STUDIES ON SOME LOCALLY AVAILABLE TREE LEAVES AND THEIR SYSTEM OF FEEDING IN GADDI GOATS

THESIS

Submitted in partial fulfilment of the requirement for the degree

of

Master of Veterinary Science

in

ANIMAL NUTRITION

By

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Roll No. 4769

To

DEEMED UNIVERSITY

INDIAN VETERINARY RESEARCH INSTITUTE

IZATNAGAR - 243 122 (U.P.)

2012

Dedicated to....

My beloved parents

&

Guide

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Certified that the research work embodied in this thesis entitled "Studies on some locally available tree leaves and their system of feeding in Gaddi goats" submitted by Dr. Bharatbhushan M., Roll No. 4769, for the award of Master of Veterinary Science degree in Animal Nutrition at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.

It is further certified that Dr. Bharatbhushan M., Roll No. 4769, has worked for more than 21 months in this Institute and has put in more than 150 days attendance under me from the date of registration for the degree of Master of Veterinary Science of the Deemed University, as required under the relevant ordinance.

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(BharatBhushan. M)

Abbreviations

%	Percentage
@	At the rate of
µg	Microgram
µmol	Micromole
^{0}C	Degree celcius
A.M.	Ante meridiem (before noon)
A:P	Acetate to propionate ratio
ADF	Acid detergent fibre
ADG	Average daily gain
ADL	Acid detergent lignin
ALP	Alkaline phosphatase
ALT	Alanine amino transferase.
AST	Aspartate amino transferase
BSA	Bovine serum albumin
Ca	Calcium
$CaCl_2$	Calcium chloride
Cal	Calorie
CF	Crude fibre
CL	Chopped leaves
CO_2	Carbon di-oxide
$CoCl_2$	Cobalt chloride
CP	Crude protein
CPI	Crude protein intake
CSD	Chopped sun dried
CSDG	Chopped sun dried and ground
CSDGL	Chopped sun died and ground leaves
CSDL	Chopped sun dried leaves
CSDTH	Chopped sun dried tremal hay
CT	Condensed tannins
d	Day
DAM	Diacetylmono-oxime

DCP	Digestible crude protein
DE	Digestible energy
dl	Decilitre
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DOM	Digestible organic matter
EDTA	Ethylenediaminetetra acetic acid
EE	Ether extract
FAO	Food and Agricultural Organisation
F-C	Folin-ciocalteu
$FeCl_3$	Ferric chloride
FL	Fresh leaves
FR	*Ficus roxburghii*
g	Gram
GLC	Gas liquid chromatography
GNC	Groundnut cake
h	Hour
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
Hr	Hour
HT	Hydrolysable tannins
IVDN	*In vitro* degradable nitrogen
IVGPT	*In vitro* gas production technique
J	Joule
K	Kilo
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
l	Litre
Mcal	Megacalories
ME	Metabolisable energy
mg	Milligram
$MgSO_4$	Magnesium sulphate

ml	Milli litre
mm	Milli metre
mmol	Millimole
$MnCl_2$	Manganese chloride
N	Normality
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bi- carbonate
NaOH	Sodium hydroxide
NDF	Neutral detergent fibre
NFE	Nitrogen free extracts
NH_3	Ammonia
NH_3-N	Ammonia nitrogen
$NH_4H\ CO_3$	Ammonium bi-carbonate
nm	Nano metre
NTP	Non-tannin phenols
NWHR	North west himalayan region
O	Ortho
OD	Optical density
OM	Organic matter
OMD	Organic matter digestibility
P.M.	Post meridiem (after noon)
PEG	Poly ethylene glycol
PF	Partitioning factor
PP	Potassium permanganate
ppm	Parts per million
PSM	Plant secondary metabolites
PVPP	Polyvinylpolypyrrolidone
QL	*Quercus leucotrichophora*
R^2	Coefficient of determination
rpm	Revolution per minute
RUSITEC	Rumen simulation technique
S	Standard
SEM	Standard error of mean
SRL	Strained rumen liquor

STP	Serum total protein
t	Tonne
TA	Total ash
TC	Total carbohydrate
TCA	Trichloroacetic acid
TDN	Total digestible nutrients
TP	Total phenols
TT	Total tannins
TTP	Total tannin phenols
TVFA	Total volatile fatty acids
UT	Untreated tremal
UTTH	Urea treated tremal hay
VFA	Volatile fatty acids
$W^{0.75}$	Metabolic body weight
w/w	Weight by weight
WA	Wood ash

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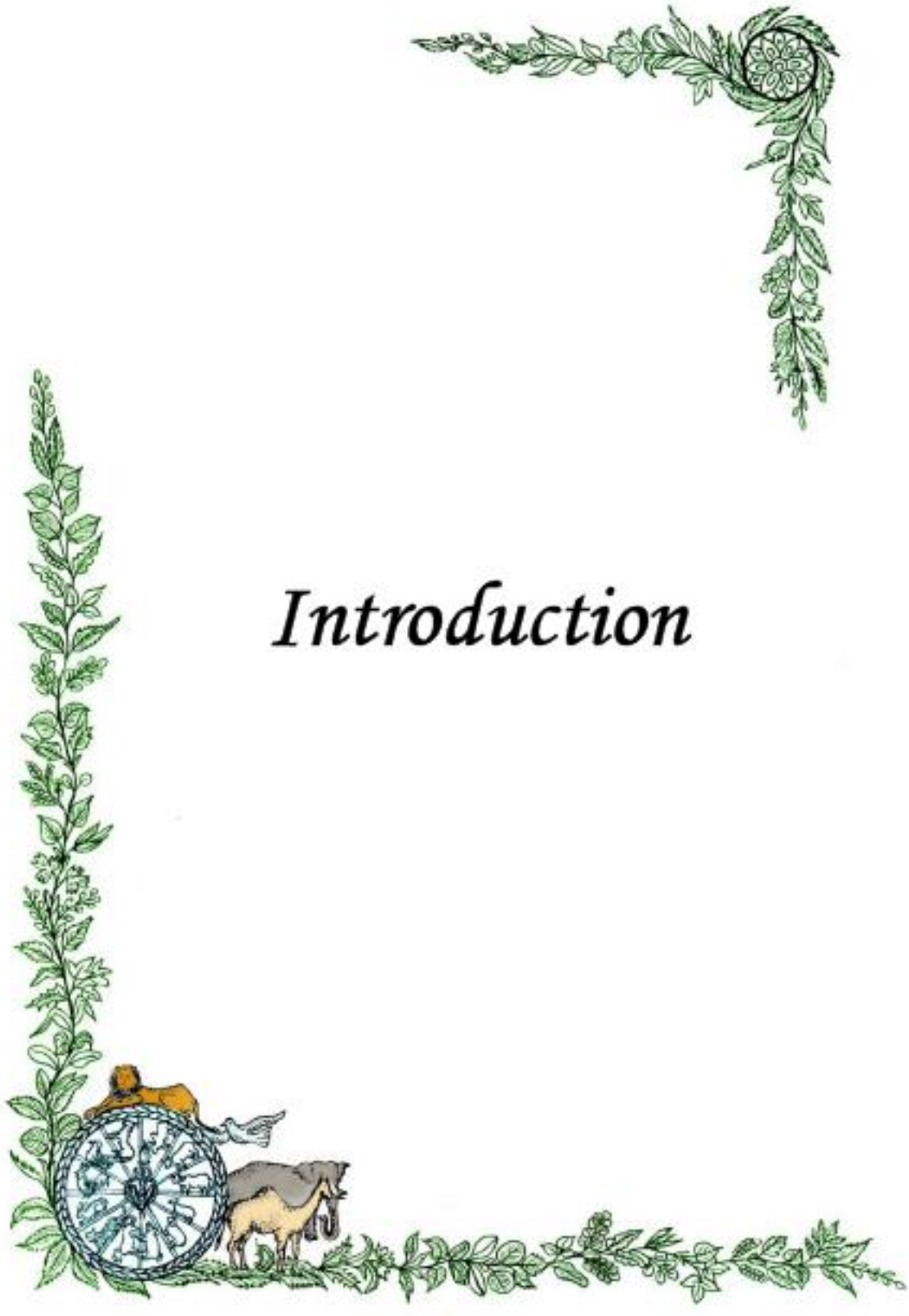


Introduction

Introduction

Inadequate availability of straws and conventional concentrates is one of the major constraints for rearing large and small ruminants in the North West Himalayan Region (NWHR). Development of alternate feeds out of locally available tree leaves may partially solve the problem of animal feeding, particularly, during scarcity period for maintenance of ruminants. Most of the tree leaves available for the feeding of animals contain varying amounts of anti-nutritional factors viz. tannins, saponins, alkaloids etc.

Grewia optiva (biul) is a good quality tree forage available adequately for feeding of small and large ruminants in the area. The Digestible Crude Protein (DCP) and Total Digestible Nutrients (TDN) values of biul are about 15.0 and 53.0 (% on DM basis) in cattle (Negi *et al.*, 1979). There are some other tree leaves like *Ficus roxburghii* and *Quercus leucotrichophora* available in this region in adequate quantity. However, their nutritive values are unsatisfactory due to high concentration of anti-nutritional factors, especially, tannins (Barman and Rai, 2000; Singh and Bhat, 2001). Tannins characteristically bind proteins. The strength and nature of binding depends on the chemical nature of the reactive phenolic groups (Spencer *et al.*, 1988). The affinity of tannins for protein increases with the increase in molecular size of tannins (Kumar and Horigome, 1986). When present in large quantities, tannins reduce forage quality and adversely affect herbivore nutrition by reducing intake and protein

digestibility by inhibiting rumen microbes and digestive enzymes (Makkar *et al.*, 1988; Jones *et al.*, 1994; Reed, 1995; Lowry *et al.*, 1996; McNabb *et al.*, 1998; Barry and McNabb, 1999; Min *et al.*, 1999; Silankove *et al.,* 2001; Min and Hart, 2003; Sharma *et al.*, 2008; Belewu and Olajide, 2010).

Strategies for overcoming the detrimental effects of feeding tannin rich feeds are thus suggested in number of studies (Bhar *et al.*, 1996; Makkar, 2003). Recently, a number of reviews have discussed the potential of tannins as modifiers of rumen microbial fermentation and ruminant production (Wallace *et al.,* 2002; Wallace, 2004; Calsamiglia *et al.,* 2007; Hart *et al.,* 2008; Patra and Saxena, 2009; Alonso-Diaz *et al.,* 2010). In small quantities, tannins, especially condensed tannins (CT), are useful as they prevent bloat, protect proteins and prevent establishment of gastrointestinal parasites (Coop and Holmes, 1996; Niezen *et al.*, 1998; Molan *et al.*, 2000a, b; Hoskin *et al.*, 2000; Athanasiadou *et al.*, 2000). Many other beneficial biological properties of tannins (viz. antioxidant, antimicrobial, antiviral and anthelmintic property etc.) have also been reported by different workers (Serrano *et al.,* 2009).Thus efforts are being made to bring the level of tannin at its optimum, to overcome its anti-nutritional effects and to promote its beneficial effect in animal production and health.The contents of anti-nutritional factors (viz., tannins, saponins) are relatively less in *Grewia optiva* and its palatability is very good. Therefore, biul is in great demand in the NWHR for feeding of ruminants. Due to the limited availability of biul, development of alternate feeding systems based on available alternate tree leaves has become one of the promising areas of animal nutrition research in NWHR. Use of tree leaves with optimum level of tannins is thus a promising area to develop an alternate diet for hill ruminants.

The detannified tree leaves have not been evaluated sufficiently by animal feeding experiments. Detannification studies have not yet been reported on *F. roxburghii. F. roxburghii* (Tremal) and *Q. leucotrichophora* (bun) are adequately available even during scarcity period *i.e.*, winter months. Thus, the present study has been planned with the following objectives:

OBJECTIVES

1) Detannification of *Q. leucotrichophora* and *F. roxburghii* leaves by physical and chemical methods in different combinations.

2) Evaluation of the detannified tree leaves by *in vitro* and *in vivo* experiments in growing goats for development of alternate diets.

Review of Literature

Review of Literature

India has the largest (15%) livestock population of the world, which is thriving only on 2% of global area. India ranks 10th in the list of nations with most forest cover in the world with 68 million ha of forest and tree cover (FAO, 2010). A major constraint to animal production in developing countries like India is the scarcity and fluctuating quantity and quality of the year-round feed supply. Lack of adequate year-round feed resources is probably the most important factor contributing to low animal production (Ben Salem and Smith, 2008; Kawas *et al.,* 2010). By the year 2020, world population is expected to reach eight billion and most of the population growth will occur in developing countries (FAO, 2010).Under these circumstances, food grains are required exclusively for human consumption rather than animal feeding.

With increasing demand for livestock products as a result of rapid growth in the world economies and shrinking land area, future hopes of feeding the millions and safeguarding their food security will depend on better utilization of unconventional feed resources that do not compete with food for human beings. The pressure on cultivable land for food grains, pulses and oilseeds is increasing day by day leaving no scope for future expansion of area under fodder crops. This has resulted in huge gap in the demand and supply of the fodder. Thus, locally available tree leaves and forages seem to be a viable alternative for the feeding of hill sheep and goats.

FEEDING OF TREE LEAVES

In Himachal Pradesh there exists a shortage of dry and green forages to the extent of 35.0 and 57.0%, respectively. Every year, on an average about 7450t of wheat straw is imported from the neighbouring states (Vashist *et al.*, 2000). In this context, fodder trees are of great importance as a major feed resource, especially, during lean period (Jiban, 2000). Tree forages form an integral part of ruminant feeds in the high altitudes of the Himachal Pradesh, Jammu and Kashmir, and Uttaranchal (Singh and Misri, 2004) (Table.1).

Trees have the advantage that they can be planted on hills, on wasteland and at the edges of ponds, and canals, etc. Trees can also be grown on boundaries where regular crops cannot be grown. Moreover, the importance of plantation is growing day by day for the protection of environment and for prevention of soil erosion. Thus, the trees in hill states have the potential to produce as much green fodder per unit area as agricultural fodder crops in the plains. Some of the tree leaves have fairly good amount of nutrients (Table. 3 and 4).

Table 1. Commonly available tree leaves in North West Himalayan Region (NWHR) and their pattern of use (Singh and Misri, 2004)

Common Name	Botanical	Time of use
Biul/Dhaman	*Grewia optiva*	November to February
Tremal	*Ficus roxburghii*	September to February
Bun	*Quercus leucotrichophora*	September to February
Khair	*Acacia catechu*	September to January
Ohi	*Albizia chinensis*	March to June
Sirin	*Albizia lebbeck*	September to December
Kachnar	*Bouhinia variegata*	August to December
Khirak	*Celtis australis*	September to February
Shahtut	*Morus alba*	May to October
Bhera	*Terminalia belerica*	September to January

Table 2. Anti-nutritional factors in tree leaves/shrubs (Kumar, 1992)

Sl. No.	Anti-nutritional substances	Species
1.	Polyphenolic compounds	
	Tannins	All vascular plants
	Lignins	All vascular plants
2.	Glycosides	
	Cyanogens	*Bambusa bambos*
	Saponins	*Bassia latifolia*
3.	Phytohemagglutinins	
	Ricin	*Ricinus communis*
	Robin	*Robinia pseudoacacia*
4.	Non-protein amino acids	
	Mimosine	*Luceana leucocephala*
	Indospecine	*Indigofera spicata*
5.	Alkaloids	
	N-methyl-β-phenethylamine	*Acacia berlandieri*
	Sesbanine	*Sesbania vesicaria*
6.	Triterpenes	
	Azadirachtin	*Azadirachta indica*
	Limonin	*Azadirachta indica*
7.	Oxalate	*Acacia aneura*

Table 3. Nutritive value (% DM) of some tree leaves available in NWHR

Species	DCP	TDN	Reference
G. optiva	15.0	53.0	Negi *et al.*, 1979
A. catechu	24.0	46.33	Singh *et al.,* 1992
A. lebbeck	11.59	49.30	Banerjee, 1988
B. variegata	4.98	47.92-55.54	Sen and Ray, 1971 and Sharma *et al.,* 1968
M. alba	7.84	48.35	SubbaRao *et al.*, 1971
T. belerica	0.86	54.45	Banerjee, 1988
F. bengalensis	-	57.05	Mia *et al.,* 1960
F. glomerata	6.69	53.82	Majumdar and Momin, 1960
F. religiosa	7.05	38.27	Ram and Ray, 1943
F. lactor	-	48.11	Ram and Ray, 1943
Q. semecarpifolia	4.9	49.3	Singh *et al.,* 1999

Table 4. Proximate principles (% DM) of some tree leaves available in NWHR

Species	CP	CF	NFE	EE	TA	Ca	P	Reference
G. optiva	19.0	22.1	43.9	2.2	14	3.3	0.2	Negi *et al.,* 1979
A. catechu	13.0	21.9	46.7	3.0	9.8	2.5	0.1	Pal *et al.*, 1979
A. lebbeck	16.8	26.5	36	2.8	7.1	1.1	0.1	Banerjee, 1988
B. variegata	15.9	33.0	51.8	3.9	12.3	4.1	0.4	Sen and Ray, 1971
M. alba	27.6	17.2	52.6	5.9	10.9	-	-	SubbaRao *et al.*, 1971
F. roxburghii	13.8	4.5	36.8	39.7	11.3	2.83	0.25	Devarajan, 1999
F. bengalensis	11.2	29.9	51.6	5.1	16.9	4.1	0.4	Mia *et al.,* 1960
F. glomerata	15.2	16.5	59.0	2.9	18.4	3.0	0.4	Majumdar & Momin, 1960
F. religiosa	13.9	21.4	50.5	3.8	14.2	3.9	0.4	Ram and Ray, 1943
F. lactor	15.7	25.9	61.1	3.4	-	3.5	0.4	Ram and Ray, 1943
Q. leucotrichophora	10.57	4.09	32.40	46.77	6.17	1.71	0.20	Sharma *et al.,* 2008

Table 5. Composition of tannins (% on DM basis) of some tree leaves available in NWHR

Species	Tannins	Reference
G. optiva	0[a], 2.30[b]	Negi *et al.,* 1979[a], Devarajan, 1999[b].
A. catechu	1.5	Mandal *et al.*, 1997
A. lebbeck	0.2	Mandal *et al.*, 1997
B. variegata	1.2	Mandal *et al.*, 1997
F. roxburghii	2.5[c], 5.1[d]	Upreti and Shrestha, 2006[c]; Devarajan, 1999[d].
F. bengalensis	1.0	Mandal *et al.*, 1997
F. glomerata	0.8	Mandal *et al.*, 1997
Q. leucotrichophora	4.9[e], 5.0[f], 5.82[g]	Negi *et al.,* 1979[e], Devarajan 1999[f], Sharma *et al.*, 2008[g].

These tree leaves contain varying amounts of anti nutritional factors viz. tannins, saponins, alkaloids etc (Kumar, 1992) (Table. 2).Tannin being the major one, drew much more attention of the animal nutritionists and biochemists for development of balanced diets out of these tree leaves (Table. 5).

TREE LEAVES TAKEN UP FOR THE PRESENT STUDY

Tree leaves chosen for the present study are *F. roxburghii*, *Q. leucotrichophora and G. optiva*.

1. *Ficus roxburghii*

Synonyms: *Ficus auriculata* Lour, *Ficus macrophylla* Roxb.

Common names: *Demur, Doomoor* (Bengali); *Timbal, Gular, Tremal* (Himachali); *Trimmal, Timal, Timla* (Hindi); *Karrekan* (Nepali); *Trimbal, Trimal, Timal, Daduri, Urmul* (Punjabi).

