 level of PEG. The IVOMD also increased significantly ($P<0.05$) from 57.64 percent in control to 73 percent in 0.25 level of PEG treatment. It showed increasing trend with increased level of treatments. Highest IVOMD were observed at 0.25 level of PEG treatment which is due to tannin binding effect of PEG as a result

digestibility of organic matter increased significantly ($p<0.05$). Similar trend was observed in IVCPD which increased significantly ($P<0.05$) from 50.05 percent in control to 61.78 percent in 0.25 level of PEG treated babul pods. Dubey and Rai (2006) found similar patterns of digestibility with increasing levels of PEG: tannin ratio for the treatment of babul pods from 0.25 to 0.5. They noticed that increasing level of PEG from 0.25 to 0.50 improved IVDMD, IVOMD, IVCPD and IVGP in Babul pods. The best level of treatment was 0.5:1m, PEG: tannin ratio.

IVGP was significantly increased ($P<0.05$) from 129.51ml/g in 0.05 to 173.23ml/g substrate in 0.25 level of PEG treatment as compared to control (81.18 ±7.24). Increasing level of PEG treatment resulted in subsequent increase in IVGP. In present study, increase in IVGP improved from 37.9 to 67.26 percent. Similar pattern was observed in the rate of gas production in PEG treated samples.

4.1.4 Effect of calcium hydroxide with polyethylene glycol treatment on *in vitro* digestibility of treated Babul pods

In vitro digestibility of nutrients in treated Babul pods are presented in Table 4.4. The IVDMD ranged from 62.08 per cent in calcium hydroxide treated samples to 69.89 per cent in calcium hydroxide plus PEG at 0.25 level of combined treatment. Increasing level of treatment resulted in respective increase in dry matter digestibility. Similar trend was observed for IVOMD and IVCPD with increasing level of treatment. Maximum IVOMD (74 per cent) and IVCPD (67.65%) were at 0.25 level of combined treatment. Additional solubilization effect of PEG treatment observed in this experiment after calcium hydroxide treatment which resulted further increase in digestibility of nutrients. Maximum digestibility was observed at 3 percent Ca $(OH)_2$ plus 0.25 level of PEG treatment.

In 3 percent Ca $(OH)_2$ with PEG treatment of babul pods increased the IVGP from 161.74±9.34 ml/g substrate in 0.05 level to 175.46±11.20 in 0.10 level and then reduced to 168.57±8.13 ml/g substrate in 0.25 level of

Table 4.3: Effect of PEG l treatment on *in vitro* digestibility (%) and gas production of babul pods

Particulars	Levels	IVDMD	IVOMD	IVCPD	IVGP (ml/g)	IVGP (ml/g/h)
Control	Untreated	50.00±7.17	57.64±2.05	50.05±2.15	81.18± 7.24	1.04±0.29
PEG: Tannin	0.05	61.07±4.69	66.88±3.75	52.69±1.96	129.51±10.63	1.71±0.26
	0.10	60.90±5.00	67.23±3.75	55.26±2.92	136.43±10.24	1.80±0.32
	0.15	60.40±4.54	68.14±2.80	57.64±1.86	144.36± 8.73	1.91±0.25
	0.20	61.68±4.54	70.15±3.53	58.72±1.96	168.01±10.63	2.24±0.24
	0.25	64.73±4.69	73.23±3.75	61.78±1.96	173.36±10.63	2.32± 0.19
	SEm	2.43	2.02	1.69	12.98	1.71
	CD	7.47**	6.22**	5.20**	39.99**	0.14*

Table 4.4: Effect of Calcium and PEG l treatment on *in vitro* digestibility (%) and gas production of babul pods

Particulars	Levels	IVDMD	IVOMD	IVCPD	IVGP (ml/g)	IVGP (ml/g/h)
Ca(OH)2	3%	62.08±5.00	69.08±3.81	55.42±3.17	131.42±10.16	1.73±0.27
$Ca(OH)_2$ + PEG (PEG: Tannin)	0.05	63.50±5.81	66.48±3.53	56.52±1.96	161.74±10.63	2.15±0.23
	0.10	67.93±5.81	73.78±3.53	62.10±2.15	175.46±10.63	2.34± 0.32
	0.15	60.84±6.18	70.64±3.53	61.87±2.15	166.47± 8.73	2.16±0.24
	0.20	66.72±5.94	71.91±3.53	66.95±2.24	147.14±10.63	1.95±0.24
	0.25	69.89±5.81	74.15±3.53	67.65±2.15	128.57±10.63	1.69±0.24
	SEm	1.18	1.61	1.48	17.50	4.53
	CD	NS	NS	NS	NS	NS

* Significant at P<0.05; * *Significant at P<0.01

PEG with 3 percent Ca(OH)2 treatment. In 3 percent Ca $(OH)_2$-treated group IVGP increased significantly ($P<0.05$) to 131.42±8.45 ml/g as compared to control (81.18 ml/g substrate). In present study, increase in IVGP was improved by 81.94 percent in 3 percent Ca $(OH)_2$ treatment over untreated and from 87.15 to 93.20 in 3 percent $Ca(OH)_2$ with PEG combination treatment. Therefore, in the present study, higher levels of PEG (0.20 and 0.25) and 3 percent $Ca(OH)_2$ with PEG treatment combination at levels (0.10 to 0.25) improved IVDMD, IVOMD, IVCPD and IVGP significantly ($P<0.05$). The best result was obtained at 3 percent $Ca(OH)_2$ and PEG treatment at 0.10 level as compared to all other treatment levels.

The overall effect of treatments and levels showed that a combination of 3 percent $Ca(OH)_2$ with PEG treatment were more effective than ($P<0.05$) the rest of treatments and levels in IVDMD, IVOMD, IVCPD and IVGP. The trend of gas production kinetic showed that the average gas production of each treatment (ml/g) was significantly ($P<0.05$) increased in the 1^{st} stage kinetics (0-24 h incubation) as compared to second stage kinetics (24–48h incubation.) IVGP ml/g/hr also followed similar trend.

The highest gas production (ml/g) was observed in 3 percent $Ca(OH)_2$ with PEG combination at 0.25 level (70 ml/g) in the first stage kinetics at 12 h incubation. But overall gas production was highest (175 ml/g) at 0.10 level of PEG with 3 percent $Ca(OH)_2$ treatment.

In the first stage kinetics (0-24 hrs) IVGP (ml/g) was lower in untreated Babul pods while, in second stage kinetics, the gas production remained higher due to slow release of bound organic matter to the fermenting bottles as compared to the treated groups.

4.1.5 Effect of PEG treatment on tannin partioning and its degradation in Babul pods

Partitioning of different components of tannins of treated babul pods is presented in Table 4.5. The PEG treatment significantly ($P<0.05$) reduced the tannin content of treated Babul pods as compared to native Babul pods. In PEG treated samples, TP, TT, CT and HT ranged from 8.33 to 14.13 percent, 5.88 to 11.14 percent, 0.24 to 0.54 percent and 5.63 to 10.59 percent respectively as compared to control (23.08, 18.37, 1.43, and 17.8 percent, TP, TT, CT and HT respectively. Tannin content of 3 percent $Ca(OH)_2$ treated Babul pods was 5.92, 3.12, 0.33 and 2.79percent, TP, TT, CT and HT respectively on DM basis.