F. roxburghii is one of very popular deciduous woody trees, having a short trunk, which soon divides into a few stout laterals, which further- branch irregularly, spreading in all directions; height, 10-12 metres; bark, smooth, gray, with a tinge of yellow. It can be seen growing in the forests, cultivated fields and in the grasslands. In Himachal Pradesh, it is a very common plant growing at elevations up to 1,700 metres (Parmar and Kaushal, 1982). *F. roxburghii* leaves contain 87.64% OM, 13.81% CP, 4.33% EE, 13.08% CF, 56.42% NFE, 12.36% ash; and 5.10% TP, 4.50% TT, 0.90% HT and 4.20% CT (on DM basis) (Devarajan, 1999). Sharma, *et al.*, 2000 reported the values of CP, EE, CF, TA and NFE in *F. roxburghii* on DM basis as 12.70, 4.79, 13.59, 21.74 and 47.18%, respectively.

2. *Quercus leucotrichophora*

Synonym: *Quercus incana*

Common names: Black jack oak, *Banj* Oak, Himalayan White Oak, *Banj, Bun* (Hindi); *Vari, Ring* (Himachali); *Phanal* (Kumaoni).

It is an evergreen tree with a height up to 25m, found in the Himalayas. It is a large or medium sized tree, with leathery dull green leaves, sharply toothed leaves, 6-16 cm long. Leaves have

dense white-woolly hairs on the underside with a greenish-white underside. The species name *leucotrichophora* means carrying white hairs. The flowers come out in catkins (slim cylindrical flower clusters). The acorns are said to contain a peanut like core when broken (Parmar and Kaushal, 1982). Leaves contain 9.56% CP, 4.8% EE, 31.30% CF, 18.40% NFE and 5.2% total ash on DM basis (CSIR, 1969). The values of OM, CP, EE, CF, NFE and total ash (on DM basis) in *Q. leucotrichophora* have been reported to vary from 91.89-95.43, 8.09-10.73, 2.45-5.38, 13.46-36.67, 48.40-49.50 and 4.76-20.19%, respectively (Sen *et al.*, 1978; Sinha *et al.*,1989; Singh *et al.,*1992; Devarajan,1999; Anandan and Dey, 2000). The TP, TTP, HT and CT in *Q. leucotrichophora* in per cent (on DM basis) have been reported as 5.00, 3.70, 1.30, and 4.25, respectively (Devarajan, 1999).

3. *Grewia optiva*

Synonym: *Grewia oppositifolia* Buch.-Ham.

Common names: Behel, Bewal, Bhengal, Bhimal, Bihul, Biul, Biung, Dhaman (Hindi); Phusre (Nepali).

G. optiva is a small to medium-sized deciduous tree, 9-12 m in height; crown spreading, branches smooth, pale silvery-brown; bark dark brown, thick and roughish, exfoliating in small woody scales. This is a tree of the subtropical climate. Plants are often cultivated in the Himalayas. It tolerates frost which is common during autumn and winter (Brandis, 1984). The leaves are rated as good fodder and trees are heavily lopped for this purpose in the winter months when usually no other green fodder is available. The green leaves constitute about 70% of the total green weight of branches. Leaf fodder yield is reported to be 11 ton/ha from 2-year-old plants, green fodder yield from mature trees is reported

to be 12-30 kg. Leaves are fairly rich in protein (19% CP) and other nutrients (2.2% EE, 43.9% NFE) and do not contain tannins (Pal *et al.*, 1979). However, Devarajan (1999) reported the presence of 2.30% TT and 88.67, 24.38, 3.79, 15.32, 11.33, 45.18 and 60.50 % OM, CP, EE, Ash, NFE and TCHO (% on DM basis), respectively.

TANNINS

Tannins are polyphenolic substances with the ability to bind proteins in aqueous solution. Based on their chemical structures, tannins are classified into two major groups: hydrolysable and condensed tannins (Zucker, 1983). Further, on the basis of sugar content, polymerization and degree of esterification, tannins are divided into three groups: hydrolysable (HT) (gallotannins and ellagitannins), condensed (CT) and complex (catechin gallate) tannins (Bhat *et al.,* 2009).

1. Hydrolysable tannins (HTs) which have a sugar core linked to gallic acid (gallotannins) or ellagic acid (ellagitannins) by esterification (Haslam *et al.,* 1961).
2. Condensed tannins (CTs) which are formed by the monomer, flavan-3-ol or a derivative thereof. These are more resistant to the microbial attack than the hydrolysable tannins (Lewis and Starkey, 1969).
3. Catechin gallates hold an intermediate position as they share the properties of both the hydrolysable tannins and the condensed tannins (Bhat *et al.,* 1998).

1. Hydrolysable tannins

Hydrolysable tannins are polyphenolic plant constituents derived from mono- to pentagalloyllated β–D-glucopyranose (Figure 1).

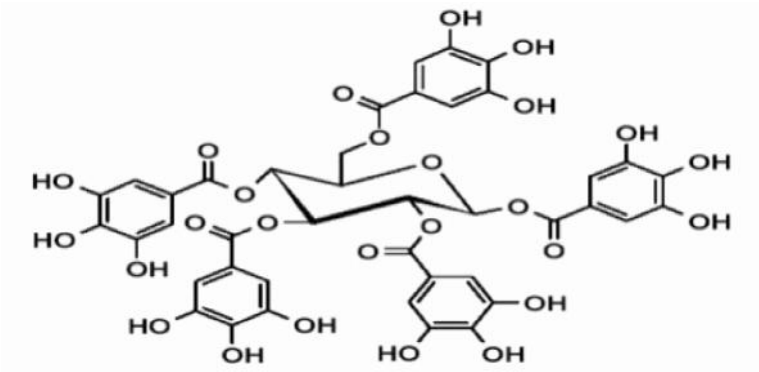


Figure 1. A hydrolysable tannin molecule with a glucose core

2. Condensed tannins

Condensed tannins are also known as proanthocyanidins, and consist of phenols of the flavon type flavonoids. They are also called flavonals because they are polymers of flavan-3-ols such as catechin or flavan-3, 4-diols known as leucocyanidins. A very interesting difference between condensed tannins and hydrolysable tannins is the fact that condensed tannins do not contain any sugar moieties (Figure 2).

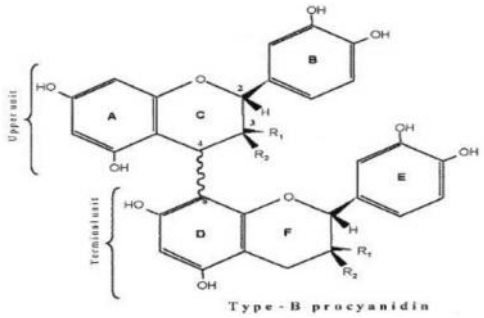


Figure 2. Condensed tannin (Proanthocyanidins)

3. Catechin tannins

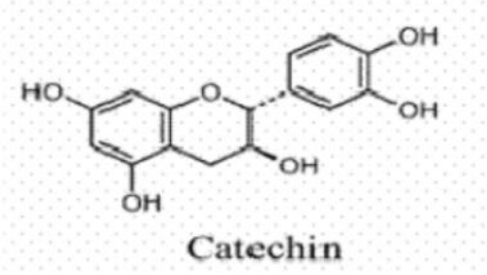


Figure 3. Catechin tannin

An intermediate group also exists that combines both the characteristics of hydrolysable tannins and condensed tannins. This family of tannins is called the catechin tannins (Figure 3). The catechin tannins are most abundant in tea leaves (Graham, 1992).

Table 6. Different types of tannins (Haslam & Tanner, 1970)

	Hydrolysable Tannins	Condensed Tannins	Catechin Tannins
1.	Gallotannins – yield gallic acid and glucose on hydrolysis.	Polymeric proanthocyanidins – yield monomers of flavonoids e.g. flavan –3, 4-diols and flavan-3-ols.	Catechin and epicatechin gallates – yield catechin, epicatechin and gallic acid on hydrolysis.
2.	Ellagitannins – yield ellagic acid and glucose on hydrolysis		

Among the plant secondary metabolites, probably tannins are the most extensively researched and reviewed secondary metabolites in terms of their effects on ruminants (Makkar *et al.*, 1987; Mangan, 1988; Kumar and Vaithiyanathan, 1990; Reed, 1995; Aerts *et al.*, 1999a; McSweeny *et al.*, 2001; Singh and Bhat, 2001; Makkar, 2003, Min *et al.*, 2003; Wallace, 2004; Puchala *et al.*, 2005; Mc Allister *et al.,* 2005; Paul *et al.,* 2006; Maia *et al.,* 2007; Kamra *et al.,* 2008; Vasta *et al.,* 2009; Grainger *et al.,* 2009; Patra *et al.,* 2006 ,2008, 2009; Patra and Saxena, 2011).

Wide distribution of tannins in nutritionally important forage trees, shrubs and legumes, and cereal grains has limited their utilization as feedstuffs. It is well known that very high levels of tannins intake by ruminants can impair nutrient utilization, produce toxicity and even cause death (Garg *et al.*, 1992; Plumee *et al.,* 1998). At low to moderate concentrations (2.5-5 % on DM basis), tannins especially

CT have been shown to exert positive effect by protecting dietary protein from ruminal degradation, subsequently increasing its availability at intestinal level for digestion and absorption which may enhance Dry Matter Intake (DMI) (kg/day) and body weight gain of the experimental animals (Sharma *et al.*, 2008). Recent studies showed that tannins could also influence other aspects of rumen metabolism including methanogenesis and efficiency of microbial protein synthesis. The demarcation between positive and negative effect on utilization of nutrients lies on ability of tannins to form complexes with protein and other macromolecules, microbes and biological membranes in gastrointestinal tract of ruminants (Figure 4).

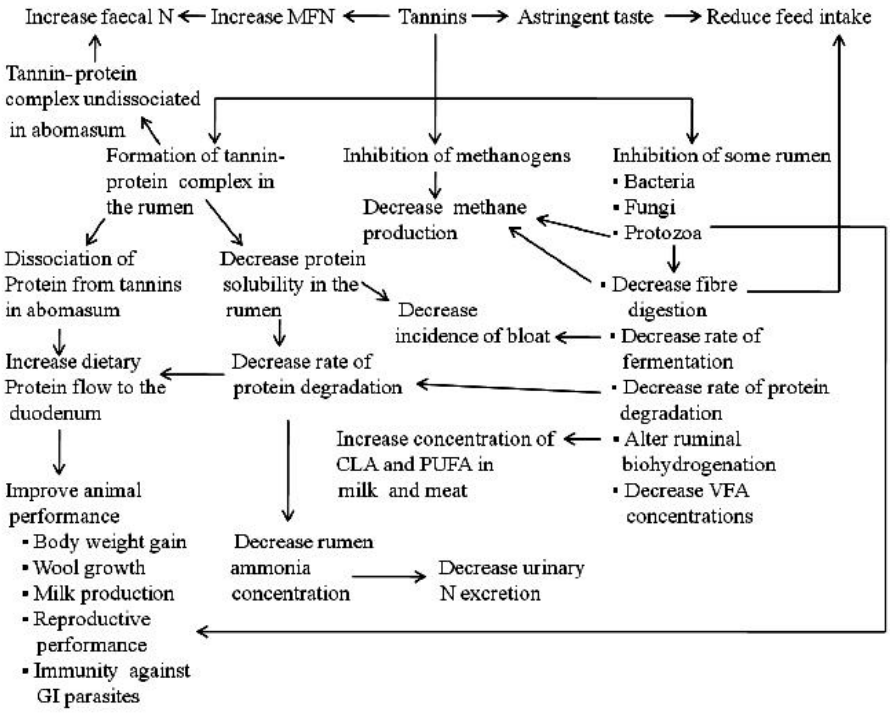


Figure 4. Schematic representation of the effects of tannins on ruminal metabolism and performance of ruminant (Patra and Saxena, 2011)

EFFECTS OF TANNINS ON NITROGEN METABOLISM

Increased flow of protein from rumen is one of the important factors for determining the productivity in ruminants which depends on decreased proteolysis by rumen microorganisms and increased

efficiency of microbial protein synthesis. Generally it is known that tannins decrease the rate of protein degradation in the rumen (Figure 4). Al-Dobaib (2009) reported decreased rate of protein degradation of lucerne hay by addition of quebracho tannins (2 - 3% on DM basis). Puchala *et al.,* (2005) also reported that ruminal ammonia nitrogen concentrations (3.7 and 9.9 mg/dl) were lower in goats fed CT containing pasture *Sericea lespedeza* than crabgrass/tall fescue. Min *et al.,* (2003) reported that CTs progressively increased the duodenal non ammonia nitrogen, where as rumen microbial protein outflow was little affected. Some more *in vitro* studies have also shown improvement of microbial protein synthesis (Bhatta *et al.,* 2001). Quebracho tannins at 1 and 2 % level (on DM basis) also increased microbial protein synthesis in sheep fed with Lucerne based diet, but no effect was reported at 3% level (on DM basis) (Al-Dobaib, 2009). However, no improvement on protein metabolism (such as protein degradation and post-ruminal protein supply) was observed in sheep fed quebracho tannins at 0 - 6 % (on DM basis) in a lucerne based diet (Komolong *et al.,* 2001). Komolong *et al.,* (2001) fed the quebracho tannins as liquid drench.

The reduction of protein degradation in the rumen may occur due to the formation of tannin- protein complexes in the rumen and inhibition of the growth and activities of proteolytic bacterial populations. Both CTs and HTs interact with proteins by forming hydrogen bonds between the phenolic groups of tannins and carboxyl groups of aliphatic and aromatic side chains of proteins and through hydrophobic interaction (Mueller-Harvey, 2006). The binding strength of the tannin-protein interactions determines the responses of tannins on protein digestibility in the digestive tract (Mueller-Harvey, 2006).

Plant protein degradation and effects on rumen microbes depend upon the chemical structure and molecular weight of tannins (Foo *et*

al., 1996, Foo *et al.*, 1997 and McAllister *et al.*, 2005). Gallic acid, which is a precursor of gallotannins, did not affect *in vitro* protein degradation of alfalfa protein, whereas tannic acid and quebracho tannins showed strong protein protection from ruminal degradation, indicating the similarity in biological effect of both HTs and CTs (Getachew *et al.*, 2008). At concentrations of 0.25 – 1.75 mg/ mg of total soluble protein, CT of *Lotus pedunculatus* was more effective than *Lotus corniculatus* in protecting plant protein from degradation (Aerts *et al.*, 1999) which suggests that kinetics of tannins may change depending on the source. Similarly, the CTs from *Calliandra calothyrsus* and *Flemingia macrophylla* had more effect on the degradation of soybean protein than CT from *Luceana leucocephala.* Tannins in *L. pedunculatus* and in *Desmodium ovalifolium* protected protein in the rumen and dissociated from the complexed protein in the abomasum and intestines (Waghorn *et al.*, 1994 and Waghorn, 1990). However, the inclusion of quebracho CT (1 and 2 % on DM basis) in diets of cattle (Beauchemin *et al.,* 2007) and feeding of *Quercus viciifolia* forage (Aufrere *et al.,* 2008), C. *calothyrsus* and *F. macrophylla* legume shrubs (Tiemann *et al.,* 2008) to sheep reduced apparent protein digestibility. Tannins may also interact with digestive enzymes (Van Leeuwen *et al.,* 1995) and the epithelium of digestive tracts (Mbatha *et al.,* 2002). If significant amount of tannins reach the duodenum, reduce amino acid absorption from the intestine (Silanikove *et al.,* 2001). Komolong *et al.,* (2001) reported that increasing concentrations of quebracho CT lowered the apparent digestibility and absorption of amino acids at the small intestine in sheep fed on the lucerne-based diet. Dietary tannins have also been implicated to increase metabolic faecal nitrogen excretion which may also account for a reduction of apparent protein digestion (Patra, 2010). Proteins also differ greatly in their affinity for phenolics. Proteins that have a high molecular weight, together with an open and flexible

tertiary structure bind strongly with phenolics (Asquith and Butler, 1986). The CT-protein complex is not dissociated by rumen microbes (McSweeney *et al.,* 1999), whereas the HT-protein complex is degraded as a result of depolymerisation of tannin polymers by cleaving the ester linkages between glucose and the phenolic subunits by the enzymes (tannin acylhydrolases and esterases) secreted by the rumen microbes (Skene and Brooker, 1995 and McSweeney *et al.,* 2001). The tannin-protein complex is dissociated in the abomasum at pH <3.5 and in the intestine at pH >7 and protein is available for digestion in the small intestine (Jones and Mangan, 1977; Waghorn, 1990; Barry and McNabb, 1999 and Mueller-Harvey, 2006). However, tannins differ in their ability to bind protein at rumen pH and thus reversibility of the process post-ruminally may also differ (Patra and Saxena, 2011).

EFFECTS OF TANNINS ON CARBOHYDRATE DIGESTION

Although the nature of binding of tannins with carbohydrate is not determined, high concentrations of CTs in *L pedunculatus* (9.5 and 10.6 % on DM basis) reduced rumen digestion of readily fermentable carbohydrate and hemicellulose (Barry and Manley, 1984). However, carbohydrate digestion in sheep fed on *L. corniculatus* containing 2.5-3.5 % CT (DM basis) was not affected (Waghorn *et al.,* 1987). Inclusion of quebracho tannins (containing 75% CT) at a dosage of 2.25 % to lucerne hay decreased the fibre digestibility; whereas no effect was noted at dosages of 0.75 and 1.5 % of CT on DM basis (Al-Dobaib, 2009). Tiemann *et al.,* (2008) reported that replacement of a *Vignaunguiculata* legume (tannin-free) with *C. calothyrsus* and *F. macrophylla* depressed the organic matter and fibre digestibility in sheep. *Terminalia chebula* containing high concentration tannins also decreased *in vitro* DM degradability (Patra *et al.,* 2006). However, when *T. chebuala* was fed to sheep at 1% of total DM intake, the digestibility of nutrients and fibre fractions increased (Patra *et al.,*

2008). Higher concentrations of tannins in diets, which remain free after binding with proteins, may depress fibre digestion by complexing with lignocelluloses, thus, preventing microbial digestion (Barry and Manley, 1986) or by directly inhibiting cellulolytic microorganisms or both (Patra and Saxena, 2009). Makkar *et al.,* (1995) studied different sources and dosages of tannins on the rate of fermentation of hay in an *in vitro* study. The decrease in rate of fermentation of DM was 3% and 17% for *Q. incana,* 6% and 21 % for *D. cinerea, and* 7% and 27% for *A. barteri* at 0.47 and 0.93 mg/ml tannins in the medium, respectively. It indicates that different tannins exhibit different effects on DM digestibility at the same inclusion level. The reduced rate of carbohydrate digestion, especially fibre, may decrease total volatile fatty acid concentrations in the rumen (Patra *et al.,* 2006 and Beauchemin *et al.,* 2007).