Percent tannin degradation (TD) in treated Babul pods was improved significantly ($P<0.05$) in all the treatment groups. Percent tannin degradation for PEG treated samples ranged from 37.90 to 67.26 as compared to control.

4.1.6 Effect of calcium hydroxide and PEG treatment on tannin partioning and its degradation in Babul pods

Partitioning of different components of tannins of treated babul pods is presented in Table 4.6. In combined 3 percent $Ca(OH)_2$ with PEG treated samples for TP, TT, CT and HT values ranged from 4.9 to 5.92, 2.74 to 2.42, 3.05 to 3.38, 0.22 to 0.32 and 2.72 to 3.2 percent TP, TT, CT and HT respectively.

In 3 percent $Ca(OH)_2$ treated samples, percent degradation was 81.93. The reduction in tannin contents was significantly higher ($P<0.05$) in PEG with samples. Percent degradation increased from 87.15 to 93.20 as the level of treatment increased from 0.05 to 0.25 as compared to native babul pods.

Table 4.5: Effect of PEG treatments of babul pods on partitioning of tannins and its degradation (%DM)

Treatment	Level	Total phenol	Total tannin	Condensed tannin	Hydrolysable tannin	Tannin degradation
Control	Untreated	21.32±0.86	18.37±1.06	1.43 ±0.03	17.48 ±0.64	0.00±4.53
PEG: Tannin	0.05	14.13 ±0.48	11.14±0.48	0.54 ±0.03	10.59 ±0.47	37.90 ±2.80
	0.10	11.10 ±0.48	8.35 ±0.48	0.48 ±0.03	7.87 ±0.47	53.49 ±2.80
	0.15	9.72 ±0.48	6.78 ±0.48	0.43 ±0.03	6.34 ±0.47	62.18 ±2.80
	0.20	9.99 ±0.48	7.43 ±0.48	0.34 ±0.03	7.09 ±0.47	58.51 ±2.80
	0.25	8.33 ±0.48	5.88 ± 0.48	0.24 ±0.03	5.63 ±0.47	67.26 ±2.80
	SEm	0.57	0.55	0.04	0.54	3.32
	CD	1.75**	1.68**	0.13**	1.67**	10.23**

Table 4.6: Effect of PEG treatments of babul pods on partitioning of tannins and its degradation (%DM)

Treatment	Level	Total phenol	Total tannin	Condensed tannin	Hydrolysable tannin	Tannin degradation
$Ca(OH)_2$	3%	5.92 ±0.48	3.12 ± 0.48	0.33 ±0.03	2.79 ±0.47	81.94 ±12.80
$Ca(OH)_2$ + PEG (PEG: Tannin)	0.05	5.70 ±0.48	3.38 ±0.48	0.32 ± 0.03	3.05 ±0.47	87.15 ±2.80
	0.10	4.90 ±0.48	3.50 ± 0.48	0.29 ±0.03	3.20 ±0.47	89.06 ±2.80
	0.15	5.45 ±0.48	3.24 ±0.48	0.25 ±0.03	2.98 ±0.47	93.06 ±2.80
	0.20	5.33 ±0.48	3.01 ± 0.48	0.25 ±0.03	2.76 ±0.47	93.20 ±2.80
	0.25	5.52 ±0.48	3.05 ± 0.48	0.32 ±0.03	2.72 ±0.47	91.99 ±2.80
	SEm	0.40	0.41	0.03	0.40	2.17
	CD	NS	NS	NS	NS	NS

* Significant at P<0.05; * *Significant at P<0.01

Alam *et al.* (2005) reported that treatment of Alberia leaves with Ca $(OH)_2$ improved tannin degradation from 86.36 to 96.82 percent as compared to control. They also found 3 percent Ca $(OH)_2$ treatment at 20% moisture level was optimum to improve nutritive value of treated Babul pods and tannin degradation. Similar observation was reported by Rubenza *et al.* (2003) between gas production and *in vitro* nutrients digestibility in East Africa browses.

4.1.7 The correlation coefficients between in vitro nutrient digestibility and tannin contents of treated babul pods

The correlation coefficients between percent tannin compositions, in vitro gas production, digestibility coefficients and percent tannin degradation are presented in Table 4.7.

Higher positive correlation ($P<0.05$) were observed between IVGP and nutrient digestibility coefficients in treated Babul pods and the correlation between IVOMD and IVGP and IVCP and IVGP were highly significant ($P<0.01$). The *in vitro* digestibility parameters were negatively correlated ($p<0.01$) with TP, TT, CT and HT. IVGP was positively correlated ($P<0.01$) with OM, CP, IVCPD, IVOMD, and IVDMD. There was negative correlation observed between tannin contents of treated Babul pods and IVGP. Tannin degradation percent (TD) was positively correlated to OM and CP ($P<0.05$) and to percent tannin composition ($P<0.001$). The positive correlation between tannin degradation and in vitro OM digestibility and in vitro gas production is indicative that as the extent of tannin degradation increases the digestibility of nutrients increases. Therefore, the degradation of tannins as a result of chemical treatment is of great significance in improving the nutrient utilization of tanniniferous feeds.

Table 4.7*:* Correlation coefficients between tannins and *in vitro* digestibility coefficients

	IVDMD	IVOMD	IVGP	IVCPD	TP	TT	CT	HT	%TD
IVDMD	1.00								
IVOMD	0.98**	1.00							
IVGP	0.54**	0.51**	1.00						
IVCPD	0.96**	0.97**	0.57**	1.00					
TP	-0.90**	-0.89**	-0.56 **	-0.88 **	1.00				
TT	-0.91**	-0.90**	-0.54 **	-0.88**	0.99**	1.00			
CT	-0.79**	-0.78 **	-0.66**	-0.79**	0.92**	0.90	1.00		
HT	-0.91**	-0.90 **	-0.53**	-0.89**	0.99**	0.89 **	0.88	1.00	
%TD	0.91**	0.89**	0.53**	0.88**	0.99**	0.89 **	0.99**	0.99	1.00

** Significant at P<0.01

Lactation studies

4.2 Influence of feeding Babul pods (*Acacia nilotica*) on nutrient utilization and milk production in lactating goats

Twenty-four lactating goats were divided into 4 of 6 each in completely randomized block design (CRD). The animals were supplemented with four different diets in the ratio of 50:50 conc: roughage. Babul pods (*Acacia nilotica*) @ 0, 42.0, 63.0 and 63.0 percent were incorporated in concentrate mixture, which were equivalent to tannin concentration of 0, 4, 6 and 6 percent in the total mixed ration (TMR) of I, II, III &IV respectively.

Native babul pods were supplemented in group II while in group III and IV, it was treated with 3 percent Ca $(OH)_{2,}$ and 3% $Ca(OH)_2$ + PEG (1.8%) and supplemented. Green chopped maize fodder was provided as a source of carotene component to meet the vitamin A requirement of the animal.

4.2.1 Chemical composition of experimental diets

The chemical composition of concentrate mixtures and green maize fodder is given in Table 4.8. Organic matter content was ranged from 90.01 percent in concentrate mixture IV to 92.5 percent in concentrate mixture III, while other concentrate showed the variation with in the range. Crude protein (CP) contents of concentrate mixture ranged from 18.82 percent in concentrate mixture III to 19.05 percent in concentrate mixture II. Ether extract contents of the mixture were 4.46, 2.58, 2.35 and 3.01 percent in concentrate mixture I to IV.

Crude fibre (CF) ranged from 10.9% in concentrate mixture I to 13.55 percent in concentrate mixture III. Neutral detergent fibre contents varied from 35.24 percent in concentrate mixture III to 42.34 in concentrate mixture I. Nitrogen free extract (NFE) content were similar between

Table 4.8: Chemical composition (%DM) of concentrate mixtures used during metabolic trial

Parameters	T1	T2	T3	T4	Maize fodder
Organic Matter	92.07±2.41	91.9±1.24	92.5±1.33	92.1±0.81	89.75± 2.34
Crude Protein	18.83±0.34	19.05±0.21	18.82±0.33	18.4±0.24	9.64 ±0.74
Crude Fibre	10.9±0.26	11.98±0.33	13.55±0.29	12.47±0.52	29.66 ±1.13
Ether Extract	4.46±0.12	2.58±0.31	2.35±0.26	3.01±0.23	2.14 ±0.03
Nitrogen Free Extract	57.81±0.24	58.29±0.42	57.78±0.34	58.22±0.27	49.31± 0.34
Neutral Detergent Fibre	42.34±2.34	35.65±4.32	37.24±3.33	35.83±1.43	43.22± 0.21
Acid Detergent Fibre	17.8±0.21	20.45±0.32	24.33±0.45	20.13±0.16	23.02 ±1.43
Hemicelluloses	24.34±0.08	15.2±0.24	12.91±0.13	15.7±0.5	20.20 ±0.84
Total ash	7.93±0.22	8.1±0.22	7.5±0.22	7.9±0.22	9.25± 0.63

T1: Farm concentrate mixture
T2=43.3 % babul pods in concentrate mixture; T3=63.3 %babul pods in concentrate mixture treated with 3% calcium hydroxide; T4=63 .3%babul pods in concentrate mixture treated with 3% calcium hydroxide followed by 1.8% PEG treatment

treatment groups. Maize fodder contained 89.75, 9.64, 29.66, 1.14 and 43.22 percent OM, CP, CF, EE and NDF respectively. Chemical composition of concentrate mixture during metabolic trial ranged OM (91.9 to 92.5%), CP (18.4 to 19.05%), CF (10.9 to 13.55%), EE (2.35 to 4.46%), NFE (58.22 to 58.81%), NDF (35.83 to 42.34%), ADF (17.8 to 24.3%), HC (12.91 to 24.34%) and total Ash (7.5 to 8.1%) in all treatment groups.

Total mixed rations have similar trends of composition in all the treatment groups (Table 4.9). Chemical composition of total mixed ration containing different level of Babul pods is presented in Table 4.9. OM content varied from 90.56 percent in TMR III to 91.88 TMR II. CP contents of the TMRS were 14.83, 14.70, 14.00, 14.75 percent in TMR I – IV, respectively. Crude fibre contents ranged from 20.28 percent in TMR IV to 21.06 percent in TMR I. EE contents of the ration were 2.58, 2.61, 2.49 and 2.55 in TMR I, II, III and IV respectively. NDF contents varied from 40.00percent in TMR II to 42.64 percent in TMR I

4.2.2 Intake of different nutrients during metabolic trial

The intake of different nutrients such as DMI and DOMI differ significantly between treatments (Table 4.10). However, the DIMI under treated or untreated babul pod group remained similar and lower than control diet but digestible ether extract intake was significantly higher in TMI III and IV than TMR I and II. The intake of other nutrients such as CP crude fibre NFE, NDF, ADF and hemicellulose remained similar amongst the treatment groups.

4.2.3 Effect of feeding of Babul pods on nutrient digestibility in lactating goats

Data on digestibility of nutrients are presented in Table 4.11. Dry matter digestibility was 58.82, 59.71, 59.67 and 61.79 percent in group I to IV, respectively which remained similar amongst treatment groups. OM digestibility ranged from 63.10 percent group I to 65.61 percent in-group

Table: 4.9 Chemical compositions (%DM) of total mixed ration containing untreated/treated babul pods

Particularies	OM	CP	CF	EE	NFE	NDF	ADF	HC	Total ash
TMR I (0% babul pods)	90.69±1.23	14.83±0.43	21.06±0.22	2.58±0.13	52.4 ±0.52	42.64±0.34	18.54±0.54	18.36±0.43	9.31±0.25
TMR II (22.2% babul pods)	91.88±1.43	14.70±1.23	20.75±0.15	2.61±0.23	53.83±0.36	40.0±60.18	23.3±50.67	12.99±0.45	8.12±0.23
TMR III* (33.3%babul pods)	90.56±2.02	14.00±0.21	20.83±0.22	2.49±0.22	53.24±0.21	44.41±0.53	28.34±0.56	10±0.24	9.44±0.33
TMRIV (33.3%babul pods)**	91.17±2.43	14.75±0.18	20.28±0.19	2.55±0.45	53.59±0.25	41.29±0.65	24.37±0.43	13.74±0.21	8.83±0.15

TMRII containing babul pods is equivalent to 4% tannins while TMR III &IV were equivalent to 6% tannins respectively

*Babul pods treated with 3% calcium hydroxide,** babul pods treated with both lime and polyethylene glycol

Table 4.10: Effect of feeding treated babul pods on nutrients intake by Lactating goats (metabolic trial)

Particulars	TMRI	TMR II	TMRIII	TMRIV	SEm	CD
Body weight (kg)	40.51 ± 0.50	42.08 ± 0.42	38.92±0.53	40.59 ± 0.36		NS
Metabolic body size (kg)	16.04± 0.11	16.50 ± 0.15	15.57 ± 0.14	16.07 ± 0.16		NS
DMI (kg/day)	1.21	1.09	1.09	1.14	0.06	NS
DMI (kg/100 kg body weight)	3.32 ± 0.09	2.97 ± 0.09	2.95 ± 0.09	3.07 ± 0.09	0.02	NS
CP intake(kg/d)	0.21± 0.04	0.18 ±0.02	0.17± 0.06	0.19± 0.01	0.01	NS
DEEI(g/d)	8.66 ± 0.29	9.24 ± 0.45	9.80 b± 0.33	11.02 ± 0.18	0.15	0.33*
DCFI(kg/d)	0.15± 0.01	0.12± 0.04	0.09± 0.08	0.13± 0.09	0.01	0.03*
DNFEI(kg/d)	0.44± 0.04	0.43± 0.07	0.42± 0.03	0.43± 0.02	0.03	NS
DNDFI(kg/d)	0.33± 0.03	0.27± 0.08	0.31± 0.01	0.32± 0.02	0.04	NS
DADFI(kg/d)	0.09± 0.02	0.11± 0.01	0.14± 0.03	0.14± 0.06	0.03	NS
DHCI(kg/d)	0.23± 0.01	0.18± 0.05	0.17± 0.04	0.18± 0.08	0.0.1	NS

Table 4.11: Effect of supplementation of treated babul pods on digestibility coefficient (%DM) of nutrients in lactating goat