EFFECTS OF TANNINS ON RUMINAL METHANOGENESIS

Tannin-containing forages and tannin extracts have been demonstrated to decrease methane production both *in vitro* and *in vivo* (Sliwinski *et al.,* 2002 a, b; Carulla *et al.,* 2005; Min *et al.,* 2005; Puchala *et al.,* 2005; Tavendale *et al.,* 2005; Min *et al.,* 2006; Patra *et al.,* 2006; Beauchemin *et al.,* 2007; Animut *et al.,* 2008; Patra *et al.,* 2008). Tannins present in *C. calothyrsus* and *O. viciifolia* reduced methane release per gram of organic matter degraded in Rusitec (McMahon *et al.,* 1999 and Hess *et al.,* 2003). Woodward *et al.,* (2002) investigated the feeding of *Hedysarum coronarium* (sulla) on methane emissions and milk yield in Friesian and Jersey dairy cows. Cows grazing on *H. coronarium* had higher daily dry matter intake (13.1 vs. 10.7 kg DM) and daily milk solid production (1.07 vs. 0.81 kg) than grazing perennial ryegrass pasture though the total daily methane emission was similar (253.9 vs. 260 g). Cows fed on *H. coronarium* produced less methane per kg DM intake (19.5 vs. 24.6 g) and per kg

milk solid yield (243.3 vs. 327.8 g). Similarly there was also a 16% reduction in methane production in lambs fed on L *pedunculatus* (9.5% CT on DM basis), which may be due to the presence of CTs (Waghorn *et al.,* 2002). Another CT containing forage, *S. lespedeza* (17.7 % CT on DM basis), decreased methane emission (7.4 vs. 10.6 g/d and 6.9 vs. 16.2 g/kg DM intake for *S. lespedeza* and crabgrass/ tall fescue, respectively) in Angora goats (Puchala *et al.,* 2005). Animut *et al.,* 2008) also reported that feeding different levels of *Kobe lespedeza* decreased methane production linearly in goats which has been attributed to the presence of CTs. Similarly, addition of CTs from *Acacia mearnsii* to forage-fed sheep (Carulla, 2005) and dairy cattle (Grainger *et al.* 2009) decreased methanogenesis substantially. Methane production was also inhibited up to 90% by inclusion of a methanol extract of pericarp of *T. chebula* (a tropical fruit), in an *in vitro* study (Patra *et al.,* 2006). Hydrolysable tannin extracts such as gallotannic acid inhibited 50% methane production (up to 700 mg/ L) (Field and Lettinga, 1987). However, addition of tannin at low dosage did not affect methane production in a number of studies (Min *et al.,* 2006 and Beauchemin *et al.,* 2007). Tavendale *et al.,* (2005) reported that methane production (ml/g DM) at 12 h for *M. sativa* (25 ml) was higher than *L pedunculatus* (17.6 ml) that contains 0.1 % CT and after addition of polyethelene glycol increased methane production for *L. pedunculatus* (17%) but not for *M. sativa*. In sheep, the inclusion of the tannin-rich browses, *C. calothyrsus* and *F. macrophylla*, reduced methane emissions (per day and per unit of feed and energy intake) by up to 24% relative to a tannin-free legume, *Vigna unguiculata,* supplemented diet; but this has been mostly due to the result of reduced organic matter and fibre digestion, as fibre digestion was also reduced in this study (Tiemann, 2008). Tannins have been shown to lower protozoal numbers (Patra and Saxena, 2009) which may also decrease protozoal-associated methanogenesis (Finlay *et al.,* 1994).

The inhibitory effects of tannins on rumen methanogenesis have been related to direct effects on methanogenic archaea, protozoal-associated methane production and indirectly through a depression of fibre digestion in the rumen (Patra and Saxena, 2011).

EFFECTS OF TANNINS ON BLOAT PREVENTION

Bloat occurs due to impaired eructation mechanism resulting in an accumulation of gas in the rumen. Free-gas or feed-lot bloat occurs sporadically in feed-lot cattle, but frothy bloat is more common in ruminants grazing legume forages (Cheng *et al.,* 1998) or immature wheat pasture (Min *et al.,*2005). Tannins have the ability to precipitate soluble protein fractions which have been implicated in the reduction of bloat grazing CT-containing legume forages (Jones *et al.,* 1973; Barry and McNabb, 1999; McMahon *et al.,* 2000). The substitution of a portion of lucerne or mixed cropping with CT-containing forages (e.g. *O. viciifolia)* may prevent incidences of bloat (McMahon *et al.,* 2000 and Frutos *et al.,* 2004). Li *et al.,* (1996) mentioned that a minimum concentration of 5% CT (on DM basis) in forages is needed to consider forages bloat-safe. Min *et al.,* (2006) reported that feeding a CT extract reduced the occurrence of bloat in steers grazing wheat pasture. Ruminal fluid supplemented with CTs and incubated with fresh wheat forage decreased the rate of gas production *in vitro* (Min *et al.,* 2005 and 2006).

EFFECTS OF TANNINS ON RUMEN MICROBIAL POPULATIONS

The mechanisms of inhibition of rumen protozoa are not known. The effects of tannins on ruminal bacteria are reported to be dependent upon the species of microorganism and type or source of tannin (Jones *et al.,* 1994 and Sivakumaran *et al.,* 2004). Jones *et al.,* (1994) studied the effects of CTs of the legume sainfoin (O. *viciifolia)* on growth and proteolysis by four strains of ruminal bacteria. They observed that

growth of proteolytic bacteria (*B. fibrisolvens, Ruminobacter amylophilus* and *Streptococcus bovis)* was reduced by CT, but had little effect on a strain of *Prevotella ruminicola.* Similarly, addition of phlorotannins at 0.5 g/l to rumen cultures inhibited the growth of *F. succinogenes,* but had minimal effect on *Ruminococcus flavefaciens* and *R. albus,* whereas the growth of *S. ruminantium, S. bovis, R. amylophilus* and *Prevotella bryantii* was stimulated (Wang *et al.*, 2009). The antimicrobial activities of tannins are related to the interactions of tannins with the extracellular enzymes secreted and the cell wall of bacteria causing morphological changes of the cell wall, tannin-induced membrane disruption, direct action on microbial metabolism, deprivation of substrates for microbial growth and chelation of cations by tannins reducing its availability to microbes (Kumar and Vaithiyanathan, 1990; Jones *et al.*,1994; Smith *et al.*, 2005 and Benchaar and Chouinard, 2009). Muhammed *et al.,* (1995) investigated the effects of tannic acid, ellagic acid, gallic acid and catechin on rumen fungus, *Neocallimastix frontalis* strain RE1. All these compounds inhibited the cellulolysis and zoospore attachment to cellulose by this fungus.

The effects of tannins on rumen protozoa are variable. Salem *et al.,* (1997) observed a linear increase in protozoal numbers in rumen fluid of sheep fed on lucern hay based diets by addition of increased proportion of *Acacia cyanophylla* foliage, which contained 4.5 % CT (on DM basis). Similarly, CT present in *L. corniculatus* and *H. coronarium* increased protozoal numbers in the rumen of sheep (Chiquette *et al.,* 1989 and Terrill *et al.,* 1992).There are many reports indicating inhibitory effect of tannins on rumen protozoa. Makkar *et al.,* (1995a,b) reported that quebracho CT (0.1 -0. 4 mg/ml media) significantly reduced the numbers of total protozoa, entodiniomorphs and holotrichs in Rusitec. Benchaar *et al.,* (2008) did not observe any

effect on protozoal numbers in dairy cattle fed quebracho tannins (CT concentrations of 70% on DM basis, 150 g/day).

EFFECTS OF TANNINS ON PERFORMANCE OF RUMINANTS

The feed intake by animals is usually reduced for diets with high concentrations of tannins, which is suggested to be due to a reduction of palatability of diets and decreased rate of digestion in the rumen (Frutos *et al.,* 2004; Mueller-Harvey, 2006 and Waghorn, 2008). Many temperate forages having tannins have been evaluated for production performances in ruminants. Hervas *et al.,* (2003) noted that the addition of quebracho tannins in the diet of sheep at 0, 0.5, 1.5 and 3.0 g/kg BW (equal to 0, 2.8, 8.3 and 16.6g % on DM basis) did not affect feed intake up to 1.5 g/ kg BW, but significantly decreased at the highest dosage (i.e. at 3.0). Similarly, quebracho CTs up to 2.0 % of DM had no influence on feed intake in cattle (Beauchemin *et al.,* 2007). It has generally been advised that tannin concentrations of greater than 5% (on DM basis) in diets may negatively affect feed intake, whereas lower concentrations of tannins have no influence on intake by ruminants (Barry and Manley, 1984; Barry *et al.,* 1986 and Aerts *et al.,* 1999). Quebracho tannins (8.93 % on DM basis) reduced DM intakes in lamb fed fresh vetch *(Vicia sativa)* than the lambs fed the same herbage without tannins (768 vs. 956 g/day), whereas tannins did not affect the DM intakes in lambs fed a concentrate-based diet (Vasta *et al.,* 2009). Terrill *et al.* (1992) observed increase in wool growth in sheep fed sulla (4-5 % CT). Similarly, increased wool growth and live weight gain was observed in sheep fed *L. corniculatus* (3 to 4 % CT) (Wang *et al.*, 1996a). In addition to increased wool growth, *L. corniculatus* also increased ovulation rate in sheep (Min *et al.*, 1999).

Wang *et al.* (1996b) investigated the influence of feeding *L. corniculatus* (4.4 % CT) to lactating ewes on milk yield and

composition. The CT had no effect on milk yield in early lactation but increased the yield of whole milk, lactose and protein by 21, 12 and 14 %, respectively, during mid and late lactation while milk fat per cent was reduced (1%). Woodward *et al.* (1999) also observed increase in milk yield and milk protein by 42 and 57%, respectively in cows fed *L. corniculatus* as compared to cows fed with PEG or other grasses. Lactating dairy cows fed diets containing 8-16% peanut skins (18.0 % CT on DM basis) resulted in higher DM intakes, milk yield and milk fat content but had lower milk protein content (West *et al.*, 1993). Inclusion of 75 g tamarind seed husk, a tannin-rich by-product (14.0 % CT on DM basis) per kg diet of cross-bred dairy cows also resulted in increased body weight gain and milk protein content in mid lactation (Bhatta *et al.*, 2000). Ramirez-Restrepo *et al.*, (2005a, b) reported higher growth, reduced parasite burden, improved reproductive performance and wool production in lambs which were grazing on *L. corniculatus* than perennial ryegrass *(Lolium* perenne)/white clover *(Trifolium repens)* pasture. Addition of CT (1 and 2% on DM basis) in steers grazing winter wheat forages increased body weight gain (Min *et al.*, 2006). However, *L. pedunculatus* containing 7.6-9.0 % CT (on DM basis) decreased rates of body weight gain and wool growth (Barry, 1985). Recently, Grainger *et al.*, (2009) reported that milk yield, fat and protein percentage of milk reduced when dairy cows were dosed daily with 163-326 g CT from *Acacia mearnsii.* In this experiment, feed intake and digestibility were also decreased by *A. mearnsii* tannins. However, Beauchemin *et al.*, (2007) did not find any significant effect on growth and intakes when cattle were fed a forage-based diet (700 g/kg) supplemented with quebracho tannins at1 and 2 % of diet. Aerts *et al.*, (1999) suggested that forages containing moderate concentrations of CT (2-4 % on DM basis) may exert beneficial effects on protein metabolism; however, high dietary CT concentrations (6-12 % on DM basis) may depress voluntary feed intakes, digestive

efficiency and animal productivity. Diets containing as low as 2.6 % tannins (on DM basis) from carob pulp *(Ceratonia siliqua)* resulted in a decrease in growth rate of lambs from 140 to less than 50 g per day (Priolo *et al.*, 2000), whereas the CT in *H. coronarium* (7.2 % on DM basis) did not affect daily gain of lambs (Douglas, 1999).

TOXICITY DUE TO TANNINS

Several authors have reported acute toxicity (Fowler and Richards, 1965) and livestock intoxication (Spier *et al.,* 1987; Garg *et al.,* 1992; Zhu *et al.,* 1992; Plumee *et al.,* 1998; Belenguer *et al.,* 2010) due to tannins at higher level. Oak leaves toxicity has been ascribed to HTs (Garg *et al.,* 1992; Plumee *et al.,* 1998). Oak poisoning has been reported from India (Negi *et al.,*1979; Lohan *et al.,*1983; Garg *et al.,* 1992), USA (Kingsbury,1964), Slovakia (Begovic *et al.,* 1978), South Africa (Nesser *et al.,*1982), China (Shi,1988), Israel (Yeruham *et al.,* 1998) and Spain (Belenguer *et al.,* 2010).

SYMPTOMS OF TANNIN TOXICOSIS

Livestock toxicity due to HTs reported by several workers (Spier *et al.,*1987; Garg, 1992; Zhu *et al.*, 1992; Plumlee *et al.,* 1998) is associated with anorexia, depression, rumen atony, hepatic and renal failure, ulcerations and severe gastroenteritis. The major lesion associated with tannin poisoning is hemorrhagic gastroenteritis, necrosis of liver, and kidney damage with proximal tubular necrosis (Dollahite *et al.*, 1962; Holliman, 1985; Filippich *et al.*, 1991).

INTERACTIONS BETWEEN PLANT SECONDARY METABOLITES

Plants contain numerous secondary metabolites and the biological activity depends on individual as well as combinations of secondary metabolites present in the plant. Very little information is available about the interactions of PSM on biological activities. Presence

of tannins and saponins in the diet of mice has been shown to abolish adverse effects of both these factors. The nullification of toxic effects of tannins was dependent on the relative proportions of tannins and saponins (Freeland *et al.*, 1985). Interactions between tannins and lectins removed inhibitory action of tannin on amylase (Fish and Thompson, 1991). However, *in vitro* studies revealed that the effect of tannins (tannic acid and quebracho tannins) and saponins (quillaja saponins) on decrease in apparent and true digestibilities and gas production is additive (Makkar *et al.*, 1995a).

DETANNIFICATION OF TREE LEAVES

A number of studies aimed at detoxification of tree leaves with the following methods have been done.

1. Physical methods

A) Chopping

Chopping facilitates the contact between plant phenolic oxidases with tannins and may reduce tannins content (Makkar and Singh, 1993; Ben Salem *et al.,* 2005).

B) Grinding

It increases surface area and thus may facilitate contact between plant phenolic oxidases and tannins, and may decrease tannins (Patel *et al.,* 2004; Vitti *et al.*, 2005).

C) Drying

Drying of mature oak leaves under different conditions have variable effect. It was reported that it had no effect on the levels of total phenols and condensed tannins when the moisture content was less and was effective for feed stuffs having high moisture content (Makkar and Singh, 1991). Ben Salem *et al.*, (1999) reported sun-

drying was more effective in reducing CT levels in acacia foliage than shade-drying (65%).

D) Storage

Various authors have reported the decrease in tannins due to storage (Russell and Lolley, 1989; Ben Salem *et al.,* 2005 and Vitti *et al.,* 2005). The rate and extent of decrease of both total phenols and condensed tannins are more in leaves on increasing the moisture level from 40% to 55% (Makkar and Singh, 1993).

2. Chemical methods

Treatment with various chemicals under alkaline conditions led to decrease in tannin content and tannin activity up to 90% in agro-industrial by-products and tree leaves. But, major disadvantage of the chemical treatments is the loss of soluble nutrients. So, steps can also be taken to utilize the soluble nutrients during chemical treatment. Various methods of chemical treatment are as follows:

A) Urea treatment

Urea is the preferred alkali for treatment due to its low cost and ease of handling (Kiangi and Kategile, 1981; Sahnoune *et al.*, 1991). Extra N it supplies increases the crude protein (CP) concentration of crop residues (McDonald *et al.,* 1995). Destabilization of tannin-protein complexes at various levels of urea (2-8%) with satisfactory results (decrease of tannins at 72-89%) has been reported by Russell and Lolley (1989), Makkar and Becker (1996) and Ben Salem *et al.,* (2005).

B) Oxidizing agents

Potassium permanganate (0.03 M) and potassium dichromate (0.02 M) decrease tannin level by about 95% (Makkar and Singh, 1992). These oxidizing agents convert tannins to quinones, which

are not capable of forming complexes with proteins and can be used for large scale detoxification of tannin rich feed stuffs because of its low cost (Makkar, 2003).

C) Ferrous sulphate

Ferrous sulphate (0.015 M) reduces tannins by 85% (Makkar and Singh, 1993). It is a tannin-complexing agent. Increase in the degree of polymerisation in the treated material could be due to binding of phenolics through ferrous ions (Deshpande *et al.,* 1986; Makkar and Singh, 1993).

D) Sodium hydroxide, Sodium carbonate and Sodium bicarbonate:

These alkalies act by oxidation of phenolics at higher pH (Makkar and Singh, 1991; Woodfolk *et al.,* 1994). Sodium hydroxide (0.05 M) is most effective, followed by sodium carbonate (0.05 M) and sodium bicarbonate (0.1 M). The reduction in tannins in oak leaves using these alkalies ranged between 70 and 90% (Makkar and Singh, 1991).

E) Wood ash

Wood ash, a source of alkali potentially available to farmers, is effective in reducing tannin contents. Wood ash has alkaline pH and has capacity for precipitation of tannins. Various levels of wood ash (1-24%) have been evaluated (Makkar and Singh, 1992; Mukuru, 1992; Kyarisiima *et al.,* 2004; Ben Salem *et al.,* 2005). Ten per cent solution of oak wood ash and pine wood ash decreased the content of total phenols, condensed tannins and protein precipitation capacity by 66, 80, 75% and 69, 85, 80%, respectively in oak leaves (Makkar and Singh, 1992). Kyarisiima *et al.* (2004) reported that treatment of high tannin sorghum with 5% wood ash extract was effective in reducing the tannin level. However, these treatments also remove nutrients, so their overall effectiveness is unclear (Makkar and Singh, 1993).

F) Polyethylene glycol (molecular weight 4000 or 6000, PEG)

Tannins have higher affinity to PEG than proteins. Incorporation of PEG has been shown to have beneficial effects in monogastrics (Rayudu *et al.,* 1970; Armstrong *et al.,* 1973; Marquardt *et al.,* 1977; Horigome *et al.,* 1984; Yu *et al.,* 1996a, b); but, both beneficial and adverse effects in ruminants. Incorporation of PEG in the diet has beneficial effects, particularly, for feedstuffs which are rich in tannins (condensed tannin content: 5–10%) (Pritchard *et al.,* 1988; Kumar and Vaithiyanathan, 1990; Silanikove *et al.,* 1994, 1996, 1997; Stienezen *et al.,* 1996; Barahona *et al.,* 1997). Inactivation of tannins using PEG increase voluntary feed intake, availability of nutrients and decrease microbial inhibition in degrading the tannin rich feed; this in turn increase the performance of animal (Horigome *et al.,* 1984; Yu *et al.,* 1996a, b). However, it was reported addition of PEG with *Lotus corniculatus* (containing condensed tannin at 2 to 4%) decreased wool growth, weight gain (Wang *et al.,* 1994, 1996a); reproduction (Barry *et al.,* 2001) and milk yield (Wang *et al.,* 1996b). This was attributed to substantially lower absorption of amino acids from the intestine due to increase in the digestibility of proteins in the rumen (Waghorn *et al.,* 1987). However, increased protein flow to the duodenum was also reported on feeding *L. corniculatus* and *L. pedunculatus* that contain moderate levels (2.5-5%) of tannins compared to low-tannin cultivars (0-2.5%), or to diets in which the tannins have been deactivated by the addition of PEG (John and Lancashire, 1981; Barry and Manley, 1984; Waghorn *et al.,* 1987; McNabb *et al.,* 1993). Adverse effect of depressing voluntary feed intake and digestibility of N, organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) has been reported in sheep fed on diets supplemented with PEG treated detannified leaves (Kumar and Vaithiyanathan, 1990; Norton and Ahn, 1997; Palmer and

McSweeney, 2000; Getachew *et al.,* 2001). Use of PEG for detannification of animal feeds has been observed to be a successful in most of the experiments but field application has not been done due to unfavourable cost benefit ratio (Ben Salem *et al.,* 2005).

3. Other methods

Solid-state fermentation

Makkar *et al* (1994) reported decrease in tannin content of oak leaves by fungus, *Sporotricum pulverulentum*. Gamble *et al* (1996) observed reduction in tannin content of *Sericee lespedeza* (*Lespedeza cuneata*) by about 65% in 10 to 20 days through solid-state fermentation technique using white rot fungi, *Ceriporiopsis subvermispora* and *Cyathus steroreus*.

CURRENT STATUS OF WORK

Theoretical working concept for detannification and utilization of oak leaves has been presented by Makkar and Singh (1992) along with some chemical and physical methods of detannification of oak leaves. But, due to insufficient work with practicable solution, the challenges of oak toxicity, conservation and processing of locally available oak and tremal leaves are still a problem to be overcome by the nutritionists and biochemists. Looking into the applicability under field condition, efforts have been made by different workers to detannify these leaves through different physical and chemical treatments. Detailed *in vitro* studies though have been done on the detannification of *Q. leucotrichophora* leaves resulting in substantial decrease in tannin content. The detannified leaves have not been evaluated sufficiently by animal feeding experiments. Detannification studies have not yet been reported on *F. roxburghii* leaves. *F. roxburghii* (Tremal) and *Q. leucotrichophora* (Bun) are though adequately available even during scarcity period *i.e.*, winter months; but, the nature, quality and

quantity of tannins/polyphenolics are different in these leaves, which in turn affect the utilizatiuon of nutrients differently. Thus, further work on optimising the tannins/polyphenolics content through different processing techniques is needed to utilize these leaves at least as maintenance roughages.