Particularizes	TMR I	TMR II	TMR III	TMR IV	SEm	CD
Dry Matter	58.82±1.75	59.71±1.75	59.07±1.75	61.79±1.75	2.68	NS
Organic Matter	63.11±1.61	64.63 ±1.61	63.62± .61	65.61±1.61	2.69	NS
Crude Protein	69.46±1.48	72.99±1.48	77.06±1.48	77.55±1.48	3.52	NS
Ether Extract	77.89±0.84	81.34±0.84	83.58±0.84	86.8 ±0.84	1.68	5.03*
Crude Fibre	53.82±1.91	49.96±1.91	45.03±1.91	50.56±1.91	3.16	NS
Nitrogen Free Extract	63.95±1.87	66.53±1.87	65.01±1.87	64.50±1.87	0.27	NS
Neutral Detergent Fibre	62.38 ±1.74	56.57 ±1.74	56.38 ±1.74	59.64±1.74	3.05	NS
Acid Detergent Fibre	45.88±2.25	40.16±1.74	40.21±2.25	44.05±1.74	3.26	NS
Hemicelluloses	66.56±1.76	75.85±1.76	77.97 ±1.76	77.30 ±1.76	2.65	7.93*

IV and remained similar amongst the treatment groups. The digestibility coefficients of CP and EE were significantly higher (P<0.05) in group III and IV as compared to group I. But Crude fibre digestibility was lower in treated groups than control and the respective values were 53.82, 49.96, 45.63 and 50.38 in-groups I to IV. However, the values remained similar among the treatment groups. NDF digestibility ranged from 56.38 percent in group III to 62.38 percent in group I and was significantly higher (P<0.05) in group I as compared to the others. NFE digestibility ranged from 63.95 percent in group I to 66.53 percent in group II. ADF digestibility was 45.88, 40.16, 40.21, 44.05 percent in groups I to IV, respectively. It was interesting to note that hemi cellulose digestibility was significantly improved in all the treated diets as compared to control.

4.2.4 Effect of feeding treated babul pods on plan of nutrition and efficiency of nutrient utilization in lactating goats

Dry matter intake (kg/100 kg B Wt.) ranged from 2.95 in group III to 3.32 in group I. Crude protein intake (g/d) was 210, 180, 170 and 190 in group II to IV and I respectively. Digestible crude protein intake (g/d) was 146.12, 131.36, 130.72 and 148.00 in group I to IV respectively. Similar trends followed in NDF intake. On the other hand, DCP intake was higher in group IV (148 g/d) as compared to group II or III.

TDN intake (kg/d) ranged from 0.73±0.03 in group III to 0.79±0.03 in group I and TDN intake (kg/w 0.75) was 49.35, 45.54, 46.95 and 49.78 in groups I - IV, respectively which were also not affected by supplementation of Babul pods. Similar trend was obtained in CP and DCP intakes in different groups. Lactating goats in untreated Babul pods supplemented group (I – II) performed similar as those without Babul pods (control) which implies tannin in Babul pods at 4 percent in diet did not affect the intake of CP, DCN and TDN in lactating goats.

Table- 4.12 Effect of feeding untreated/treated babul pods on Plane of nutrition/Feed conversion efficiency and 5energy and protein utilization efficiency in lactating goats

Particulars	TMR-I	TMR-II	TMR-III	TMR-IV	SEm	CD
DMI (kg/day)	1.21	1.09	1.09	1.14	0.06	NS
DCP intake g/d	145	137	136	149	0.01	NS
DOMI(kg/d)	0.75	0.71	0.70	0.75	0.01	NS
TDN intake, kg/d	0.79	0.75	0.73	0.80	0.10	NS
Milk yield(kg/d)	1.14	1.09	1.13	1.25	0.28	NS
DMI,(kg/kg milk yield)	1.75	1.61	1.60	1.39	0.06	0.35*
DMI(kg/kg FCM)	1.75	1.58	1.61	1.39	0.05	0.35*
Milk protein yield(kg/d)	0.034	0.035	0.036	0.039	0.01	1.18*
Milk energy yield(Mcal)	1.00	1.43	2.12	1.11	0.41	NS
Gross energetic efficiency (%)	36.5	39.38	33.78	41.04	5.38	NS
GP efficiency (%)	38.08	39.75	32.15	41.61	5.12	NS

*P<0.05, NS = non-significant

The effect of feeding untreated/tread babul pods on feed conversion efficiency, energy and protein utilization efficiency were presented on Table4:15

4.2.5 Effect of feeding treated babul pods on milk yield and composition in lactating goats (during metabolic trial)

Milk yield and composition during metabolic trial was presented on Table 4:13.Milk yield and composition were similar among all during the metabolic trial period .However, milk protein yield and milk and total solid yield were significantly higher in TMR-IV as compared to other groups. This was attributed to marginal increase in milk production in this groups as compared to others.

4.2.6 Effect of feeding treated of Babul pods on Nitrogen balances in lactating goats

Balance of nitrogen in lactating goats during metabolic trial is presented in Table 4.14.

Nitrogen intake (g/d) was 33.75, 30.16, 28.63 and 30.64 in TMR I to IV, respectively. Intake of nitrogen was significantly higher ($P<0.05$) in group I as compared to group III. This is because the CP and OM intake of this group was slightly higher. Fecal N (g/d) out put was 7.51, 7.68, 6.55 and 6.77 in group I to IV, respectively. Urinary N (g/d) excretion was 10.43, 9.56, 9.92 and 8.4 in groups I to IV, respectively. It was significantly lower ($P<0.05$) in group IV as compared to group I. Milk nitrogen secretion (g/d) was 5.19, 5.58, 5.98, 6.14 in group I to IV, respectively. Total nitrogen loss (g/d) was not different significantly in all groups. It ranged from 21.32 (g/d) in TMR IV to 23.13 in TMR I.

Nitrogen absorbed as percent intake was significantly higher ($P<0.05$) in TMR I (28.56 g/d) than TMR II (24.57 g/d), group III (22.6 g/d) and TMR IV (24.51 g/d), respectively. Lower nitrogen intake in TMR II to IV as compared to group I was due to low DMI and N intake in TMR II to IV than control. Low intakes of both N and DM in TMR II, III and IV were due to presence of tannin in

the ration which might result in reduced palatability of the offered feed due to astringent effect. More over, fecal N-excretion was higher ($P<0.05$) in TMR II, III and IV as compared to TMR I, which might be due to incomplete breakage of tannin protein complex by the treatments or presence of fiber bound nitrogen with tannin in Babul pod supplemented groups. Higher fecal excretion of N was also reported by Alam *et al.* (2005) when *Albezia* leaves supplemented to basal hay and what bran based diet in goats. Fecal excretion of nitrogen was also higher ($P<0.05$) in TMR III and IV as compared to TMR II which might be due to high level of babul pods in TMR III and IV, respectively.

Part of the effect of reduction in nitrogen balance was attributed to the relatively lower N intake in both group II and group III as compared to group I and IV. However, the net protein utilization Efficiency was higher ($P<0.01$) in group IV as compared to groups I, II and III, respectively.

On the other hand, Rai and Shukla (1977) found negative nitrogen balance by incorporation of 20 percent salseed meal in the diet of lactating cows. However, Alam *et al.* (2005) reported positive nitrogen balance in $Ca(OH)_2$ treated group, PEG treated group and PEG and $Ca(OH)_2$ groups fed on treated Albezia leaves. More over, Ally and Kunjikutty (2003) also reported positive nitrogen balance in goats fed on tree leaves containing 3.4 and 5.5 percent tannin.

4.2.7 Effect of Babul pod supplementation on mineral balance

4.2.7.1 Calcium Balance

The intake, excretion, secretion and balance (g/d) of calcium is presented in Table 4.15. Calcium intake (g/d) was higher in TMR I, III and IV as compared to TMR II. This was due to lower DMI of TMR II and higher calcium intake by calcium hydroxide treatments in TMR III and IV as well as higher DM intake in group I as compared to TMR II. Average calcium balance (g/d) in TMR I to IV was

Table 4.13: Effect of feeding babul pods on milk yield and composition in lactating goats during metabolic trial

Particulars	TMR-I	TMR-II	TMR-III	TMR-IV	SEm	CD
Milk yield(kg/d)	1.14±0.34	1.09±0.45	1.13±0.23	1.25±0.19	0.28	NS
Protein (%)	2.97±0.12	2.95±0.13	2.94±0.17	2.96±0.16	0.23	NS
Fat (%)	3.92±0.11	3.89±0.14	3.86±0.18	3.91±0.15	0.19	NS
SNF(%)	8.30±0.23	8.29±0.25	8.22±0.18	8.28±0.20	0.26	NS
TS(%)	12.22±0.81	12.38±0.74	12.17±0.45	12.31±0.43	0.40	NS
Milk nutrient yield						
Protein(g/d)	34.12	35.22	36.18	39.24	0.22	0.35*
Fat(g/d)	48.4	48.8	49.6	51.0	0.11	NS
SNF(g/d)	94.86	93.52	95.31	106.96	8.74	NS
TS(g/d)	139.81	136.58	139.58	138.09	11.65	1.80*

*= Significant at P<0.05; ** = Significant at P<0.01

Table 4.14: Effect of treated babul pods on nitrogen balance during metabolic trial in lactating goats

Particularies	TMRI	TMRII	TMRIII	TIV	SEm	CD
N intake, g/d	33.75±0.92	30.16±0.92	28.63±0.92	30.64±0.92	1.63	5.01*
Fecal N, g/d	7.5	7.68	6.55	6.77	0.91	NS
Urine, g/d	10.43±0 .37	9.56± 0.37	9.92±0.37	8.4 ±0.37	1.05	NS
Milk N, g/d	5.19±0.30	5.58±0.30	5.98±0.30	6.14±0.30	0.88	NS
Total N out go, g/d	23.13±0.60	22.83±0.60	22.45±0.60	21.32±0.60	1.56	NS
N_balance,g/d	10.61±.01	7.32.01	6.17 ±1.01	9.32 ±1.01	2.12	NS
Absorbed N as %intake	28.56±1.03	24.57±1.03	22.6±1.03	24.51±1.03	5.86	NS
Net protein utilization (%)	42.05 ±1.90	40.85[a]±1.90	41.24±1.90	48.97 ±1.90	4.08	NS
Biological value (%)	59.23 ±2.02	55.56±2.02	53.75[a]±2.02	62.84 ±2.02	4.30	NS

*= Significant at P<0.05; ** = Significant at P<0.01

8.80, 2.04, 8.86 and 27.73, respectively and was significantly higher ($P<0.05$) in TMR I, III and IV as compared to TMR II.

Singh (1979) reported inclusion of Salseed meal at the rate of 45 percent (5.75% tannin) in the concentrate mixture of lactating buffaloes reduced calcium balance.

However, Rai and Shukla did not found effect on calcium balance when Salseed meal was supplemented at 10 and 20 percent in the concentrate mixture of lactating cows. In present study the calcium balance was positive in all the experimental groups. Ally and Kunjkutty, (2003) reported positive calcium balance in goats fed on tree leaves containing 3.4 and 4.5 percent tannin. Barman (2004) also reported positive calcium balance in both growing as well as lactating cow fed on 4% tannins in diets through babul pods.

4.2.7.2 Phosphorus balance

Data on phosphorus intake, excretion, secretion and balance (g/d) are given in Table 4:15. Phosphorus intake was 7.51, 7.05, 6.50 and 7.06 in group I, II, III and IV, respectively. The intake of P was not affected by the supplementation of Babul pods. However, Fecal phosphorus excretion was higher in group III and IV as compared to groups I, II but urinary excretion was slightly lower in group I and III than other groups which might be due to residual tannin in this group may bind phosphorous and increased fecal excretion. Milk secretion of phosphorus was similar in all groups irrespective of difference in daily milk production. Total phosphorus loss was significantly higher in group III and IV as compared to group I and II.

The overall phosphorus balance remained similar in different group and it was positive in all the dietary groups. The similar trend was noticed for absorbed phosphorus as percent intake in different groups.

The above findings corroborate the findings of Barman (2004) who also found positive phosphorus balance in lactating cows fed on babul pods to the extent of 4% tannin in their diet as well as 6% tannin in diets of growing calves. Thereby it is of indicative that supplementation of babul pods containing 4% tannins in diet did not alter phosphorus balance in ruminants.

4.2.8 Effect of feeding treated Babul pods on fortnight nutrient intake and utilization.

Fortnightly nutrient intake during lactation trial is presented in Table 4.16. The overall body weight during 8 fortnight experimental period revealed that higher body weight was achieved in TMR II than other dietary treatment groups. However, other groups remained similar to each other. The respective average body weights were 40.51, 42.08, 38.91 and 4.59 kg/animal with usual variation in body weight during the experimental period. Similar trend was observed when the data were converted into metabolic body size and the respective values were 16.03, 16.49, 15.57, 16.08 kg/animal in groups I to IV.

The overall DMI (Kg/d) in different treatments remained similar amongst each other and the values were 1.19, 1.15, 1.17 and 1.20 for TMR I to IV, respectively during whole experiment and the respective values for DMI, kg/ 100kg BW were 3.26, 3.11, 3.39 and 3.25 in TMR I to IV. Similar values were obtained with in different dietary groups with regard to DCP intake 145, 137, 136, 149 (g/d), DCP intake 9, 8, 8, 9 (g/kg W 0.75), TDN intake 0.75, 0.75, 0.73, 0.80 (kg/d) and TDN intake 49, 45, 47, 49 (g/kg w).

4.2.9 Effect feeding treated Babul pods on milk yield, composition and milk nutrient yield during whole lactation period

The overall milk yield during and fortnight period showed a decreasing trend as the lactation period progressed in all the treatment groups. Similar trend was also noticed for FCM production over the period

Table 4.15: Effect of supplementation of babul pods on calcium and phosphorous balances in lactating goats

Particularies	**Treatments**					
Calcium	TMR-I	TMR-II	TMR-III	TMR-IV	SEm	CD
Ca intake, g/d	13.20	4.97	11.70	12.32	1.76	5.75*
Fecal Ca, g/d	2.96	1.20	1.74	2.57	10.49	NS
Urine Ca, g/d	0.05	0.031	0.03 2	0.03	0.004	0.014**
Milk Ca, g/d	1.38	1.07	1.07	1.98	0.35	NS
Total Ca out go, g/d	4.39	2.92	2.84	4.59	0.35	NS
Ca balance, g/d	8.80	2.04	8.86	7.73	1.75	1.71*
Absorbed Ca as %intake	30.24	21.55	29.96	29.74	0.09	NS
Phosphorous						
P intake, g/d	7.51	7.05	6.50	7.06	1.48	NS
Fecal P, g/d	1.43	1.88	3.22	2.27	0.27	0.87*
Urine P, g/d	0.38	0.51	0.13	0.53	0.07	0.22**
Milk P, g/d	1.00	1.07	1.09	1.14	0.004	NS
Total P out go, g/d	2.81	3.47	4.44	3.95	029	0.97*
P balance, g/d	3.69	3.58	3.06	3.11	1.38	NS
Absorbed P as %intake	6.08	5.16	3.28	4.79	0.08	NS