Materials and Methods

Materials and Methods

The present experiment was carried out at I.V.R.I Regional Station, Palampur (H.P) located at an altitude of 1291m above mean sea level with latitude and longitude 32.6 ºN and 76 ºE, respectively. A brief description of experimental techniques followed during the course of study is presented in this chapter. The experiment was carried out in 3 phases.

PHASE I

3.1 COLLECTION OF TREE LEAVES

Fresh leaves of *Ficus roxburghii* (FR) and *Quercus leucotrichophora* (QL) were lopped from the forest area of Palampur. Efforts were made to collect the leaves from the same tree to avoid the variability of leaves in stage of maturity, chemical composition and tannin content. Leaves were packed in gunny bags, brought to the Institute, 100 g samples each of FR and QL leaves were taken for proximate analysis

3.2 PHYSICAL AND CHEMICAL TREATMENT OF LEAVES

3.2.1 Physical treatment

Leaves were divided into 4 parts, each containing 2.5 kg, for physical processing as follows.

- Untreated leaves [UL, Control]
- Chopping [CL]

- ☞ Chopping and sun drying (for 3 days) [CSDL]
- ☞ Chopping, sun drying (for 3 days) and grinding [CSDGL]

Enough care was taken to avoid mixing of sample with soils/ sands during the collection and sun drying. 50 gm samples of each physically processed leaves were then taken separately for tannin assays.

3.2.2 Chemical treatment

The remaining leaves were divided into 16 parts and subjected to chemical treatment by overnight soaking of leaves in water along with respective chemical/GNC/wood ash.

Preparation of chemical solution and GNC/wood ash suspensions for chemical treatment.

The chemical treatment was done in trays. The leaves were soaked in water in stainless steel tray and respective chemicals/GNC/ wood ash were added (w/w, on DM basis). While soaking, proper care was taken for thorough mixing of leaves with the treatment material. Wood ash suspension was prepared by burning pine wood collected from local forest (about 2kg) and pH of wood ash solution was recorded. Treated material was kept in an oven at temperature, 49 ± 1^0C, till it was dried completely.

Treatments	Control	UL	CL	CSDL	CSDGL
Potassium permanganate	0.0%	0.1%, 0.2%, 0.4%	-do-	-do-	-do-
Urea	0.0%	0.5%, 1.5%, 2.5%	-do-	-do-	-do-
Pine wood ash	0.0%	2.5%, 5.0%,10.0%	-do-	-do-	-do-
Groundnut cake	0.0%	1.7%, 3.4%,7.5%,	-do-	-do-	-do-

250 g samples of each of chemically processed leaves were taken for estimation of proximate principles and tannin assays respectively.

3.3 SAMPLE PREPARATION FOR ESTIMATION OF PROXIMATE PRINCIPLES AND TANNIN ASSAYS

Each of 250 gm of leaves (physically processed and chemically treated) including control leaves were oven dried (49 ± 1^0 C) and ground to pass through a 2 mm sieve. The ground samples were stored in airtight plastic jars and used for estimation of proximate principles and tannin assays (total phenolics, total tannins, condensed tannins and hydrolysable tannins).

3.3.1 Proximate composition

Standard methods as described in AOAC (1990) were followed for the determination of proximate composition. The details of estimation for the individual parameters are as follows:

3.3.1.1 Dry matter (DM)

Representative samples of feed ingredients were weighed in moisture cups and kept overnight in a hot air oven at 100^0 C. Dried samples were weighed and DM calculated as follows:

$$DM\ (\%) = a/b \times 100$$

where, a = weight of the sample after oven drying
b = fresh weight of the sample

3.3.1.2 Nitrogen

Nitrogen content of the samples was determined by the standard Kjeldahl method using Tecator digestion unit and Kjeltek Auto 1026 distillation unit (Tecator, Sweden). The titration was done using standard N/100 sulphuric acid and a digital burette (Digitrate, U.K.).

3.3.1.3 Ether extrac (EE)

A known quantity of ground sample was taken in the thimble (Whatman) and extracted for 7-8 h with petroleum ether (B.P. 40-60^0 C) in fat extraction apparatus. The extracted oil along with oil flask

and thimble were dried at 100^0 C to a constant weight. The ether extract was estimated as the difference in the weight of oil flask with and without oil and also as the loss of weight in sample in the thimble due to extraction. The average of both these values was taken as ether extract content of the sample.

3.3.1.4 Total ash

A known quantity of ground sample was taken in a preweighed silica basin and charred over the heater to make it smoke free. The crucible along with the sample was ignited at 600^0 C for 3 h. The residue on ashing was taken as total ash and was expressed on DM basis.

Calcium and phosphorus estimation

Methods for the estimation of Ca and P in feed, faeces and urine samples are discussed below.

Preparation of mineral extract

Weighed amount of feed or faeces sample was taken in a pre-weighed silica crucible, which was subjected to decarbonization followed by ashing at 550 - 600^0C in muffle furnace for 3 hrs. The residue left in the crucible was taken as total ash. This ash sample was digested with dilute HCl on a hot plate for 30 minutes and then cooled. The digested sample was then filtered through Whatman filter paper No. 1 in a 250 ml volumetric flask followed by washing with hot distilled water, till it was free from acid and volume was made up to the mark.

Mineral extract of urine samples was prepared by drying the urine samples in silica crucibles on hot plate, then decarbonized followed by ashing in muffle furnace. Mineral extract was prepared as described for feed and faeces samples.

Calcium estimation (Talapatra *et al*., 1940)

Reagents required

- Dilute hydrochloric acid (1: 4)
- Alcoholic methyl red indicator (0.1%)
- Dilute ammonia solution
- Dilute sulphuric acid solution
- Standard N/10 potassium permanganate solution
- Saturated ammonium oxalate solution.

Procedure

An aliquot of 25 ml mineral extract was transferred into a 250 ml beaker and to it 10 ml saturated ammonium oxalate solution was added with constant stirring. Then, two drops of methyl red indicator were added and pH was adjusted by adding dilute HCl so as to get faint pink colour. Beaker contents were heated on a hot plate for about 30 minutes and kept overnight for proper precipitation of calcium oxalate. On the next day, beaker contents were filtered through Whatman filter paper No. 40 with minimum five washings using hot distilled water. After that, filter paper containing whitish calcium oxalate precipitate was transferred into a beaker and dissolved by adding dilute sulphuric acid, followed by heating and titration against standard, N/10 KMnO4 solution. Calcium in the sample was calculated as follow:

$$\text{Ca (\%)} = \frac{\text{N/10 } KMnO_4 \text{ used (ml) x 0.002 x dilution factor}}{\text{Wt of sample (DM basis)}} \text{ x 100}$$

Estimation of Phosphorus (AOAC, 2000)

Reagents required

a) Molybdo-vandate reagent

Ammonium molybdate tetrahydrate (40 g) was dissolved in 400 ml hot distilled water in a beaker and the content was cooled. In

another flask, 2 g of ammonium metavandate was dissolved in 250 ml hot distilled water, cooled and 250 ml of 70% perchloric acid ($HClO_4$) was added. Molybdate solution was gradually added to vandate solution and volume was made up to 2 liters mark with distilled water.

b) Phosphorus standard solution

- Stock solution (2 mg P/ml) - 0.8788 g Potassium dihydrogen phosphate (KH_2PO_4) was dissolved in distilled water and volume was made up to 100 ml.
- Working solution (0.1 mg P/ml) – 5 ml stock solution was diluted to 100 ml.

Preparation of standard curve

Different volumes of working standard solution were taken in the test tubes so as to have 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg P and volume was made to 6 ml by adding distilled water. Then, 4 ml of molybdo-vandate reagent was added to all the test tubes and mixed. After 10 minutes, absorbance was taken at 400 nm in a spectrophotometer and regression equation was developed.

Estimation of phosphorus in feed, faeces and urine samples

Suitable aliquots of mineral extract were taken in test tubes, and to this distilled water were added to make the total volume to 6 ml. Then 4 ml of molybdo-vandate reagent was added in each tube, mixed and absorbance was taken at 400 nm after 10 minutes. The amount of phosphorus present in the samples was determined by using regression equation.

3.3.1.5 Organic matter (OM)

Organic matter was caluculated by subtracting total ash from 100.

3.3.1.6 Total carbohydrates

Total carbohydrate was calculated by subtracting the sum of percentage of CP and EE from OM.

3.3.2 Fiber composition

Fiber composition was determined as per the methods of Robertson and Van Soest (1989).

3.3.2.1 Neutral detergent fiber (NDF)

A known quantity of ground sample (0.5 or 1.0 g) was taken in spoutless beaker and a known quantity of neutral detergent solution (50 or 100 ml) was added to it. The beaker along with the contents was heated to boil and refluxed for 60 min. The contents were filtered through a preweighed Gooch crucible under vacuum with 3-4 washings of hot distilled water and a final washing of acetone. The crucibles were dried to a constant weight at 100^0 C and weighed. Cell wall contents or NDF was calculated as follows:

$$NDF(\%) = \frac{\text{(wt. of crucible + cell wall contents) - wt. of crucible}}{\text{Wt. of sample on DM basis}} \times 100$$

3.3.2.2 Acid detergent fiber (ADF)

A known quantity of ground sample (0.5 or 1.0 g) was taken in spoutless beaker and a known quantity of acid detergent solution (50 or 100 ml) was added to it. The beaker along with the contents was heated to boil and refluxed for 60 min. The contents were filtered through a preweighed Gooch crucible under vacuum with 3-4 washings of hot distilled water and a final washing of acetone. The crucibles were dried to a constant weight at 100^0 C and weighed. Acid detergent fiber was calculated as follows:

$$ADF(\%) = \frac{(X - Y)}{S} \times 100$$

Where, X = weight of oven dried crucible including ADF
Y = weight of empty oven dried crucible
S = sample weight on DM basis

3.3.2.3 Acid detergent lignin (ADL)

To the acid detergent residue in a Gooch crucible with glass rod was added around 30 ml of 72% sulphuric acid (w/w) was added. The crucibles were kept in a glass tray and 72% sulphuric acid was added from time to time so that all particles remained submerged in the acid. The contents were stirred occasionally. After 3 h, the contents were filtered under vacuum with 6-7 washings of hot distilled water. The washings were continued till the contents were acid free. The crucibles were dried overnight at 100° C, weighed and ignited in a muffle furnace at 550° C for 3 h. The loss in weight due to ashing was taken as lignin content in the sample.

$$\text{ADL (\%)} = \frac{L}{S} \times 100$$

Where, L = loss in weight due to ashing after 72% H_2SO_4 treatment
S = sample weight on DM basis

Hemicellulose was estimated as the difference between NDF and ADF while cellulose was the difference between ADF and ADL (Jung and Vogel, 1986). Total carbohydrate (TC) content was the sum of CF and NFE (Nitrogen free extracts).

3.3.3 Total phenolics, hydrolysable and condensed tannins

The methods for quantification of tannins were based on chemical properties of tannins. i.e, the method for total tannins is based on oxidation-reduction principles (tannins are reducing agents) and that for condensed tannins is based on oxidative depolymerisation of condensed tannins (proanthocyanidins) to anthocyanidins in butanol-HCl-Fe^{+++} mixture (Makkar, 2003).

Extraction of tannins

200 mg of selected feed sample was taken in a 50 ml conical flask. Before extraction of tannins, the pigments and fats from the sample were removed by 20 ml of diethyl ether containing 1% ascorbic acid. This mixture was shaken thoroughly and kept for 5 min. Diethyl ether was then decanted. After removal of diethyl ether by drying the residue at 30^0 C, 10 ml of 70% aqueous acetone was added to the defatted and depigmented residue. The flask was shaken for 2 h in an orbital shaker (30^0 C, 130 rpm). The contents were centrifuged for 20 min at 5,000 g. The supernatant was collected and stored at 4^0 C, if not immediately processed for analysis of tannins.

3.3.3.1 Analysis of total phenols

Reagents

- Folin-Ciocalteu (F-C) reagent (1N): Commercially available Folin-Ciocalteu reagent (2N) was diluted (1:1) with the distilled water and kept at 4^0 C.
- Sodium carbonate (Na_2CO_3) - 20%.
- Standard (S) tannic acid solution (0.5 mg/ml, was freshly prepared).

Suitable aliquots of the tannin-containing extract were taken in test tubes and volume made upto 1.0 ml with distilled water. Folin-Ciocalteu reagent (0.5 ml) and 20% sodium carbonate reagent (2.50 ml) were added to the tannin samples. The contents of the tubes were thoroughly mixed using vortex mixer and kept at room temperature for 40 min. Absorbance was recorded at 725 nm. The amount of the total phenols was calculated as tannic acid equivalent from the calibration curve (Figure 5) and the total phenolic content (%) was expressed on DM basis.

3.3.3.2 Analysis of non-tannin phenols

100 mg of commercially available insoluble polyvinylpolypyrrolidone (PVPP), which binds tannins, was weighed into 100 x 12 mm test tube. One ml distilled water and then 1 ml tannin containing extract were added to it and vortexed. The tube was kept at 4^0 C for 15 min, vortexed again, then centrifuged (3000 rpm for 10 min.) and supernatant was collected. This supernatant had only simple phenolics other than tannins. For measuring phenolic content of the supernatant, the procedure used for the estimation of total phenolics was followed.

The standard curve for tannic acid using Folin-Ciocalteu reagent was drawn using following protocol.

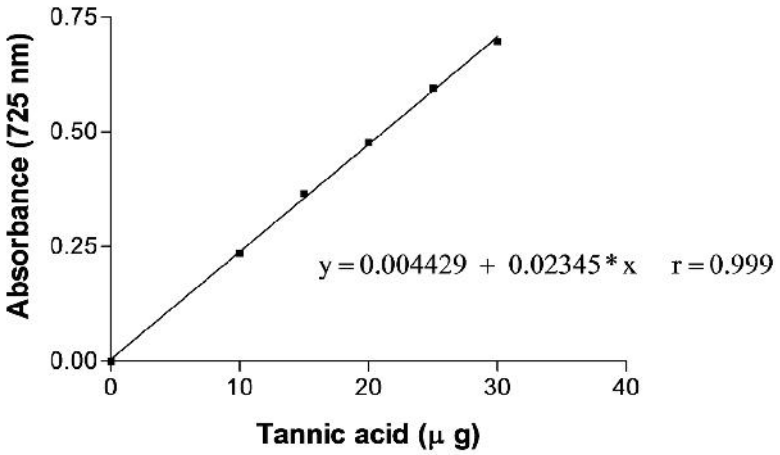


Figure 5. Standard curve for tannic acid

3.3.3.3 Determination of condensed tannins

Reagents

- Butanol-HCl reagent (butanol-HCl 95:5 v/v): Mixed 950 ml of n-butanol and 50 ml concentrate HCl (37%).
- Ferric reagent (2% ferric ammonium sulphate in 2 N HCl): Dissolved 2 g ferric ammonium sulphate in 100 ml of 2 N HCl (16.6 ml of conc. HCl is made to a final volume of 100 ml with water) and stored in an amber coloured bottle.

Tannin extract (0.5 ml) was taken in glass test tube, butanol-HCl reagent (3.0 ml) and ferric reagent (0.1 ml) were added to it. The contents of the tubes were thoroughly mixed using a vortex mixer. The mouth of the tubes were covered with glass marbles and put in a heating block (98 ± 2° C) for 60 min. The tubes were cooled and absorbance was recorded at 550 nm against a blank which comprised of an unheated mixture of the above reagents. Condensed tannins (% DM) as leucocyanidin equivalent was calculated by the formula:

$$\frac{A_{550nm} \times 78.26 \times \text{dilution factor}}{\%DM}$$

PHASE II

Among processed tree leaves maximum level of detannification was achieved in *F. roxburghii* (tremal) 0.5% (on DM basis) urea treated tremal. Therefore, all the levels of urea treated tremal leaves used in the phase I (0.5 %, 1.5% and 1.5%) were selected for evaluation of *in vitro* digestibility, VFA, NH_3-N and methane production (Menke *et al.*, 1979) and Untreated leaves were taken as control.

3.4 *IN VITRO* GAS PRODUCTION

The amount of gas which is released when feedstuffs are incubated *in vitro* with rumen liquor is closely related to digestibility and therefore to the energetic value of feedstuffs for ruminants (Menke *et al.,* 1979, Steingass, 1983).Therefore on the basis of gas production, quality of feeds can be evaluated.

Requirements

1. Glass syringes, 100 ml, graduated 1/1, with capillary attachment.
2. Incubator
3. Water bath
4. Silicone tube

5. Shut off clips
6. Carbon dioxide grade 1.
7. Glass wares of general use.
8. Chemicals

Micromineral solution[a]

$CaCl_2.2H_2O$	13.2 g
$MnCl_2.4H_2O$	10.0 g
$CoCl_2.6H_2O$	1.0 g
$FeCl_3.6H_2O$	8.0 g

Dissolved in 100 ml of water.

Rumen buffer solution[b]

NH_4HCO_3	4.0 g
$NaHCO_3$	35.0 g

Dissolved in 1000 ml of water.

Macromineral solution[c]

Na_2HPO_4 anhydrous	5.70 g
KH_2PO_4 anhydrous	6.20 g
$MgSO_4.7H_2O$	0.60 g

Dissolved in 1000 ml of water.

Resazurine solution[d] 0.10 % w/v

Items	30 syringes	45 syringes	60 syringes
Solution:I			
Distilled water	365 ml	550 ml	730 ml
Micromineral solution[a]	0.1 ml	0.15 ml	0.185 ml
Rumen buffer solution[b]	183 ml	275 ml	365 ml
Macromineral solution[c]	183 ml	275 ml	365 ml
Resazurin solution[d]	0.95 ml	1.45 ml	1.90 ml
Solution:II			
1N NaOH	1.6 ml	2.4 ml	3.1 ml
$Na_2S.7H_2O$	220 mg	330 mg	440 mg
Distilled water	37 ml	55 ml	73 ml
Solution:III			
Rumen liquor	330 ml	500 ml	660 ml

Procedure

Incubations were carried out in 100 ml calibrated syringes (Haberle, Germany) according to the method described by Menke *et al.* (1979). For each sample, 9 syringes were used [3 each for blank, blank + standard (wheat straw), blank+ test sample]. Respective sample of 200mg was taken in the each syringes except blank. The substrate /sample was weighed on a plastic boat with removable stem and was placed into the bottom of the glass syringe without sticking to the sides of the syringe. The piston was lubricated with petroleum jelly and pushed inside the glass syringe.The syringes were kept in oven at 39°C until incubation.

Rumen liquor was collected from fistulated male cattle maintained on a roughage based diet (1.0 kg concentrate mixture in equal proportions at 10.00 a.m and 4.00 p.m and wheat straw *ad lib*) before morning feeding into a pre-warmed thermo-flask and brought to the laboratory. The rumen liquor was bubbled with CO_2 for about 2 minutes and filtered through 4 layers of muslin cloth. Solution I (medium) was warmed and bubbled with CO_2 for few minutes. Then solution II (reducing agent) was added. This medium mixture solution was prewarmed to 39°C and bubbled with CO_2. Once the medium mixture solution became colourless, the required amount of filtered rumen liquor was added. The proportion of medium mixture solution to rumen liquor was 2:1. After 15 minutes, 30 or 40 ml of incubation medium was injected to the syringes using autopipette. The syringes were shaken gently, residual air or air bubbles if any was removed and the outlet was closed.