*= Significant at P<0.05; ** = Significant at P<0.01

Table 4.16: Effect of supplementation of treated babul pods on fortnightly body weight, dry matter intake, feed conversion efficiency, DCP and TDN intakes in lactating goats

Attributes	Group	Fortnight								
		1	2	3	4	5	6	7	8	Mean± SE
Body weight (kg)	T1	38.33±1.42	39.41±1.41	40.58^{ab}±1.6	40.66±1.42	41.85±1.31	41.39±1.25	40.861.63	40.66±1.61	40.51±0.44
	T2	39.91^{b}±1.5	40.9 ^{b}±1.22	42.33^{ab}±1.6	43.25^{a}±1.6	43.16^{a}±1.4	42.98^{ab}±1.5	42.0^{ab}0±1.6	42.00^{ab}±1.2	42.08±0.55
	T3	37.33±1.61	38.08±1.68	38.91±1.12	39.3 ±1.41	39.58±1.37	39.91±1.28	39.91±1.41	38.91±1.32	38.91±0.64
	T4	38.75±1.11	40.03±1.61	40.83±1.34	41.08±1.32	41.66±1.50	41.81±1.21	40.88±1.62	40.08±1.91	40.59±0.52
	SEm	0.86	1.18	1.40	1.64	0.77	1.28	1.39	1.28	0.43
	CD									1.23**
Met.Body size (kg)	T1	15.39±0.42	15.72±0.36	16.07±0.37	16.09±0.31	16.44±0.35	16.30±0.72	16.15±0.32	16.08±0.62	16.03±0.10
	T2	15.86±0.37	16.17±0.34	16.58±0.34	16.84±0.22	16.82±0.62	16.78±0.34	16.48±0.32	16.47±0.32	16.49±0.21
	T3	15.09±0.35	15.32±0.32	15.57±0.22	15.70±0.38	15.78±0.34	15.87±0.32	15.57±0.32	15.62±0.42	15.57±0.41
	T4	15.52±0.62	15.90±0.12	16.14±0.12	16.22±0.24	16.39±0.12	16.43±0.28	16.16±0.32	15.92±0.37	16.08±0.15
	SEm	0.32	0.35	0.41	0.47	0.43	0.38	0.36	0.35	0.42
	CD									NS
DMI(kg/d)	T1	1.21±0.04	1.37±0.04	1.11±0.05	1.09±0.03	1.25± 0.04	1.09± 0.06	1.20±0.01	1.18±0.06	1.27
	T2	1.16± 0.03	1.34 ± 0.06	1.15 ±0.07	1.11±0.05	1.24 ±0.01	1.12± 0.05	1.27 ±0.04	1.22 ± 0.04	1.23
	T3	1.23± 0.01	1.24± 0.03	1.12±0.04	1.10 ± 0.04	1.23 ±0.09	1.10± 0.06	1.15 ± 0.03	1.25 ± 0.03	1.16
	T4	1.618 ±0.02	1.36± 0.01	1.17±0.02	1.22±0.02	1.25±0.02	1.11±0.02	1.16±0.05	1.28 ± 0.02	1.34
	SEm	0.21	0.06	0.03	0.06	0.01	0.01	0.05	0.04	0.03
	CD									0.08**
DMI(kg/100kg BW)	T1	3.51±0.14	3.79±0.15	3.01±0.18	2.96±0.18	3.22±0.16	3.42±0.17	3.60±0.14	3.32 ±0.11	3.26 ^{b} ±0.08
	T2	3.25±0.12	3.57±0.10	2.96±0.12	2.77 ±0.16	3.08±0.22	3.18±0.18	3.38 ±0.13	3.16±0.16	3.11± 0.09
	T3	3.67±0.16	3.86±0.14	3.21±0.15	3.09±0.12	3.39 ±0.17	3.50±0.14	2.74±0.12	3.39±0.12	3.39^{a}±0.04
	T4	3.37±0.11	3.68±0.12	3.11±0.14	2.98±0.11	3.24±0.12	3.27±0.15	3.43 ±0.11	3.32±0.14	3.25^{b}±0.07
	SEm	0.02	0.01	0.12	0.11	0.02	0.31	0.13	0.12	0.03
	CD									0.11*

4.16 continued

DCP Intake (g/d)	T1	144.12±5.34	145.22±3.44	148.12±5	143.15±3.7	147.33±5.75	145.24±3.73	149.23±5.37	145.12±3.22	145.12±2.13
	T2	133.22±4.53	136.70±5.21	136.75±5.74	131.15±5.4	138.72±4.37	136.79±5.44	130.12±5.73	135.94±5.41	136.72±2.04
	T3	136.23±3.72	134.22±2.74	138.22±5.74	133.02±3.74	136.45±3.74	131.21±4.74	143.02±4.23	138.31±3.74	136.32±1.98
	T4	146.21±5.74	149.11±5.14	144.25±5.74	149.21±5.14	151.42±4.24	145.41±5.64	148.42±5.74	147.31±6.74	149.21±2.03
	SEm	5.65	5.65	5.65	4.85	5.42	5.35	4.64	5.32	2.01
	CD									NS
TDN intake, kg/d	T1	0.78±0.03	0.79±0.02	0.80±0.07	0.79±0.04	0.77±0.08	0.81±0.05	0.79±0.07	0.78±0.03	0.79±0.004
	T2	0.75±0.04	0.74±0.01	0.73±0.03	0.77±0.09	0.73±0.07	0.75±0.02	0.75±0.03	0.74±0.08	0.75±0.007
	T3	0.74±0.04	0.76±0.06	0.72±0.02	0.75±0.04	0.73±0.04	0.72±0.09	0.71±0.01	0.70±0.01	0.73±0.005
	T4	0.80±0.02	0.81±0.05	0.83±0.05	0.82±0.02	0.81±0.01	0.82±0.01	0.79±0.02	0.80±0.02	0.80±0.009
	CD									NS

in all the treatment groups. The overall mean values for milk yield (Kg/day) were 1.20, 1.18, 1.24 and 1.33 in TMR I to IV, respectively. The usual decreasing trend over lactation period of 8 fortnights was from 1.48 to 1.10, 1.42 to 1.03, 1.48 to 1.16 and 1.49 to 1.22 kg/day/animal in the groups I to IV, respectively. The FCM yield was 1.21, 1.17, 1.24 and 1.34 kg/day in the respective treatments. (Table-4.17)

The yields of fat and proteins (g/day) followed the similar trend in different treatments. The respective values were 48.51, 48.58, 49.70 and 53.86 g/day in groups I to IV, respectively.

The data on SNF and TS revealed that the values in group I and II are quite similar however, the same was found higher in groups III and IV but the values between first two groups (I and II) and last two groups (III and IV) remained similar (Table-4.17). The results clearly showed that there was no difference between in TMR I and TMR II) as well as in TRM III and IV. That means that control as well as native babul pods performed in a similar way in production performance while TMR III and TMR IV were found better in production performance where time treatment as well as lime plus PEG treatments was done. The addition in organic Ca through calcium hydroxide treatment of babul pods provide more calcium for lactating goats which helps in better milk production performance in goats.

4.2.10 Effect of supplementation of treated babul pods on milk composition (%) at fortnight interval in lactating goats

The detail results with regard to percentage of protein, fat, SNF and Total Solids in different fortnights have been presented in the Table 4.18. The data clearly showed that all the parameters study under present investigation have similar values and remained statistically non significant amongst all the treatment groups.

Table 4.17: Effect of feeding untreated/treated babul pods on average fortnightly milk yield and composition in lactating goats

Attributes	Group	Fortnight								
		1	2	3	4	5	6	6	8	Overall
Milk yield, kg/d	T1	1.48±0.09	1.30±0.13	1.22±0.21	1.19±0.21	1.13±0.08	1.15±0.16	1.05±0.12	1.10±0.11	1.20±0.05
	T2	1.42±0.08	1.37±0.21	1.25±0.09	1.20 ±0.30	1.12±0.11	1.12±0.13	1.01±0.13	1.03±0.11	1.18±0.03
	T3	1.48±0.11	1.44±0.08	1.25±0.10	1.22±0.12	1.12±0.21	1.15±0.06	1.04±0.14	1.16±0.11	1.24±0.04
	T4	1.49±0.12	1.48±0.13	1.38±0.12	1.35±0.11	1.27 ±0.14	1.28±0.09	1.18±0.10	1.22±0.11	1.33±0.02
	SEm	0.09	0.08	0.10	0.09	0.07	0.08	0.10	0.11	0.03
	CD									NS
4%FCM (kg/d)	T1	1.55±1.12	1.28±1.11	1.24±1.20	1.18±1.12	1.12±1.15	1.13±1.12	1.03±1.12	1.11±1.13	1.21±0.28
	T2	1.48±1.13	1.38±1.14	1.26±1.18	1.22±1.10	1.17±1.12	1.09±1.20	1.02±1.11	1.01±1.14	1.17±0.29
	T3	1.57±1.14	1.42±1.12	1.24±1.16	1.21±1.11	1.14±1.10	1.12±1.16	1.03±1.08	1.15±1.12	1.24 ±0.30
	T4	1.52±1.10	1.49±1.17	1.40±1.10	1.36±1.09	1.28±1.14	1.25±1.12	1.19±1.14	1.22±1.17	1.34 ±0.31
	SEm	1.08	1.07	1.09	1.06	1.05	1.06	1.09	1.08	0.24
	CD									NS
Fat (g/d)	T1	63.96±4.34	51.11±3.38	50.27±4.52	47.28±4.28	44.72±5.48	44.94±4.43	41.13±3.28	44.67±3.24	48.51^{b}±1.32
	T2	61.09±3.98	55.98±4.41	51.18±3.48	49.51±5.08	45.88±4.97	42.75±5.08	41.51±2.44	40.70±4.78	48.58^{b}±1.28
	T3	65.44±4.47	56.76±5.57	49.86±5.47	48.59±4.48	45.55±4.68	42.27±4.46	41.25±6.08	45.86±4.53	49.70^{b}±1.54
	T4	61.67±5.26	60.04±4.38	56.35±4.38	54.99±4.48	51.64±5.48	49.14±4.43	42.85±4.41	49.27±4.38	53.86^{a}±1.59
	SEm	3.30	4.38	3.32	3.66	4.75	3.39	3.32	3.32	1.34*
	CD									1.26
Protein (g/d)	T1	43.14±3.15	37.98±3.26	35.83±4.26	33.30±3.24	33.03±4.26	35.19±3.27	31.47±3.46	30.56±3.45	35.06±1.14
	T2	41.45±3.36	40.08±3.26	37.52±3.15	35.38±4.24	30.57±3.15	34.83±2.36	30.92±4.26	29.80±3.26	35.07±1.13
	T3	46.16±3.08	41.92±2.98	36.82±3.33	35.24±4.20	31.67±2.26	33.11±3.15	32.41±2.97	32.96±2.65	36.29±0.95
	T4	45.28±3.26	42.66±3.15	41.21±3.26	37.90±3.26	42.84±3.26	40.01±4.26	35.42±3.12	32.96±4.00	40.0±1.15
	SEm	3.16	4.14	3.18	2.17	2.13	4.16	3.13	3.19	1.09
	CD									NS

Table 4.17: continued…

	Group	Fortnight								
		1	2	3	4	5	6	7	8	Overall
SNF (g/d)	T1	120.74±9.23	108.16±10.21	122.31±9.24	99.91±8.23	94.14±10.24	94.86±9.03	86.28±8.73	91.99±9.22	99.77±3.43
	T2	116.94±9.23	113.79±9.43	102.05±10.21	100.40±10.24	92.99±9.63	93.52±8.23	84.28±9.25	84.79±8.46	98.81±4.26
	T3	121.91±9.23	119.35±9.25	103.76±9.46	100.87±8.27	94.41±10.28	95.31±9.43	85.71±8.29	96.80±10.52	123.41±2.25
	T4	121.54±9.23	122.30±9.16	103.64±9.64	111.62±9.23	106.02±10.23	106.96±9.09	97.92±9.28	100.12±9.64	110.11±3.46
	SEm	8.26	7.46	8.53	7.56	9.07	8.74	6.56	6.74	3.32
	CD									9.82*
Total solids (g/d)	T1	184.71±13.47	159.27±13.66	152.32±13.38	147.19±14.68	138.87±13.46	139.81±13.60	127.42±14.66	136.66±13.45	148.28^{b}±3.83
	T2	78.03±14.36	169.78±14.56	154.93±11.67	149.91±13.67	138.89±12.64	136.27±12.64	125.81±12.62	125.49±12.64	147.39^{b}±4.45
	T3	187.35±12.65	176.11±12.73	153.51±12.66	149.46±12.76	139.96±11.47	139.58±11.36	126.97±13.56	142.66±13.62	151.95^{b}±5.46
	T4	183.21±14.55	182.34±14.65	170.70±13.76	166.61±13.06	157.66±12.06	156.09±10.64	145.77±13.06	149.39±11.66	163.97^{a}±4.88
	SEm	12.98	12.98	11.94	13.92	14.78	11.65	12.90	12.43	3.84
	CD									10.00*

*= Significant at P<0.05; ** = Significant at P<0.01

Table 4.18: Effect of supplementation of treated babul pods on milk composition (%) at fortnight interval in lactating goats