The level of piston was recorded and the syringes were placed in a water bath (39° ± 0.5°C). The incubation medium was bubbled with CO_2 and maintained at 39° ± 0.5°C. The syringes were shaken at

an interval of every 30 minutes for first 2 h from the start of the incubation and thereafter at every 2 h up to 10 h of incubation. For estimating the rate and extent of gas production, the volume of gas produced was recorded at 0^{th}, 1^{th}, 3^{rd}, 6^{th} and 24^{th} h. On termination of incubation, gas production and methane was measured by GLC. Syringe contents were centrifuged at 4500 rpm for 25 minutes. The supernatant was used for estimation of metabolites like ammonia nitrogen and volatile fatty acids.

3.4.1 True degradability

True degradability (TD) was estimated as per the method outlined by Goering and Van Soest (1970) with modifications. The incubations were carried out in 100 ml graduated glass syringes. The sample weight was increased to 400 mg to increase the residue so as to reduce the analytical error inherent to gravimetric determinations. To maintain pH and buffering for higher short chain fatty acid production, double strength buffer was used. The increase in buffer volume was matched by an equal increase in the volume of distilled water so as to keep the osmolality similar to the one in routine incubations. Consequently 40 ml buffered rumen liquor was injected in to each syringe as compared to 30 ml in routine incubations. Blank and standard hay samples (400 mg) were also included in each run. After 24 h incubation, the contents of the syringe were directly transferred in a 500 ml spoutless beaker. The syringe was washed with 40 ml of double strength Nuetral detergent solution (NDS) and washings added to the beaker. The contents in the beaker were refluxed for 1 h, filtered under vacuum through preweighed Gooch crucibles. NDF content of the residue was determined. True digestibility was calculated as follows.

$$TD = \frac{\text{Wt. of sample - wt. of residual NDF}}{\text{Wt of sample}} \times 100$$

3.4.2 Ammonia nitrogen

To 5 ml of rumen fluid sample, 2 ml of 1N NaOH was added and steam distilled using Kjeltec Analyzer (Tecator, Sweden); and the NH3 evolved was collected in boric acid and titrated against 0.01N H_2SO_4.

Calculation

1 ml 0.01 H_2SO_4 = 0.00014 g nitrogen

$$\text{Ammonia Nitrogen (mg)/100 ml rumen liquor} = \frac{R^* 0.00014^* D^*100}{v^*A}$$

Where, R = Reading of titer.
D = Dilution (volume made in volumetric flask)
v = Initial volume of rumen liquor taken for digestion.
A = Aliquot taken (5 ml)

Nitrogen of sample = Nitrogen of sample – Nitrogen of blank.

3.4.3 Volatile fatty acids (VFA)

Total and individual VFA in the rumen fluid samples were determined using Gas liquid chromatography (Nucon 5765, Nucon Engineers, New Delhi) equipped with flame ionization detector and glass column packed with chromosorb –101 (length 4'; o.d ¼"; i.d. 3 mm; mesh range 80-100). Analytical conditions for fractionation of VFA were as follows: Injection port temperature, 250°C; column temperature, 175°C; detector temperature, 260°C. The flow rate of carrier gas (nitrogen) was 40 ml/min; hydrogen 30ml/min; air 300 ml/min. Injection volume was 1 µl. The injection was performed by means of 10 µl Hamilton syringe (Hamilton Company, Nevada, USA). The concentration of various VFA (mmol/l) in the standard mixture were, acetic acid, 65.07; propionic acid, 20.01; iso-butyric acid, 2.95; butyric acid, 8.06; iso-valeric acid, 1.96, and valeric acid, 2.01. The mixture consisted of 0.8 ml VFA standard, 0.1 ml 25% metaphosphoric acid and 0.1 ml pivalic acid (5 mg/ml). Pivalic acid (0.1 ml) was added to rumen fluid samples (obtained after incubation) (0.8 ml rumen liquor and 0.1 ml metaphosphoric acid)

and centrifuged at 10,000 g for 5 minutes in a Microfuge centrifuge (Remi). The supernatant (1 µl) was injected into gas chromatograph for analysis of VFA. Pivalic acid was included as internal standard while metaphosphoric acid was used to deproteinize the sample. The peaks were identified by comparision of above standard and the response factor obtained using standard VFA mixture for each fraction was used to quantify VFA fractions (mmol/l) in the sample. The analysis and calculations were performed using Aimil chromatography data system (WINACDS). The amount of VFA produced (mmol) was calculated after deducting the corresponding blank values.

3.4.4 Methane

The chromatograph system and the analytical conditions followed for methane estimation were same as those for VFA except that the column was made up of stainless steel and packed with porapak-q (length 6'; o.d. 1.8"; i.d. 2 mm; mesh range 80-100). The temperature of injection port was 150 ° C; column 60 ° C; detector 130 ° C. Injection volume was 100 µl. The injection was performed by means of 500 µl Hamilton GASTIGHT syringe (Hamilton, Nevada, USA). The standard gas used for methane estimation composed of 50% methane and 50% carbon dioxide (SPANCAN Calibration gas, Spantech, Surrey, England). The peak was identified by comparision of above standard and the response factor obtained was used to calculate methane concentration in the gas sample. The volume of methane produced (ml) was calculated by multiplying the gas produced (ml) by the concentration of methane in the sample. The methane produced from the substrate after 24 h incubation was calculated by correcting the corresponding blank values.

Caluculations

Per cent digestible organic matter (DOM) and metabolizable energy (ME) per kg DM was estimated from the gas volume and/or chemical composition as suggested by Menke and Steingass (1988), Steingass and Menke (1986) and Krishnamoorthy *et al.* (1995).

- DOM (%) = 14.88 + 0.889 * gas(ml/200 mg DM) + 0.45 * CP + 0.65 * Ash

For concentrates

- ME digestible organic matter (DOM) and metabolizable energy (ME) (MJ/kg) = 1.06 + 0.157 * gas(ml/200 mg DM) + 0.0084 * CP + 0.022*EE – 0.0081* Ash

Menke and Steingass (1988)

For roughages

- ME (MJ/kg) = 2.20 + 0.136* gas(ml/200 mg DM) + 0.0057 *CP + 0.0029 *EE^2

Steingass and Menke (1986)

Common equations applicable for both roughage and concentrate.

- ME (MJ/kg) = 2.43 + 0.1206* gas(ml/200 mg DM) + 0.0069 *CP + 0.0187*EE

Krishnamoorthy *et al.* (1995).

Where, CP, ash and EE are expressed as per cent in DM

PHASE III

3.5 *IN VIVO* STUDIES

3.5.1 Animals, housing and management

Twelve growing female gaddi goats (6 to 12 months old) procured from local farmers were housed in a well ventilated shed with

arrangements for individual feeding and watering. All the animals were dewormed and fed with a basal ration to adopt them for stall feeding. After 1 month, the goats were divided into 3 groups of 4 animals each.

3.5.2 Feed and dry matter intake

Ficus roxburghii CSD (chopped sundried and 0.5% urea treated), which gave maximum level of OMD%/ME value in phase II, was used for development of alternate diet.Three diets based on dry *Grewia Optiva* (control), untreated *Ficus roxburghii* and *Ficus roxburghii CSD (chopped sundried and 0.5% urea treated)* tree leaves *ad lib* was provided to goats along with the supplementation of concentrates (maize grain 60%, groundnut cake 37%, mineral mixture 2% and salt 1%) for supporting 40 g average daily gain (Mandal *et al.,* 2005). The weighed amount of tree leaves were offered twice a day in the morning (9.00 a.m.) and evening (4.00 p.m.) and the left over of both the periods was recorded for assessment of daily feed intake. Daily dry matter (DM) intake was assessed from feed intake by daily determination of DM of both feed and residue. Adequate amount of clean drinking water was provided to all the goats.

1.3 Live weight gain

Animals were weighed at weekly intervals in the morning prior to offering of feed and water to assess the live weight gain.

1.4 Metabolism trial

Metabolism trial was conducted in two phases during 11th and 12th week of experiment taking two goats of each dietary treatment per phase involving 6 days collection of faeces and urine. Faeces voided by the individual animal in a total period of 24 hr (from 10 AM to 10 AM) was collected separately in previously weighed polythene

bag. It was weighed and the amount of faeces was quantified. After thorough mixing a suitable aliquot of faeces was taken for DM estimation. The faeces samples were pooled for 6 days and stored for further laboratory analysis. A separate aliquot of faeces mixed with 25% sulphuric acid was preserved daily for 6 days in previously weighed wide mouth plastic containers for nitrogen estimation.

Representative samples of feed offered and residue left during the digestibility trials were also collected daily for the estimation of DM and pooled over 6 days, for further chemical analysis.

Weight of freshly voided faeces and its moisture content was recorded over the period of 24 h. The moisture content of faeces was recorded by keeping adequate amount of the representative sample in hot air oven at 100 °C for 8 h and the total DM voided was worked out. The faeces was also analysed for its chemical constituents (ash, CP, EE, TCHO, NDF, ADF and lignin) by using standard methods as described earlier. The urine was analysed for urinary nirogen and ash.

2. Blood biochemical constituents

Collection of blood and separation of serum

About 15 ml of blood was collected from each kid by puncturing jugular vein in the morning (before watering and feeding) at zero day and subsequently at 90 days interval. Blood collected was kept in slanting position for 45 minutes and later brought to laboratory and centrifuged at 3000 rpm for 3 minutes to separate serum, which was collected into small plastic vials (5 ml) and stored at -20^{0}C for further analysis.

2.1. Glucose

Glucose in the serum was estimated by O-toluidine method (Sastry *et al.*, 1999), where in glucose forms a green coloured complex

with O-toluidine in hot acetic acid, the intensity of which is read photometrically.

2.2 Total protein and albumin

Serum total protein (STP) and albumin were estimated by Biuret and BCG dye binding method (Dumas *et al.*, 1971). Serum protein binded to copper ions in an alkaline medium of Biuret reagent and produced a purple colour complex, whose absorbance at 555nm was proportional to protein concentration. Serum albumin binded to bromocresol green in acidic condition and produced green colour, whose absorbance was measured at 630nm and the concentration, was expressed as g/dl blood serum.

2.3 Serum globulin

It was calculated by subtracting serum albumin from STP and expressed as g/dl blood serum.

2.4 Creatinine

Creatinine content in the serum was determined by the alkaline picrate method of Bonses and Taussky (1945), where the creatinine in the protein-free solution was allowed to react with alkaline picrate to produce a red colour complex, which was subsequently measured colorimetrically at 520 nm.

2.5 Blood urea nitrogen

Blood urea nitrogen was determined by diacetylmono-oxime (DAM) method as described by Wybenga *et al.*, (1971). Urea reacted with DAM in an acidic medium to produce a pinkish colour complex. The colour was intensified by using thiosemicarbazide and a cadmium salt. The absorbance of coloured complex at 520nm was proportional to the urea concentration (mg/dl) of sample. The values were multiplied with 0.467 to get the urea nitrogen content (mg/dl).

2.6 Calcium

Calcium in serum was estimated by the method of Trinder (1960). Calcium present in the sample was precipitated with naphthylhydroxamic acid. The precipitate was dissolved in EDTA reagent and calcium from this solution was complexed with colour reagent to give a coloured complex, which was measured colorimetrically at 450nm.

2.7 Phosphorus

Phosphorus in serum samples was determined (Gomorri, 1942), using Crest Biosystems kit. Phosphate ions in an acidic medium reacted with ammonium molybdate to form a phosphomolybdate complex. This complex reacted with metol and was reduced to a molybdenum blue complex. Intensity of the molydbenum blue complex formed was directly proportional to the amount of inorganic phosphorus present in the sample.

2.8 Aspartate aminotransferase (AST)

AST in blood serum was determined as per the method given by Reitman and Frankel (1957) using diagnostic kit (Glaxo, manufactured by Sigma Diagnostic Pvt. Limited, Baroda, India). SGOT catalyzes the transfer of amino group from L-asparate to a-ketoglutarate with formation of oxaloacetate and glutamate. The oxaloacetate so formed is allowed to react with 2, 4 DNPH to form 2,4-dinitrophenyl hydrazone derivative, which is brown in colour in alkaline medium. The absorbance of this hydrazone derivative is correlated to AST activity by plotting a calibration curve using pyruvate standard.

2.9 Alanine aminotransferase (ALT)

ALT was estimated by the method described by Reitman and Frankel (1957) using diagnostic kit (Glaxo, manufactured by Sigma

Diagnostic Pvt. Limited, Baroda, India). SGPT catalyzes the transfer of amino group from L-alanine to a-ketoglutarate with formation of pyruvate and glutamate. The pyruvate so formed is allowed to react with 2, 4-dinitrophenyl hydrazine to produce 2,4-dinitrophenyl hydrazone derivative, which is brown in colour in alkaline medium. The absorbance of this hydrazone derivative is correlated to SGPT activity by plotting a calibration curve using pyruvate standard.

2.10 Total bilirubin

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration (Pearlman and Lee, 1974)

2.11 Total cholesterol

The cholesterol ester is hydrolysed by cholesterol esterase to produce cholesterol and free fatty acid. In the presence of cholesterol oxidase, to produce cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase hydrogen peroxide couples with 4-aminoantipyrine and phenol to produce quinoneimine dye. Absorbace was measured at 505 nm and is proportional to the triglyceride concentration in the sample (Herbert, 1984).

2.12 Alakaline phosphatase

At alkaline pH alkaline phosphatase catalyses the hydrolysis of colourless p-nitrophenyl phosphate to yellow coloured p-nitrophenol and phosphate this change in colour was measured kinetically at 405 nm and was proportional to the Alakaline phosphatase activity (Tietz *et al.*, 1983).

2.13 Triglyceride (mg/dl)

Triglycerides are hydrolysed by lipoprotein lipase to produce glycerol and free fatty acid. In the presence of glyceerol kinase,

adenosine triphosphate phosphorylates glycerol to produce glycerol 3-phosphate and adenosine diphosphate glycerol 3-phosphate is further oxidized by glycerol 3- phosphate oxidase to produce dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase hydrogen peroxide couples with 4- aminoantipyrine and chlorophenol to produce red quinoneimine dye. Absorbace was measured at 505 nm and was proportional to the triglyceride concentration in the sample (Allain, 1974).

STATISTICAL ANALYSIS

All the data generated in the above experiments were statistically analyzed using SAS (2003) computer package. For comparison of groups, Generalized Linear Model ANOVA procedure and Duncan's multiple range tests (Steel and Torrie, 1980) were used.

Results

4 Results

The studies described in previous section are presented here.

PHASE I

4.1 DETANNIFICATION OF *QUERCUS LEUCOTRICHOPHORA* (BUN) AND *FICUS ROXBURGHII* (TREMAL) LEAVES BY DIFFERENT PHYSICAL AND CHEMICAL TREATMENTS

Chemical composition of tree leaves

The values of OM (Organic matter), CP (Crude protein), EE (Ether extract), TCHO (Total carbohydrate), NDF (Neutral detergent fibtre), ADF (Acid detergent fibre), cellulose, hemi cellulose, total ash, calcium and phosphorous contents of *Q. leucotrichophora* and *F. roxburghii* leaves (% on DM basis) are presented in the Table 7.

Table 7 : Chemical composition of tree leaves

Attributes	Tree leaves	
	Q. leucotrichophora	*F. roxburghii*
Proximate Composition		
OM	94.02	92.38
CP	10.53	13.86
EE	4.02	4.34
TCHO	79.47	74.18
Cell wall constituents		
NDF	48.89	60.78
ADF	31.51	52.72
Cellulose	20.54	36.80
Hemi cellulose	17.38	8.06

Minerals		
Ash	5.98	7.62
Calcium	1.89	2.38
Phosphorous	0.27	0.22

4.1.1 Physical treatment of *Q. leucotrichophora leaves*

Effect of physical treatments on different polyphenols of *Q. leucotrichophora* leaves is presented in Table 8. Fresh *Q. leucotrichophora* leaves contained 7.26 % total phenols (TP), 1.12 % non tannin phenols (NTP), 6.15 % total tannins (TT), 1.62 % condensed tannins (CT) and 4.52 % hydrolysable tannins (HT). All the physical treatments [chopping, chopping and sun drying (CSD), chopping sun drying and grinding (CSDG)] were effective ($P < 0.0001$) in reducing all, the phenolic contents in oak leaves (Table 8).

Among the treatments chopping followed by sun drying and grinding (CSDG), reduced the phenolic contents to the maximum level followed by other two treatments [i.e. chopped and chopping followed by sun drying (CSD)]. HT was reduced by CSDG ($P < 0.0001$) at maximum level (i.e. 58.13 %) followed by total tannin (TT) (i.e. 40.79 %), total phenol (TP) (i.e. 40.76 %), condensed tannin (CT) (14.83 %) and non tannin phenols (i.e. 8.18 %). Chopping was effective ($P < 0.0001$) in reducing the TP, NTP, TT, CT and HT to the extent of 31.73, 3.77, 36.93, 12.86 and 45.42 %, respectively; whereas chopping followed by sun drying (CSD) reduced ($P < 0.0001$) the respective polyphenols, to the extent, 28.91, 7.26, 32.96, 14.03 and 39.60%, respectively.

4.1.2 Effect of physical along with different chemical treatments on polyphenol content of *Q. leucotrichophora* leaves

4.1.2.1 Effect of different physical treatments followed by potassium permanganate (PP) treatment on polyphenol contents of *Q. leucotrichophora* leaves

Polyphenol content of physico-chemically (PP) treated oak leaves has been presented in Table 9. When the oak leaves were treated

Table 8 : Effect of physical treatment on phenolic contents (% on DMB) in *Q. leucotrichophora* leaves

Physical Treatments	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	$7.26^{A} \pm 0.03$	$1.12^{A} \pm 0.01$	$6.15^{A} \pm 0.04$	$1.62^{A} \pm 0.02$	$4.52^{A} \pm 0.06$
Chopped	$4.96^{C} \pm 0.02$	$1.08^{B} \pm 0.01$	$3.88^{C} \pm 0.02$	$1.41^{B} \pm 0.01$	$2.47^{C} \pm 0.02$
Chopped sun dried (CSD)	$5.16^{B} \pm 0.02$	$1.04^{C} \pm 0.01$	$4.12^{B} \pm 0.02$	$1.39^{B} \pm 0.003$	$2.73^{B} \pm 0.01$
Chopped sun dried ground (CSDG)	$4.30^{D} \pm 0.05$	$1.02^{C} \pm 0.005$	$3.27^{D} \pm 0.05$	$1.38^{B} \pm 0.002$	$1.89^{D} \pm 0.05$
Overall mean ± SE	5.42 ± 0.23	1.06 ± 0.008	4.35 ± 0.23	1.45 ± 0.02	2.90 ± 0.21
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Per cent reduction on physical treatment				
Chopped	$31.73^{B} \pm 0.25$	$3.77^{B} \pm 0.46$	$36.93^{B} \pm 0.30$	$12.86^{B} \pm 0.50$	$45.42^{B} \pm 0.52$
Chopped sun dried	$28.91^{a} \pm 0.24$	$7.26^{a} \pm 0.46$	$32.96^{a} \pm 0.27$	$14.03^{a} \pm 0.17$	$39.60^{a} \pm 0.33$
Chopped sun dried ground	$40.76^{A} \pm 0.68$	$8.18^{A} \pm 0.45$	$46.79^{A} \pm 0.75$	$14.83^{A} \pm 0.13$	$58.13^{A} \pm 0.1$
Overall mean ± SE	33.80 ± 1.25	6.40 ± 0.52	38.89 ± 1.44	13.90 ± 0.26	47.72 ± 1.91
P Value	< 0.0001	< 0.0001	< 0.0001	0.002	< 0.0001

Means bearing different superscripts with in a column (A, B, C) differ significantly ($P < 0.0001$)

physically followed by chemically with potassium permanganate, the overall reduction of TP, NTP, TT, CT and HT was 61.76 (29.78-65.79), 30.05 (9.39-41.69), 67.60 (33.61-72.48), 32.94 (9.39-50.69) and 79.95 (42.14-93.40)%, respectively. The reduction in the concentration of these polyphenols due to physical treatments varied from 54.05 to 65.52, 23.66 to 36.19, 58.18 to 72.30, 26.06 to 41.08 and 69.61 to 85.17 %, respectively; and due to chemical treatments (PP) (including water), varied from 55.30 to 65.43, 22.79 to 35.01, 61.30 to 71.03, 13.48 to 40.06 and 78.35 to 82.07 %, respectively.

Extent of overall reduction was maximum in case of HT (79.95 %) followed by TT (i.e. 67.60 %), TP (61.76 %), CT (32.94 %) and was lowest in case of NTP (30.05%). Both physical and chemical (including water) treatments had their effect in reducing ($P < 0.0001$) all the polyphenols, but among the physical treatments, reduction of TP, TT and CT was maximum ($P < 0.0001$) due to CSDG and was lowest in fresh leaves treated only with water. Similarly, among the chemical treatments, 0.4% potassium permanganate reduced all the polyphenols to the maximum extent followed by 0.2% and 0.1% PP and was minimum through water treatment.

Interaction between physical and PP treatments was also found to be very significant ($P < 0.0001$). On comparison between 16 groups of different treatment combinations (Physical × Chemical), it was observed that the reduction of TP was maximum in 4^{th} (fresh 0.4% PP), NTP in 12^{th} (CSD 0.4 % PP), TT in 14^{th} (CSDG 0.1% PP), CT in 16^{th} (CSDG 0.4% PP) and HT in 5^{th} (chopped with water) treatment. The values of maximum reduction of TP was comparable among 4^{th}, 12^{th}, 13^{th}, 14^{th}, 15^{th} and 16^{th} treatments; NTP was comparable between 4^{th} and 12^{th} treatments; TT and CT was comparable among 14^{th}, 15^{th} and 16^{th} treatments. Reduction of all the polyphenols was found to be at minimum ($P < 0.0001$) in first treatment (fresh × 0.0 % PP).

Table 9 : **Effect of different physical and chemical (potassium permanganate) treatments on phenolic contents of *Q. leucotrichophora* leaves**

Physical Treatment	Chemical Treatment	Polyphenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0%PP (Water)	$5.10^{A} \pm 0.02$	$1.01^{A} \pm 0.01$	$4.08^{A} \pm 0.02$	$1.47^{A} \pm 0.01$	$2.62^{A} \pm 0.03$	$29.78^{G} \pm 0.29$	$9.39^{J} \pm 0.70$	$33.61^{J} \pm 0.36$	$9.39^{G} \pm 0.52$	$42.14^{J} \pm 0.58$
	0.1%PP	$2.94^{B} \pm 0.03$	$0.69^{HI} \pm 0.01$	$2.25^{B} \pm 0.04$	$1.13^{D} \pm 0.03$	$1.12^{B} \pm 0.03$	$59.47^{F} \pm 0.45$	$38.19^{BC} \pm 0.49$	$63.41^{I} \pm 0.60$	$30.07^{D} \pm 1.95$	$75.28^{I} \pm 0.73$
	0.2%PP	$2.85^{C} \pm 0.03$	$0.68^{I} \pm 0.005$	$2.17^{C} \pm 0.03$	$1.12^{DE} \pm 0.03$	$1.05^{B} \pm 0.05$	$60.73^{E} \pm 0.39$	$38.89^{B} \pm 0.45$	$64.78^{H} \pm 0.48$	$31.16^{CD} \pm 1.93$	$76.75^{I} \pm 1.06$
	0.4%PP	$2.45^{G} \pm 0.02$	$0.67^{J} \pm 0.005$	$1.79^{FGH} \pm 0.02$	$1.08^{E} \pm 0.03$	$0.71^{G} \pm 0.04$	$66.21^{A} \pm 0.24$	$40.53^{A} \pm 0.44$	$70.94^{CDE} \pm 0.30$	$33.60^{C} \pm 2.00$	$84.26^{D} \pm 0.78$
Chopped	0.0%PP (Water)	$2.63^{E} \pm 0.01$	$0.88^{B} \pm 0.01$	$1.74^{HIG} \pm 0.01$	$1.44^{A} \pm 0.01$	$0.30^{J} \pm 0.02$	$63.76^{C} \pm 0.20$	$20.73^{I} \pm 0.50$	$71.66^{BCD} \pm 0.21$	$10.81^{G} \pm 0.50$	$93.40^{A} \pm 0.38$
	0.1%PP	$2.72^{D} \pm 0.02$	$0.86^{C} \pm 0.003$	$1.85^{EF} \pm 0.02$	$1.02^{F} \pm 0.01$	$0.83^{CDEF} \pm 0.02$	$62.58^{D} \pm 0.26$	$23.15^{H} \pm 0.25$	$69.82^{EF} \pm 0.31$	$37.06^{B} \pm 0.33$	$81.49^{EFG H} \pm 0.46$
	0.2%PP	$2.65^{DE} \pm 0.02$	$0.85^{C} \pm 0.004$	$1.80^{GF} \pm 0.02$	$1.01^{F} \pm 0.01$	$0.79^{EFG} \pm 0.02$	$63.50^{CD} \pm 0.30$	$24.18^{H} \pm 0.39$	$70.72^{DE} \pm 0.33$	$37.68^{B} \pm 0.39$	$82.50^{DEF} \pm 0.50$
	0.4%PP	$2.59^{EF} \pm 0.02$	$0.82^{D} \pm 0.01$	$1.77^{GHIF} \pm 0.02$	$1.02^{F} \pm 0.01$	$0.76^{FG} \pm 0.03$	$64.29^{BC} \pm 0.29$	$26.58^{G} \pm 0.78$	$71.21^{BCDE} \pm 0.28$	$37.31^{B} \pm 0.81$	$83.30^{DE} \pm 0.60$
Chopped sun dried	0.0%PP (Water)	$2.73^{D} \pm 0.02$	$0.78^{F} \pm 0.003$	$1.94^{D} \pm 0.02$	$1.37^{B} \pm 0.003$	$0.57^{H} \pm 0.02$	$62.45^{D} \pm 0.26$	$30.23^{E} \pm 0.31$	$68.37^{G} \pm 0.34$	$15.32^{F} \pm 0.19$	$87.32^{C} \pm 0.48$
	0.1%PP	$2.63^{E} \pm 0.01$	$0.72^{G} \pm 0.006$	$1.91^{DE} \pm 0.01$	$0.98^{F} \pm 0.002$	$0.93^{C} \pm 0.01$	$63.80^{C} \pm 0.20$	$35.72^{D} \pm 0.54$	$68.97^{FG} \pm 0.20$	$39.67^{B} \pm 0.10$	$79.41^{H} \pm 0.24$
	0.2%PP	$2.61^{E} \pm 0.01$	$0.70^{HG} \pm 0.01$	$1.90^{DE} \pm 0.02$	$0.99^{F} \pm 0.01$	$0.92^{CD} \pm 0.02$	$64.09^{C} \pm 0.19$	$37.13^{CD} \pm 0.68$	$69.06^{FG} \pm 0.31$	$39.16^{B} \pm 0.37$	$79.71^{GH} \pm 0.43$
	0.4%PP	$2.51^{G} \pm 0.02$	$0.65^{J} \pm 0.004$	$1.86^{EF} \pm 0.02$	$0.99^{F} \pm 0.01$	$0.86^{CDE} \pm 0.03$	$65.45^{A} \pm 0.28$	$41.69^{A} \pm 0.32$	$69.84^{EF} \pm 0.31$	$38.63^{B} \pm 0.67$	$80.95^{FGH} \pm 0.62$
Chopped sun dried and ground	0.0%PP (Water)	$2.52^{GF} \pm 0.04$	$0.77^{F} \pm 0.004$	$1.75^{HIG} \pm 0.04$	$1.32^{C} \pm 0.004$	$0.43^{I} \pm 0.04$	$65.23^{AB} \pm 0.53$	$30.83^{E} \pm 0.32$	$71.55^{BCD} \pm 0.59$	$18.40^{E} \pm 0.25$	$90.54^{B} \pm 0.77$
	0.1%PP	$2.51^{G} \pm 0.04$	$0.85^{C} \pm 0.01$	$1.66^{J} \pm 0.04$	$0.83^{G} \pm 0.001$	$0.83^{DEF} \pm 0.04$	$65.39^{A} \pm 0.53$	$23.81^{H} \pm 0.57$	$73.02^{A} \pm 0.66$	$48.76^{A} \pm 0.07$	$81.65^{EFG} \pm 0.88$
	0.2%PP	$2.49^{G} \pm 0.04$	$0.80^{E} \pm 0.01$	$1.69^{IJ} \pm 0.04$	$0.82^{G} \pm 0.001$	$0.87^{CDE} \pm 0.04$	$65.66^{A} \pm 0.52$	$28.47^{F} \pm 0.59$	$72.48^{AB} \pm 0.67$	$49.35^{A} \pm 0.06$	$80.71^{FGH} \pm 0.90$
	0.4%PP	$2.48^{G} \pm 0.04$	$0.77^{F} \pm 0.008$	$1.71^{HIJ} \pm 0.04$	$0.80^{G} \pm 0.002$	$0.91^{CD} \pm 0.04$	$65.79^{A} \pm 0.52$	$31.24^{E} \pm 0.72$	$72.14^{ABC} \pm 0.68$	$50.69^{A} \pm 0.12$	$79.77^{GH} \pm 0.94$

Table 9 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.34^{A} ± 0.22	0.76^{C} ± 0.03	2.57^{A} ± 0.18	1.20^{A} ± 0.04	1.37^{A} ± 0.15	54.05^{C} ± 2.97	31.75^{B} ± 2.71	58.18^{D} ± 3.02	26.06^{D} ± 2.18	69.61^{D} ± 3.40
Chopped	2.65^{b} ± 0.01	0.85^{A} ± 0.01	1.79^{c} ± 0.01	1.12^{b} ± 0.04	0.67^{d} ± 0.05	63.53^{b} ± 0.18	23.66^{d} ± 0.50	70.85^{b} ± 0.19	30.71^{c} ± 2.41	85.17^{a} ± 1.03
Chopped sun dried	2.62^{b} ± 0.02	0.71^{d} ± 0.01	1.90^{b} ± 0.01	1.08^{c} ± 0.04	0.82^{b}± 0.03	63.95^{b} ± 0.24	36.19^{a} ± 0.88	69.06^{c} ± 0.18	33.19^{b} ± 2.16	81.85^{c} ± 0.70
Chopped sun dried and grinded	2.50^{c} ± 0.01	0.79^{b} ± 0.01	1.70^{d}± 0.02	0.94^{d} ± 0.05	0.76^{c} ± 0.04	65.52^{a}± 0.25	28.59^{c} ± 0.67	72.30^{a} ± 0.32	41.80^{a} ± 2.82	83.17^{b} ± 0.98
Potassium permanganate	**Potassium permanganate Treatments**					**Potassium permanganate Treatments**				
0.0%PP (Water)	3.24^{A} ± 0.22	0.86^{A} ± 0.02	2.38^{A} ± 0.21	1.40^{A} ± 0.01	0.97^{A} ± 0.20	55.30^{D} ± 3.08	22.79^{D} ± 1.83	61.30^{C} ± 3.35	13.48^{D} ± 0.77	78.35^{C} ± 4.39
0.1%PP	2.70^{b} ± 0.04	0.78^{b} ± 0.02	1.92^{b} ± 0.05	0.99^{b} ± 0.02	0.93^{b} ± 0.03	62.81^{c} ± 0.49	30.22^{c} ± 1.44	68.81^{b} ± 0.76	38.89^{a} ± 1.47	79.46^{b} ± 0.61
0.2%PP	2.65^{c} ± 0.03	0.76^{c}± 0.01	1.89^{b} ± 0.04	0.98^{b} ± 0.02	0.91^{b} ± 0.03	63.50^{b} ± 0.41	32.17^{b} ± 1.29	69.26^{b} ± 0.63	39.34^{a} ± 1.43	79.92^{b} ± 0.56
0.4%PP	2.51^{d} ± 0.02	0.73^{d} ± 0.02	1.78^{c} ± 0.02	0.97^{b} ± 0.02	0.81^{c} ± 0.02	65.43^{a} ± 0.22	35.01^{a} ± 1.35	71.03^{a} ± 0.26	40.06^{A} ± 1.44	82.07^{a} ± 0.51
Overall mean ± SE	2.78 ± 0.06	0.78 ± 0.01	1.99 ± 0.06	1.09 ± 0.02	0.91 ± 0.05	61.76 ± 0.87	30.05 ± 0.87	67.60 ± 0.94	32.94 ± 1.32	79.95 ± 1.11
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

NB: Values bearing the different superscripts (A, B, C, D etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *Q. leucotrichophora* leaves were 7.26 ± 0.03, 1.12± 0.01, 6.15± 0.04, 1.62 ± 0.02 and 4.52 ± 0.06 , respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

4.1.2.2 Effect of different physical treatments followed by urea treatment on polyphenol contents of *Q. leucotrichophora* leaves

Polyphenol content of physico-chemically (urea) treated oak leaves have been presented in Table 10. When the oak leaves were treated physically followed by chemically with urea, the overall reduction of TP, NTP, TT, CT and HT was 57.73 (29.78-66.10), 27.72 (9.39-41.42), 62.90 (33.61-70.98), 15.48 (9.39-21.24) and 79.81 (42.14-93.40)%, respectively. The reduction in the concentration of these polyphenols due to physical treatments was 48.15 to 65.25, 21.50 to 36.14, 53.09 to 70.85, 12.75 to 19.98 and 67.45 to 89.02 %, respectively; and due to chemical treatments (urea) (including water), it varied from 50.91 to 63.77, 22.79 to 34.58, 54.88 to 69.15, 13.48 to 17.47 and 69.07 to 87.60 %, respectively.

Extent of overall reduction was maximum in case of HT (79.81 %) followed by TT (i.e. 62.90 %), TP (57.73 %), NTP (29.72 %) and was lowest in case of CT (15.48 %). Both physical and chemical (including water) treatments had their effect in reducing ($P < 0.0001$) all the polyphenols, but among the physical treatments, reduction was maximum ($P < 0.0001$) due to CSDG for TP, NTP, TT, CT and HT and was lowest (for all the phenols) in fresh leaves treated only with water. NTP was reduced to the maximum extent in CSD treatments. Similarly, among the chemical treatments, 2.5 % urea treatment reduced all the polyphenols to the maximum extent followed by 1.5 % urea treatments. Extent of reduction of TP, TT and HT was minimum at 0.5 %; but for NTP and CT it was minimum in water treatment only (0.0 % urea).

Interaction between physical and urea treatments was also found to be vary significant ($P < 0.0001$). On comparison among 16 groups of different treatment combinations (Physical × chemical), it was observed that the reduction of TP and NTP was maximum in 12th (CSD 2.5 % urea); TT and HT reduction was maximum in 5th (Chopped

Table 10 : Effect of different physical and chemical (urea) treatments on phenolic contents of *Q. leucotrichophora* leaves

Physical Treatment	Chemical Tretment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0% Urea (Water)	5.10^{A} ± 0.02	1.01^{A} ± 0.01	4.08^{A} ± 0.02	1.47^{A} ± 0.01	2.62^{A} ± 0.03	29.78^{K} ± 0.29	9.39^{J} ± 0.70	33.61^{J} ± 0.36	9.39^{K} ± 0.52	42.14^{L} ± 0.59
	0.5% Urea	4.13^{B} ± 0.02	0.86^{e} ± 0.01	3.28^{B} ± 0.01	1.42^{C} ± 0.002	1.86^{B} ± 0.01	42.99^{J} ± 0.21	23.15^{H} ± 0.61	46.70^{I} ± 0.19	12.34^{J} ± 0.13	58.90^{K} ± 0.28
	1.5% Urea	3.00^{E} ± 0.01	0.85^{C} ± 0.004	2.15^{EF} ± 0.006	1.40^{D} ± 0.002	0.75^{E} ± 0.005	58.66^{G} ± 0.10	24.41^{H} ± 0.32	64.97^{EF} ± 0.10	13.42^{H} ± 0.14	83.37^{H} ± 0.11
	2.5% Urea	2.82^{G} ± 0.02	0.79^{D} ± 0.003	2.02^{G} ± 0.02	1.36^{EFG} ± 0.002	0.66^{FG} ± 0.02	61.17^{E} ± 0.23	29.06^{G} ± 0.27	67.09^{D} ± 0.27	15.85^{EFG} ± 0.10	85.38^{FG} ± 0.36
Chopped	0.0% Urea (Water)	2.63^{I} ± 0.01	0.88^{B} ± 0.01	1.74^{J} ± 0.01	1.44^{E} ± 0.01	0.30^{L} ± 0.02	63.76^{C} ± 0.20	20.73^{I} ± 0.50	71.66^{A} ± 0.21	10.81^{J} ± 0.50	93.40^{A} ± 0.38
	0.5% Urea	3.90^{C} ± 0.01	0.85^{C} ± 0.004	3.05^{C} ± 0.01	1.42^{C} ± 0.004	1.63^{C} ± 0.01	46.25^{I} ± 0.19	24.29^{H} ± 0.37	50.34^{H} ± 0.24	12.49^{I} ± 0.27	63.79^{J} ± 0.31
	1.5% Urea	2.91^{F} ± 0.01	0.79^{DE} ± 0.003	2.12^{F} ± 0.01	1.39^{D} ± 0.001	0.72^{EF} ± 0.01	59.89^{I} ± 0.09	29.32^{FG} ± 0.24	65.52^{E} ± 0.10	14.03^{H} ± 0.09	83.90^{GH} ± 0.15
	2.5% Urea	2.75^{GH} ± 0.02	0.78^{EF} ± 0.002	1.98^{GH} ± 0.02	1.36^{FG} ± 0.001	0.62^{GH} ± 0.02	62.07^{F} ± 0.22	30.53^{EF} ± 0.18	67.88^{CD} ± 0.25	16.33^{EF} ± 0.08	86.28^{EF} ± 0.33
Chopped sun dried	0.0% Urea (Water)	2.73^{H} ± 0.02	0.78^{DEF} ± 0.003	1.94^{H} ± 0.02	1.37^{E} ± 0.003	0.57^{HI} ± 0.02	62.45^{D} ± 0.26	30.23^{EFG} ± 0.31	68.37^{C} ± 0.34	15.32^{G} ± 0.19	87.32^{DE} ± 0.48
	0.5% Urea	3.67^{D} ± 0.02	0.72^{GH} ± 0.01	2.94^{D} ± 0.02	1.37^{E} ± 0.003	1.58^{C} ± 0.02	49.50^{H} ± 0.26	35.61^{CD} ± 0.54	52.12^{G} ± 0.30	15.48^{G} ± 0.18	65.14^{J} ± 0.40
	1.5% Urea	2.91^{F} ± 0.02	0.70^{I} ± 0.01	2.21^{E} ± 0.03	1.36^{EF} ± 0.001	0.84^{D} ± 0.03	59.86^{F} ± 0.28	37.30^{B} ± 0.68	64.05^{F} ± 0.40	15.75^{FG} ± 0.03	81.26^{I} ± 0.56
	2.5% Urea	2.46^{K} ± 0.03	0.66^{J} ± 0.004	1.81^{IJ} ± 0.03	1.35^{G} ± 0.001	0.45^{JK} ± 0.03	66.10^{A} ± 0.39	41.42^{A} ± 0.32	70.65^{AB} ± 0.49	16.47^{E} ± 0.06	89.99^{BC} ± 0.66
Chopped sun dried and grinded	0.0% Urea (Water)	2.52^{JK} ± 0.04	0.77^{F} ± 0.004	1.75^{IJ} ± 0.04	1.32^{H} ± 0.004	0.43^{K} ± 0.04	65.23^{AB} ± 0.53	30.83^{E} ± 0.32	71.55^{AB} ± 0.59	18.40^{D} ± 0.25	90.54^{B} ± 0.77
	0.5% Urea	2.55^{J} ± 0.04	0.73^{G} ± 0.006	1.82^{I} ± 0.03	1.30^{I} ± 0.001	0.52^{IJ} ± 0.03	64.91^{B} ± 0.53	35.25^{D} ± 0.55	70.36^{B} ± 0.56	19.69^{C} ± 0.07	88.46^{CD} ± 0.73
	1.5% Urea	2.52^{JK} ± 0.04	0.71^{HI} ± 0.007	1.81^{IJ} ± 0.04	1.29^{J} ± 0.001	0.53^{I} ± 0.04	65.23^{AB} ± 0.53	36.67^{BC} ± 0.67	70.49^{AB} ± 0.59	20.59^{B} ± 0.09	88.31^{D} ± 0.78
	2.5% Urea	2.48^{JK} ± 0.04	0.70^{I} ± 0.01	1.78^{IJ} ± 0.04	1.28^{K} ± 0.002	0.51^{IJ} ± 0.04	65.74^{AB} ± 0.52	37.28^{B} ± 0.52	70.98^{AB} ± 0.65	21.24^{A} ± 0.09	88.75^{CD} ± 0.86