Attributes	group	Fortnights								
		1	2	3	4	5	6	7	8	Overall
Protein (%)	T1	2.91±0.12	2.92±0.12	2.92±0.15	2.81±0.12	2.94±0.43	3.18±0.23	3.00±0.14	3.10±0.12	2.97±0.08
	T2	2.93±0.13	2.91±0.03	2.99±0.06	2.97±0.16	2.73±0.33	3.09±0.16	3.05±0.23	2.80±0.23	2.94±0.07
	T3	3.11±0.14	2.94±0.14	2.98±0.23	2.87±0.14	2.76±0.16	3.83±0.32	3.12±0.35	2.72±0.16	2.92±0.05
	T4	3.05±0.12	2.84±0.15	2.96±0.14	2.47±0.17	3.41±0.45	3.09±0.13	3.042±0.24	2.85±0.15	2.96±0.14
	SEm	0.16	0.18	0.17	0.14	0.13	0.19	0.13	0.12	0.039
	CD									NS
Fat (%)	T1	4.32±0.07	3.92±0.24	4.04±0.06	3.94±0.06	3.92±0.05	3.90±0.08	3.92±0.02	3.98±0.04	4.0 ±0.06
	T2	4.26±0.28	4.09±0.04	4.09±0.05	4.09±0.04	4.0±0.09	3.80±0.08	4.09±0.06	3.99±0.01	4.05±0.09
	T3	4.42±0.08	3.96±0.05	3.98±0.09	3.98±0.07	3.95±0.01	3.79±0.08	3.97±0.05	3.94±0.05	4.00±0.03
	T4	4.22±0.13	4.03±0.21	4.05±0.17	4.05±0.11	4.03±0.07	3.80±0.14	4.03±0.15	4.05±0.11	4.03±0.04
	SEm	0.09	0.04	0.08	0.05	0.09	0.03	0.01	0.04	0.07
	CD									NS
SNF (%)	T1	8.18±0.02	8.29±0.03	8.32±0.06	8.34±0.05	8.30± 0.07	8.21±0.03	8.21 ±0.07	8.31 ± 0.04	8.27±0.01
	T2	8.20±0.03	8.29±0.05	8.29±0.09	8.29±0.07	8.21 ±0.02	8.31±0.08	8.2 ± 0.08	8.30± 0.05	8.28±0.03
	T3	8.10±.04	8.23±0.08.	8.23± 0.06	8.22 ± 0.04	8.22± 0.01	8.21±0.05	8.22± 0.04	8.30± 0.07	8.23±0.02
	T4	8.15±0.01	8.23± 0.04	8. 13±.04	8.24±0.19	8.28±0.04	8.31±0.04	8.28 ±0.03	8.24 ±0.04	8.24±0.04
	SEm	0.04	0.02	0.01	0.04	0.06	0.09	0.07	0.08	0.01
	CD									NS
Total solids (%)	T1	12.57±0.04	12.20±0.07	12.37±0.05	12.28±0.08	12.22±0.09	12.11±0.04	12.13±0.07	12.34±0.04	12.28±0.04
	T2	12.46±0.02	12.38±0.01	12.38±0.04	12.38±0.07	12.38±0.02	12.38±0.07	12.1±0.01	12.38±0.02	12.35±0.03
	T3	12.61±0.04	12.19±0.07	12.24±0.05	12.20±0.08	12.17±0.04	12.02±0.08	12.20±0.07	12.24±0.06	12.23±0.05
	T4	12.37±0.08	12.2±0.08	12.29±0.09	12.29±0.08	12.31±0.05	12.12±0.06	12.31±0.06	12.29±0.01	12.28±0.02
	SEm	0.06	0.06	0.02	0.07	0.03	0.06	0.04	0.09	0.01
	CD									NS

*= Significant at P<0.05; ** = Significant at P<0.01

5. SUMMARY AND CONCLUSION

5.1 EFFECT OF CHEMICAL TREATMENTS ON CHEMICAL COMPOSITION, *in vitro* NUTRIENT DIGESTIBILITY AND PARTITIONING OF TANNINS OF BABUL PODS

Babul pods was first treated with different levels of PEG (0.05, 0.10, 0.15, 0.20, 0.25 PEG: tannin), In another trail babul pods were treated with 3 percent $Ca(OH)_2$ fallowed by different levels of PEG in combination as mentioned above levels in first trial. The result revealed that OM, CP, NDF and ADF content of the PEG treated Babul pods reduced significantly ($P<0.05$), while the total ash content increased significantly with increasing level of PEG. In the second trial the calcium hydroxide treatment has shown solubilization of organic constituents but when it was further treated with PEG, the associative effect was noticed only to aftect OM and ADF contents which were lower down while ash contents further increased due to PEG treatment over Ca $(OH)_2$ treatment.

The *in vitro* digestibility of DM, OM, CP and gas production increased ($P<0.01$) due to PEG over native babul pods. In the second trial where $Ca(OH)_2$ treatment was done, showed considerably high *in vitro* digestibility of treated babul pods but when it was further treated with PEG, could not bring appreciable changes in digestibility of any of the parameter over control.

All tannin components of Babul pods reduced significantly ($P<0.01$) as the level of PEG treatment increased from 0.05 to 0.25 PEG: tannin ratio. The tannin degradation was significantly ($P<0.01$) increased up to 67.26% with PEG treatment in first trial, whereas in second trial with calcium hydroxide showed still better degradation of all the components and the tannin degradation was to the extent of 81.94% due to lime

treatment over native babul pods but further treatment with PEG after lime treatment did not result much in the individual components of tannin but overall tannin degradation of increased from 81.94 to 93.20% due to addition of PEG over lime treatment. The trend was also similar as in *in vitro* digestibility of various nutrients.

5.2 EFFECT OF FEEDING UNTREATED/TREATED BABUL PODS ON DRY MATTER INTAKE, NUTRIENT UTILIZATION, MILK PRODUCTION EFFICIENCY AND BALANACES OF N, Ca AND P IN LACTATING GOATS

Twenty four lactating goats were divided into four groups of six each in a completely randomized design and allotted four diets. The diet consisted of green maize fodder and concentrate in 50: 50 ratio (TMR I) where as in TMR II untreated babul pods was added to the extent of 22.2% while, in TMR III, the babul pods were (33.3%) treated with 3% $Ca(OH)_2$ and TMR IV, the babul pods were (33.3%) treated with 3% $Ca(OH)_2$ + PEG (0.10 PEG : Tannin). All diets were isonitrogenous and isocaloric. The feeding trail continued up to 8 fortnights.

The data with regard to DMI, DOMI, CPI, TDNI remained similar amongst the dietary groups and did not differ significantly from one to another. Similarly, the digestibility coefficients of different nutrients such as DM, OM, CP, CF, NFE, NDF, ADF remained similar amongst different groups. However, the digestibility coefficients of EE and HC increased significantly ($P<0.05$) in treated groups II to IV than control (TMR I). The different components of milk such as fat, protein, SNF, TS in terms of percentage or yield (g/d) remained similar amongst different dietary groups.

The plane of nutrition data clearly indicated that the dietary treatments did not influence the intake of either DCP or TDN or DOMI as compared to control. The milk yield increased only in TMR IV where as it was similar in TMR I to III. The feed conversion efficiency (DMI, kg/kg milk

production) also showed similar trend and was better than control (1.75 versus 1.39 kg DMI/kg MY). Similarly the gross energetic efficiency (%) was comparatively higher in treated group than control diet but the gross protein efficiency remained similar across the dietary treatments.

The balances of N, Ca and P data showed positive balance in all the dietary treatment groups. However, N and P balance remained similar among the groups but Ca balance was found variable amongst diets.

The periodical data obtained during 8 fortnights with respect of body weight clearly indicated that the TMR II where native babul pods used had significantly higher body weight than other groups. The DMI/day data in TMR III showed lower ($P<0.01$) value than control. Other parameters remained similar amongst dietary treatment groups.

Conclusion

It is concluded that both PEG as well as lime treatment of babul pods resulted significant improvement in the digestibility of nutrients *in vitro* as well as tannin degradation. However, combination of lime with PEG was not found effective in improving the nutritive value of babul pods.

The incorporation of native babul pods was equally good as control diet in terms of performance. Therefore, inclusion of babul pods in the ration of lactating goats up to 22% in TMR (equivalent to 4% tannin in diet) could be safely made with out affecting performance of animal. In case the babul pods to be increased from 22% (4 % tannin) to 33% (6% tannin) in TMR, the treatment either with lime or PEG may be needed to neutralize the excess tannin influence on the performance of the animal.

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