Table 10 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.76^{A} ± 0.19	0.88^{a} ± 0.02	2.88^{a} ± 0.18	1.41^{a} ± 0.01	1.47^{a} ± 0.17	48.15^{d} ± 2.65	21.50^{d} ± 1.55	53.09^{c} ± 2.87	12.75^{d} ± 0.50	67.45^{d} ± 3.75
Chopped	3.05^{b} ± 0.10	0.83^{b} ± 0.01	2.22^{b} ± 0.10	1.40^{b} ± 0.01	0.82^{c} ± 0.10	57.99^{c} ± 1.44	26.22^{c} ± 0.84	63.85^{b} ± 1.69	13.42^{c} ± 0.44	81.84^{b} ± 2.30
Chopped sun dried	2.94^{c} ± 0.09	0.72^{d} ± 0.01	2.23^{b} ± 0.09	1.36^{c} ± 0.002	0.86^{b}± 0.09	59.48^{b} ± 1.29	36.14^{a} ± 0.87	63.79^{b} ± 1.50	15.76^{b} ± 0.11	80.93^{c} ± 2.03
Chopped sun dried and grinded	2.52^{d} ± 0.02	0.73^{c} ± 0.01	1.79^{c}± 0.02	1.30^{d} ± 0.004	0.50^{d} ± 0.02	65.28^{a}± 0.25	35.01^{b} ± 0.58	70.85^{a} ± 0.30	19.98^{a} ± 0.23	89.02^{a} ± 0.41
Level of Urea	**Urea Treatments**					**Urea Treatments**				
0.0% Urea (Water)	3.24^{B} ± 0.22	0.86^{a} ± 0.02	2.38^{b} ± 0.21	1.40^{a} ± 0.01	0.97^{b} ± 0.20	55.30^{c} ± 3.08	22.79^{d} ± 1.83	61.30^{c} ± 3.35	13.48^{d} ± 0.77	78.35^{c} ± 4.39
0.5% Urea	3.56^{a} ± 0.13	0.79^{b} ± 0.01	2.77^{a} ± 0.11	1.38^{b} ± 0.01	1.40^{a} ± 0.11	50.91^{d} ± 1.76	29.57^{c} ± 1.25	54.88^{d} ± 1.91	15.00^{c} ± 0.63	69.07^{d} ± 2.39
1.5% Urea	2.83^{c} ± 0.04	0.76^{c}± 0.01	2.08^{c} ± 0.03	1.36^{c} ± 0.01	0.71^{c} ± 0.03	60.91^{b} ± 0.55	31.92^{b} ± 1.14	66.25^{b} ± 0.54	15.95^{b}± 0.59	84.21^{b} ± 0.58
2.5% Urea	2.63^{d} ± 0.03	0.73^{d} ± 0.01	1.90^{d} ± 0.03	1.34^{d} ± 0.01	0.56^{d} ± 0.02	63.77^{a} ± 0.48	34.58^{a} ± 1.06	69.15^{a} ± 0.41	17.47^{a} ± 0.45	87.60^{a} ± 0.47
Overall mean ± SE	3.07 ± 0.07	0.78 ± 0.01	2.28 ± 0.07	1.37 ± 0.01	0.91 ± 0.06	57.73 ± 1.02	29.72 ± 0.80	62.90 ± 1.11	15.48 ± 0.34	79.81 ± 1.44
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C, D etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh Q. leucotrichophora leaves were 7.26 ± 0.03, 1.12± 0.01, 6.15± 0.04, 1.62 ± 0.02 and 4.52 ± 0.06 , respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

with water); CT reduction was maximum in 16th (CSDG 2.5 % urea) treatment. The maximum reduction of TP was comparable among 12th, 13th, 15th and 16th treatments; TT was comparable among 5th, 12th, 13th, 15th and 16th treatments. Reduction of all the polyphenols was found to be at minimum in 1st (fresh + 0.0 % urea) treatment.

4.1.2.3 Effect of different physical treatments followed by wood ash (WA) treatment on polyphenol contents of *Q. leucotrichophora* leaves

Polyphenol content of physico-chemically (WA) treated oak leaves has been presented in Table 11. When the oak leaves were treated physically followed by chemically with wood ash, the overall reduction of TP, NTP, TT, CT and HT was 52.98 (29.78-68.78), 24.98 (9.39-30.91), 58.15 (33.61-75.72), 21.30 (9.39-26.40) and 71.27 (41.48-93.62)% respectively. The reduction in the concentration of these polyphenols due to physical treatments was 39.68 to 66.32, 17.06 to 28.51, 43.90 to 73.26, 17.90 to 23.25 and 53.09 to 91.33 %, respectively; and due to chemical (WA) treatments (including water), it varied from 44.56 to 57.47, 21.84 to 29.35, 48.79 to 69.67, 13.48 to 25.10 and 78.35 to 76.03 %, respectively.

Extent of overall reduction was maximum in case of HT (71.27%) followed by TT (i.e. 58.15%), TP (52.98%), NTP (24.98%) and was lowest in case of CT (21.30%). Both physical and chemical (including water) treatments had their effect in reducing ($P<0.0001$) all the polyphenols, but among the physical treatments reduction was maximum ($P < 0.0001$) due to CSDG, and was lowest in fresh leaves treated only with water. Similarly, among the chemical treatments, 10% wood ash reduced all the polyphenols (except HT) to the maximum extent followed by 5% and 2.5% WA treatments. HT was reduced at maximum level (78.35 %) through water treatment, followed by 10%, 5% and 2.5% WA treatments.

Table 11 : Effect of different physical and chemical [wood ash (WA)] treatments on phenolic contents of *Q. leucotrichophora* leaves

Physical Treatment	Chemical Tretment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0% wood ash (Water)	$5.10^{A} \pm 0.02$	$1.01^{A} \pm 0.08$	$4.08^{A} \pm 0.02$	$1.47^{A} \pm 0.01$	$2.62^{A} \pm 0.03$	$29.78^{L} \pm 0.29$	$9.39^{O} \pm 0.70$	$33.61^{K} \pm 0.36$	$9.39^{H} \pm 0.52$	$42.14^{H} \pm 0.58$
	2.5% wood ash	$4.88^{B} \pm 0.01$	$0.94^{B} \pm 0.01$	$3.95^{B} \pm 0.01$	$1.30^{E} \pm 0.01$	$2.65^{A} \pm 0.02$	$32.73^{K} \pm 0.18$	$16.30^{F} \pm 0.53$	$35.83^{J} \pm 0.20$	$19.69^{D} \pm 0.83$	$41.48^{H} \pm 0.47$
	5% wood ash	$3.87^{DE} \pm 0.03$	$0.93^{B} \pm 0.003$	$2.94^{D} \pm 0.03$	$1.29^{E} \pm 0.01$	$1.65^{C} \pm 0.03$	$46.73^{IH} \pm 0.35$	$17.33^{F} \pm 0.29$	$52.17^{H} \pm 0.45$	$20.17^{D} \pm 0.83$	$63.53^{F} \pm 0.72$
	10% wood ash	$3.67^{F} \pm 0.02$	$0.84^{D} \pm 0.01$	$2.83^{E} \pm 0.02$	$1.26^{F} \pm 0.007$	$1.57^{D} \pm 0.01$	$49.47^{G} \pm 0.33$	$25.21^{D} \pm 1.20$	$53.97^{G} \pm 0.24$	$22.36^{C} \pm 0.47$	$65.20^{E} \pm 0.33$
Chopped	0.0% wood ash (Water)	$2.63^{I} \pm 0.01$	$0.89^{C} \pm 0.005$	$1.74^{J} \pm 0.01$	$1.44^{B} \pm 0.01$	$0.30^{H} \pm 0.02$	$63.76^{D} \pm 0.20$	$20.73^{E} \pm 0.50$	$71.66^{C} \pm 0.21$	$10.82^{G} \pm 0.50$	$93.40^{A} \pm 0.38$
	2.5% wood ash	$4.77^{C} \pm 0.01$	$0.86^{D} \pm 0.006$	$3.92^{B} \pm 0.008$	$1.24^{GF} \pm 0.01$	$2.68^{A} \pm 0.02$	$34.28^{J} \pm 0.18$	$23.57^{D} \pm 0.58$	$36.34^{J} \pm 0.14$	$23.52^{BC} \pm 0.79$	$40.80^{H} \pm 0.42$
	5% wood ash	$3.85^{DE} \pm 0.03$	$0.80^{E} \pm 0.006$	$3.06^{C} \pm 0.02$	$1.21^{H} \pm 0.002$	$1.84^{B} \pm 0.02$	$46.92^{IH} \pm 0.35$	$28.82^{C} \pm 0.59$	$50.30^{I} \pm 0.31$	$25.06^{A} \pm 0.14$	$59.24^{G} \pm 0.47$
	10% wood ash	$3.80^{E} \pm 0.02$	$0.77^{G} \pm 0.008$	$3.03^{C} \pm 0.02$	$1.20^{H} \pm 0.004$	$1.83^{B} \pm 0.03$	$47.71^{H} \pm 0.34$	$31.24^{A} \pm 0.72$	$50.80^{I} \pm 0.38$	$26.02^{F} \pm 0.28$	$59.57^{G} \pm 0.61$
Chopped sun dried	0.0% wood ash (Water)	$2.73^{H} \pm 0.02$	$0.78^{EFG} \pm 0.003$	$1.94^{G} \pm 0.02$	$1.37^{C} \pm 0.003$	$0.57^{F} \pm 0.02$	$62.45^{E} \pm 0.26$	$30.23^{ABC} \pm 0.31$	$68.37^{E} \pm 0.34$	$15.32^{A} \pm 0.19$	$87.32^{C} \pm 0.48$
	2.5% wood ash	$3.91^{D} \pm 0.01$	$0.85^{D} \pm 0.006$	$3.05^{C} \pm 0.01$	$1.20^{H} \pm 0.001$	$1.84^{B} \pm 0.01$	$46.20^{I} \pm 0.17$	$23.70^{D} \pm 0.58$	$50.39^{I} \pm 0.18$	$25.31^{A} \pm 0.08$	$59.26^{G} \pm 0.26$
	5% wood ash	$3.02^{G} \pm 0.04$	$0.79^{EF} \pm 0.006$	$2.23^{F} \pm 0.04$	$1.20^{H} \pm 0.001$	$1.03^{E} \pm 0.04$	$58.39^{D} \pm 0.58$	$29.08^{BC} \pm 0.59$	$63.80^{F} \pm 0.66$	$25.98^{A} \pm 0.08$	$77.27^{D} \pm 0.91$
	10% wood ash	$2.62^{I} \pm 0.02$	$0.78^{EFG} \pm 0.008$	$1.84^{H} \pm 0.02$	$1.19^{H} \pm 0.001$	$0.64^{F} \pm 0.02$	$63.90^{C} \pm 0.23$	$30.05^{ABC} \pm 0.73$	$70.13^{D} \pm 0.35$	$26.40^{A} \pm 0.04$	$85.73^{C} \pm 0.46$
Chopped sun dried and grinded	0.0% wood ash (Water)	$2.52^{J} \pm 0.04$	$0.77^{GF} \pm 0.003$	$1.75^{I} \pm 0.04$	$1.32^{D} \pm 0.004$	$0.43^{G} \pm 0.04$	$65.23^{C} \pm 0.53$	$30.83^{AB} \pm 0.32$	$71.55^{C} \pm 0.59$	$18.40^{E} \pm 0.25$	$90.54^{B} \pm 0.77$
	2.5% wood ash	$2.54^{J} \pm 0.04$	$0.85^{D} \pm 0.06$	$1.69^{IJ} \pm 0.04$	$1.25^{GF} \pm 0.001$	$0.44^{G} \pm 0.04$	$65.04^{C} \pm 0.53$	$23.81^{D} \pm 0.58$	$72.60^{BC} \pm 0.66$	$22.96^{BC} \pm 0.10$	$90.33^{B} \pm 0.88$
	5% wood ash	$2.45^{K} \pm 0.03$	$0.80^{E} \pm 0.01$	$1.64^{J} \pm 0.04$	$1.24^{G} \pm 0.002$	$0.41^{G} \pm 0.04$	$66.24^{B} \pm 0.45$	$28.47^{C} \pm 0.59$	$73.18^{B} \pm 0.60$	$23.72^{B} \pm 0.15$	$90.84^{B} \pm 0.83$
	10% wood ash	$2.27^{L} \pm 0.01$	$0.77^{GF} \pm 0.008$	$1.49^{K} \pm 0.01$	$1.20^{H} \pm 0.01$	$0.29^{H} \pm 0.01$	$68.78^{A} \pm 0.07$	$30.91^{AB} \pm 0.72$	$75.72^{A} \pm 0.13$	$25.64^{A} \pm 0.15$	$93.62^{A} \pm 0.21$

Table 11 (Contd.)

Fresh	4.38^{A} ± 0.13	0.93^{a} ± 0.01	3.45^{a} ± 0.12	1.33^{a} ± 0.02	2.12^{a}± 0.11	39.68^{d} ± 1.79	17.06^{e} ± 1.22	43.90^{d} ± 1.93	17.90^{e}± 1.09	53.09^{d} ± 2.37
Chopped	3.76^{b} ± 0.16	0.83^{b} ± 0.01	2.94^{b} ± 0.16	1.27^{b} ± 0.02	1.66^{b} ± 0.18	48.17^{e} ± 2.19	26.09^{b} ± 0.91	52.27^{c} ± 2.63	21.35^{b} ± 1.30	63.25^{e} ± 3.97
Chopped sun dried	3.07^{e}± 0.11	0.80^{e} ± 0.006	2.26^{e} ± 0.1	1.24^{e} ± 0.02	1.02^{e}± 0.12	57.74^{b} ± 1.46	28.27^{a} ± 0.62	63.17^{b} ± 1.62	23.25^{a} ± 0.96	77.40^{b} ± 2.34
Chopped sun dried and grinded	2.44^{d} ± 0.03	0.80^{e} ± 0.01	1.64^{d}± 0.03	1.25^{e} ± 0.01	0.39^{d} ± 0.02	66.32^{a}± 0.37	28.51^{a} ± 0.66	73.26^{a} ± 0.41	22.68^{a} ± 0.56	91.33^{a} ± 0.44
wood ash	wood ash *Treatments*					wood ash *Treatments*				
0.0% wood ash (Water)	3.24^{C} ± 0.22	0.86^{a} ± 0.02	2.38^{b} ± 0.21	1.40^{a} ± 0.12	0.98^{d}± 0.20	55.30^{b} ± 3.08	22.79^{c} ± 1.83	61.30^{a} ± 3.35	13.48^{c} ± 0.77	78.35^{a}± 4.39
2.5% wood ash	4.02^{a} ± 0.20	0.88^{a} ± 0.008	3.15^{a} ± 0.19	1.25^{b} ± 0.008	1.90^{a} ± 0.19	44.56^{c} ± 2.70	21.84^{c} ± 0.72	48.79^{c} ± 3.12	22.87^{b}± 0.50	57.97 ^{d} ± 4.20
5% wood ash	3.30^{b} ± 0.13	0.83^{b}± 0.01	2.47^{b} ± 0.12	1.24^{b} ± 0.008	1.23^{b} ± 0.12	54.57^{b} ± 1.73	25.93^{b} ± 1.07	59.86^{a} ± 1.95	23.74^{b}± 0.50	72.72^{c} ± 2.70
10% wood ash	3.09^{d} ± 0.14	0.79^{c} ± 0.007	2.30^{d} ± 0.14	1.21^{c} ± 0.005	1.08^{c} ± 0.13	57.47^{a} ± 1.89	29.35^{a} ± 0.65	62.67^{a} ± 2.20	25.10^{a}± 0.36	76.03^{b} ± 2.94
Overall mean ± SE	3.41 ± 0.09	0.84 ± 0.007	2.57 ± 0.09	1.27 ± 0.009	1.30 ± 0.09	52.98 ± 1.29	24.98 ± 0. 65	58.15 ± 1.45	21.30 ± 0.54	71.27 ± 1.96
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C, D etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *Q. leucotrichophora* leaves were 7.26 ± 0.03, 1.12± 0.01, 6.15± 0.04, 1.62 ± 0.02 and 4.52 ± 0.06 , respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

Interaction between physical and WA treatments were also found to be very significant (P < 0.0001). On comparison among 16 groups of different treatment combinations (Physical × chemical), it was observed that the reduction of all the polyphenols was maximum in 16th treatment (CSDG 10% WA), the values of reduction of NTP was comparable among the treatments, 8th, 9th, 12th, 13th and 16th; similarly the extent of reduction of CT was comparable among the interactive treatments, 7th, 8th, 9th, 10th, 11th, 12th and 16th treatments. Extent of reduction of polyphenols was minimum in 1st treatment (fresh × 0.0 % WA).

4.1.2.4 Effect of different physical treatments followed by ground nut cake (GNC) treatment on polyphenol contents of *Q. leucotrichophora* leaves

Polyphenol content of physico-chemically (GNC) treated oak leaves have been presented in Table 12. Overall reduction of TP, NTP, TT, CT and HT in physico-chemically treated leaves was 52.17 (29.78-69.96), 23.73 (9.39-32.63), 57.43 (33.61-77.77), 15.73 (9.39-25.08) and 72.28 (42.14-96.68) %, respectively. The reduction in the concentration of these polyphenols due to physical treatments was 38.81 to 67.69, 15.14 to 27.58, 43.22 to 75.05, 12.50 to 22.86 and 54.10 to 93.69 %, respectively; and due to GNC (including water) treatments, it varied from 48.62 to 55.30, 22.30 to 26.69, 57.35 to 61.30, 13.48 to 17.17 and 67.16 to 78.35 %, respectively.

Extent of overall reduction was maximum in case of HT followed by TT, TP, and NTP and was lowest in case of CT. Both physical and GNC (including water) treatments had their effect on in reducing (P < 0.0001) all the polyphenols; but among the physical treatments the reduction was maximum (P < 0.0001) in CSDG followed by CSD, chopped and fresh leaves treated with water (FLTW). However, the effect of GNC was variable among the phenols. The reduction of NTP and CT was maximum due to 7.5 % GNC, followed by 3.4 and 1.7 %

Table 12 : Effect of different physical and chemical [ground nut cake (GNC)] treatments on phenolic contents of *Q. leucotrichophora* leaves

Physical Treatment	Chemical Tretment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0%GNC (Water)	$5.10^{A} \pm 0.02$	$1.01^{A} \pm 0.01$	$4.08^{A} \pm 0.02$	$1.47^{A} \pm 0.01$	$2.62^{A} \pm 0.03$	$29.78^{L} \pm 0.29$	$9.39^{I} \pm 0.70$	$33.61^{H} \pm 0.36$	$9.39^{I} \pm 0.52$	$42.14^{I} \pm 0.58$
	1.7%GNC	$4.56^{B} \pm 0.02$	$0.96^{B} \pm 0.01$	$3.61^{B} \pm 0.01$	$1.45^{B} \pm 0.01$	$2.15^{B} \pm 0.01$	$37.14^{K} \pm 0.23$	$14.69^{H} \pm 1.05$	$41.33^{O} \pm 0.19$	$10.24^{H} \pm 0.51$	$52.35^{H} \pm 0.25$
	3.4%GNC	$4.07^{C} \pm 0.02$	$0.92^{C} \pm 0.01$	$3.15^{C} \pm 0.02$	$1.37^{F} \pm 0.01$	$1.79^{C} \pm 0.02$	$43.90^{J} \pm 0.26$	$18.05^{O} \pm 0.33$	$48.70^{F} \pm 0.35$	$15.68^{D} \pm 0.44$	$60.42^{O} \pm 0.49$
	7.5%GNC	$4.04^{C} \pm 0.01$	$0.91^{CD} \pm 0.01$	$3.12^{C} \pm 0.01$	$1.38^{E} \pm 0.01$	$1.74^{CD} \pm 0.01$	$44.40^{J} \pm 0.20$	$18.43^{PO} \pm 0.41$	$49.22^{F} \pm 0.25$	$14.69^{E} \pm 0.09$	$61.49^{PO} \pm 0.36$
Chopped	0.0%GNC (Water)	$2.63^{H} \pm 0.01$	$0.89^{CD} \pm 0.01$	$1.74^{F} \pm 0.01$	$1.44^{B} \pm 0.01$	$0.30^{H} \pm 0.02$	$63.76^{E} \pm 0.20$	$20.73^{PO} \pm 0.50$	$71.66^{C} \pm 0.21$	$10.81^{H} \pm 0.50$	$93.40^{B} \pm 0.38$
	1.7%GNC	$3.96^{D} \pm 0.01$	$0.84^{E} \pm 0.01$	$3.11^{C} \pm 0.01$	$1.42^{C} \pm 0.01$	$1.69^{D} \pm 0.01$	$45.48^{G} \pm 0.15$	$24.59^{E} \pm 0.58$	$49.37^{F} \pm 0.19$	$12.21^{G} \pm 0.06$	$62.58^{F} \pm 0.25$
	3.4%GNC	$3.82^{E} \pm 0.03$	$0.82^{EF} \pm 0.01$	$2.99^{D} \pm 0.02$	$1.42^{C} \pm 0.01$	$1.58^{E} \pm 0.02$	$47.43^{H} \pm 0.34$	$26.38^{DE} \pm 0.50$	$51.36^{E} \pm 0.40$	$12.55^{G} \pm 0.1$	$65.15^{E} \pm 0.55$
	7.5%GNC	$3.76^{EF} \pm 0.02$	$0.81^{EFG} \pm 0.01$	$2.95^{D} \pm 0.01$	$1.40^{D} \pm 0.01$	$1.55^{E} \pm 0.01$	$48.17^{GH} \pm 0.21$	$27.26^{CDE} \pm 0.50$	$52.06^{E} \pm 0.23$	$13.82^{F} \pm 0.1$	$65.66^{E} \pm 0.33$
Chopped sun dried	0.0%GNC (Water)	$2.73^{G} \pm 0.02$	$0.78^{GH} \pm 0.003$	$1.94^{E} \pm 0.02$	$1.37^{EF} \pm 0.003$	$0.57^{F} \pm 0.02$	$62.45^{F} \pm 0.26$	$30.23^{ABC} \pm 0.31$	$68.37^{D} \pm 0.34$	$15.32^{DE} \pm 0.19$	$87.32^{D} \pm 0.48$
	1.7%GNC	$3.96^{D} \pm 0.01$	$0.85^{E} \pm 0.01$	$3.12^{C} \pm 0.01$	$1.38^{EF} \pm 0.01$	$1.74^{CD} \pm 0.01$	$45.39^{G} \pm 0.15$	$24.46^{E} \pm 0.58$	$49.29^{F} \pm 0.19$	$15.10^{DE} \pm 0.07$	$61.43^{PO} \pm 0.25$
	3.4%GNC	$3.80^{EF} \pm 0.02$	$0.82^{EF} \pm 0.01$	$2.98^{D} \pm 0.02$	$1.37^{EF} \pm 0.01$	$1.61^{E} \pm 0.02$	$47.68^{GH} \pm 0.34$	$26.72^{DE} \pm 050$	$51.58^{E} \pm 0.40$	$15.41^{DE} \pm 0.05$	$64.44^{E} \pm 0.54$
	7.5%GNC	$3.74^{F} \pm 0.02$	$0.80^{FGH} \pm 0.01$	$2.94^{D} \pm 0.01$	$1.38^{EF} \pm 0.01$	$1.56^{E} \pm 0.01$	$48.46^{G} \pm 0.25$	$28.44^{BCD} \pm 0.54$	$52.19^{E} \pm 0.23$	$15.07^{DE} \pm 0.60$	$65.39^{E} \pm 0.31$
Chopped sun dried and grinded	0.0%GNC (Water)	$2.52^{I} \pm 0.04$	$0.77^{HI} \pm 0.004$	$1.75^{F} \pm 0.04$	$1.32^{G} \pm 0.004$	$0.43^{G} \pm 0.04$	$65.23^{D} \pm 0.53$	$30.83^{AB} \pm 0.32$	$71.55^{C} \pm 0.59$	$18.40^{C} \pm 0.25$	$90.54^{C} \pm 0.77$
	1.7%GNC	$2.43^{J} \pm 0.02$	$0.83^{EF} \pm 0.01$	$1.60^{G} \pm 0.02$	$1.25^{H} \pm 0.001$	$0.35^{H} \pm 0.02$	$66.53^{C} \pm 0.23$	$25.47^{DE} \pm 0.57$	$74.06^{B} \pm 0.32$	$23.07^{B} \pm 0.06$	$92.27^{B} \pm 0.44$
	3.4%GNC	$2.25^{K} \pm 0.02$	$0.88^{D} \pm 0.04$	$1.37^{H} \pm 0.05$	$1.22^{I} \pm 0.001$	$0.15^{I} \pm 0.05$	$69.04^{B} \pm 0.23$	$21.36^{F} \pm 3.75$	$77.77^{A} \pm 0.87$	$24.88^{A} \pm 0.29$	$96.68^{A} \pm 1.08$
	7.5%GNC	$2.18^{L} \pm 0.01$	$0.75^{I} \pm 0.01$	$1.43^{H} \pm 0.02$	$1.21^{I} \pm 0.01$	$0.21^{I} \pm 0.02$	$69.96^{A} \pm 0.18$	$32.63^{A} \pm 0.46$	$76.80^{A} \pm 0.25$	$25.08^{A} \pm 0.04$	$95.29^{A} \pm 0.34$

Table 12 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	$4.44^{A} \pm 0.01$	$0.95^{a} \pm 0.01$	$3.49^{a} \pm 0.08$	$1.42^{a} \pm 0.01$	$2.07^{a} \pm 0.07$	$38.81^{c} \pm 1.25$	$15.14^{c} \pm 0.82$	$43.22^{c} \pm 1.33$	$12.50^{c} \pm 0.60$	$54.10^{d} \pm 1.63$
Chopped	$3.54^{b} \pm 0.11$	$0.84^{b} \pm 0.01$	$2.70^{c} \pm 0.02$	$1.42^{b} \pm 0.01$	$1.28^{c} \pm 0.12$	$51.21^{b} \pm 1.53$	$24.74^{b} \pm 0.58$	$56.11^{b} \pm 1.89$	$12.35^{c} \pm 0.25$	$71.70^{b} \pm 2.63$
Chopped sun dried	$3.55^{b} \pm 0.10$	$0.81^{c} \pm 0.01$	$2.75^{b} \pm 0.01$	$1.37^{c} \pm 0.01$	$1.37^{b} \pm 0.10$	$50.99^{b} \pm 1.40$	$27.46^{a} \pm 0.50$	$55.36^{b} \pm 1.59$	$15.22^{b} \pm 0.06$	$69.64^{c} \pm 2.16$
Chopped sun dried and grinded	$2.35^{c} \pm 0.01$	$0.81^{c} \pm 0.01$	$1.53^{c} \pm 0.04$	$1.25^{d} \pm 0.01$	$0.28^{d} \pm 0.03$	$67.69^{a} \pm 0.42$	$27.58^{a} \pm 1.29$	$75.05^{a} \pm 0.57$	$22.86^{a} \pm 0.57$	$93.69^{a} \pm 0.61$
Ground Nut Cake	**Ground nut cake Treatments**					**Ground nut cake Treatments**				
0.0%GNC (Water)	$3.24^{C} \pm 0.22$	$0.86^{a} \pm 0.02$	$2.38^{c} \pm 0.21$	$1.40^{a} \pm 0.01$	$0.97^{c} \pm 0.20$	$55.30^{a} \pm 3.08$	$22.79^{b} \pm 1.83$	$61.30^{a} \pm 3.35$	$13.48^{c} \pm 0.77$	$78.35^{a} \pm 4.39$
1.7%GNC	$3.73^{a} \pm 0.16$	$0.87^{a} \pm 0.01$	$2.86^{a} \pm 0.16$	$1.37^{b} \pm 0.02$	$1.48^{a} \pm 0.14$	$48.63^{c} \pm 2.27$	$22.30^{b} \pm 0.98$	$53.51^{c} \pm 2.56$	$15.15^{b} \pm 1.03$	$67.16^{c} \pm 3.14$
3.4%GNC	$3.48^{b} \pm 0.15$	$0.86^{a} \pm 0.01$	$2.62^{b} \pm 0.15$	$1.34^{c} \pm 0.02$	$1.28^{b} \pm 0.14$	$52.01^{b} \pm 2.08$	$23.13^{b} \pm 1.17$	$57.35^{b} \pm 2.48$	$17.13^{a} \pm 0.98$	$71.67^{b} \pm 3.05$
7.5%GNC	$3.43^{b} \pm 0.15$	$0.82^{b} \pm 0.01$	$2.61^{b} \pm 0.14$	$1.34^{c} \pm 0.02$	$1.27^{b} \pm 0.13$	$52.75^{b} \pm 2.10$	$26.69^{a} \pm 1.10$	$57.57^{b} \pm 2.33$	$17.17^{a} \pm 0.96$	$71.96^{b} \pm 2.83$
Overall mean ± SE	3.47 ± 0.09	0.85 ± 0.007	2.62 ± 0.008	1.37 ± 0.007	1.25 ± 0.08	52.17 ± 1.21	23.73 ± 0.67	57.43 ± 1.37	15.73 ± 0.49	72.28 ± 1.73
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh Q. leucotrichophora leaves were 7.26 ± 0.03, 1.12± 0.01, 6.15± 0.04, 1.62 ± 0.02 and 4.52 ± 0.06 , respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

GNC; but reduction was minimum for NTP by 1.7 % GNC and for CT by 0.0 % GNC (water), where as the reduction of TP, TT, HT was maximum in simple water treatment followed by 3.4 % and 7.5 % GNC treatment. The values of reduction of TP, TT and HT were comparatively lower in 1.7 % GNC treatment.

Interactions among physical and GNC treatments were also found to be varied significant ($P < 0.0001$). On comparison among 16 groups of different combinations (Physical × GNC), it was revealed that the reduction of all the polyphenols was maximum in 16th treatment (CSDG + 7.5 % GNC). However, values of reduction in NTP were comparable among 9th, 13th and 16th; whereas, in case of TT, CT, and HT the values of phenol reduction were comparable between 15th and 16th treatments. Values of reduction of all the polyphenols were at minimum in 1st treatment (fresh + 0.0 % GNC).

4.1.2.5 Effect of different physical followed by chemical treatments on different polyphenol contents of *Q. leucotrichophora* leaves (*comparison between the chemicals*)

Effect of all the treatments (water, PP, urea, WA and GNC) along with physical treatments on polyphenol contents of oak leaves has been compared and presented in Table 13. Overall reduction of TP, NTP, TT, CT and HT was 56.36 (29.78-68.51), 28.12 (9.39-39.21), 61.57 (33.61-76.20), 23.18 (9.39-49.60) and 75.24 (42.14-94.75), respectively. Reduction of respective polyphenols due to physical treatments was 48.72 to 64.43, 24.13 to 32.43, 53.29 to 73.13, 19.13 to 28.78 and 65.43 to 89.02 %, respectively. Among the physical treatments, CSDG was most effective in reducing all the polyphenols (except NTP) at maximum level followed by CSD and chopping; NTP was reduced at maximum level through CSD followed by CSDG and chopping. Reduction of these polyphenols without chemicals was 55.30, 22.79, 61.30, 13.48 and 78.35 % through water treatment

Table 13 : Effect of different physical and chemical treatments on phenolic contents of *Q. leucotrichophora* leaves.

Physical Treatment	Chemical Treatment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
		Chemical Treatments					Chemical Treatments				
Fresh	0.0% Chemical (Water)	$5.10^{A} \pm 0.02$	$1.01^{A} \pm 0.01$	$4.08^{A} \pm 0.02$	$1.47^{A} \pm 0.01$	$2.62^{A} \pm 0.03$	$29.78^{H} \pm 0.29$	$9.39^{I} \pm 0.70$	$33.61^{H} \pm 0.36$	$9.39^{H} \pm 0.52$	$42.14^{G} \pm 0.58$
	Pot. Permn.	$2.75^{F} \pm 0.05$	$0.68^{E} \pm 0.003$	$2.07^{H} \pm 0.05$	$1.11^{K} \pm 0.02$	$0.96^{GHIJ} \pm 0.05$	$62.14^{C} \pm 0.74$	$39.21^{A} \pm 0.34$	$66.37^{D} \pm 0.84$	$34.61^{D} \pm 1.12$	$78.76^{BCD} \pm 1.07$
	Urea	$3.32^{D} \pm 0.14$	$0.83^{DE} \pm 0.01$	$2.49^{D} \pm 0.14$	$1.40^{CD} \pm 0.01$	$1.09^{EFG} \pm 0.13$	$54.28^{E} \pm 1.95$	$25.54^{EF} \pm 0.66$	$59.59^{E} \pm 2.22$	$13.87^{GH} \pm 0.36$	$75.88^{DE} \pm 2.92$
	Wood ash	$4.13^{B} \pm 0.13$	$0.90^{C} \pm 0.01$	$3.24^{BC} \pm 0.12$	$1.28^{H} \pm 0.01$	$1.96^{B} \pm 0.12$	$42.98^{G} \pm 1.79$	$19.61^{G} \pm 1.05$	$47.32^{G} \pm 1.99$	$20.74^{G} \pm 0.49$	$56.74^{F} \pm 2.64$
	GNC	$4.22^{B} \pm 0.05$	$0.93^{B} \pm 0.01$	$3.30^{B} \pm 0.05$	$1.40^{C} \pm 0.01$	$1.89^{B} \pm 0.05$	$41.82^{G} \pm 0.81$	$17.06^{H} \pm 0.55$	$46.42^{G} \pm 0.89$	$13.54^{H} \pm 0.61$	$58.09^{F} \pm 1.01$
Chopped	0.0% Chemical (Water)	$2.63^{FG} \pm 0.01$	$0.88^{C} \pm 0.01$	$1.74^{FG} \pm 0.01$	$1.44^{D} \pm 0.01$	$0.30^{KJ} \pm 0.02$	$63.76^{BC} \pm 0.20$	$20.73^{G} \pm 0.50$	$71.66^{BC} \pm 0.21$	$10.81^{IH} \pm 0.50$	$93.40^{AB} \pm 0.38$
	Pot. Permn.	$2.65^{FG} \pm 0.02$	$0.84^{D} \pm 0.01$	$1.81^{EFG} \pm 0.01$	$1.01^{L} \pm 0.05$	$0.79^{IJKL} \pm 0.02$	$63.45^{BC} \pm 0.23$	$24.64^{F} \pm 0.45$	$70.58^{BCD} \pm 0.22$	$37.35^{C} \pm 03.1$	$82.43^{DE} \pm 0.34$
	Urea	$3.19^{DE} \pm 0.12$	$0.81^{EF} \pm 0.01$	$2.38^{D} \pm 0.12$	$1.39^{CDE} \pm 0.01$	$0.99^{GHIJ} \pm 0.11$	$56.07^{DE} \pm 1.70$	$28.05^{DE} \pm 0.67$	$61.25^{E} \pm 1.89$	$14.28^{GH} \pm 0.40$	$77.99^{BCD} \pm 2.45$
	Wood ash	$4.14^{AB} \pm 0.11$	$0.81^{EF} \pm 0.01$	$3.33^{AB} \pm 0.10$	$1.22^{HJ} \pm 0.01$	$2.12^{B} \pm 0.10$	$42.97^{G} \pm 1.50$	$27.86^{DE} \pm 0.85$	$45.81^{G} \pm 1.63$	$24.87^{EF} \pm 0.36$	$53.20^{HI} \pm 2.15$
	GNC	$3.85^{C} \pm 0.02$	$0.83^{DE} \pm 0.01$	$3.02^{C} \pm 0.02$	$1.41^{C} \pm 0.01$	$1.61^{C} \pm 0.02$	$47.03^{F} \pm 0.31$	$26.08^{EF} \pm 0.39$	$50.93^{F} \pm 0.32$	$12.86^{H} \pm 0.17$	$64.47^{H} \pm 0.39$
Chopped sun dried	0.0% Chemical (Water)	$2.73^{F} \pm 0.02$	$0.78^{FG} \pm 0.03$	$1.94^{EFG} \pm 0.02$	$1.37^{EF} \pm 0.003$	$0.57^{JKL} \pm 0.02$	$62.45^{C} \pm 0.26$	$30.23^{CD} \pm 0.31$	$68.37^{CD} \pm 0.34$	$15.32^{IJ} \pm 0.19$	$87.32^{CD} \pm 0.48$
	Pot. Permn.	$2.60^{FG} \pm 0.02$	$0.70^{GH} \pm 0.01$	$1.89^{EFG} \pm 0.01$	$0.98^{IM} \pm 0.002$	$0.91^{HIJK} \pm 0.01$	$64.44^{BC} \pm 0.21$	$38.18^{AB} \pm 0.68$	$69.29^{BCD} \pm 0.18$	$39.15^{B} \pm 0.26$	$80.02^{BCD} \pm 0.30$
	Urea	$3.01^{E} \pm 0.12$	$0.69^{HI} \pm 0.01$	$2.32^{D} \pm 0.12$	$1.36^{F} \pm 0.01$	$0.96^{GHIJ} \pm 0.11$	$58.49^{D} \pm 1.67$	$38.11^{AB} \pm 0.66$	$62.27^{E} \pm 1.87$	$15.90^{I} \pm 0.12$	$78.80^{BCD} \pm 2.52$
	Wood ash	$3.16^{DE} \pm 0.13$	$0.81^{EF} \pm 0.01$	$2.37^{D} \pm 0.12$	$1.20^{J} \pm 0.01$	$1.17^{D} \pm 0.12$	$56.17^{DE} \pm 1.81$	$27.61^{DE} \pm 0.76$	$61.44^{E} \pm 2.01$	$25.90^{E} \pm 0.11$	$74.09^{G} \pm 2.70$
	GNC	$3.84^{C} \pm 0.02$	$0.82^{DE} \pm 0.005$	$3.01^{C} \pm 0.02$	$1.37^{EFG} \pm 0.01$	$1.64^{C} \pm 0.02$	$47.17^{F} \pm 0.35$	$26.54^{EF} \pm 0.50$	$51.02^{F} \pm 0.34$	$15.19^{IJK} \pm 0.05$	$63.75^{H} \pm 0.46$
Chopped sun dried and grinded	0.0% Chemical (Water)	$2.52^{FGH} \pm 0.04$	$0.77^{G} \pm 0.004$	$1.75^{EF} \pm 0.04$	$1.32^{G} \pm 0.004$	$0.43^{KL} \pm 0.04$	$65.23^{ABC} \pm 0.53$	$30.83^{C} \pm 0.32$	$71.55^{BC} \pm 0.59$	$18.40^{H} \pm 0.25$	$90.54^{ABC} \pm 0.77$
	Pot. Permn.	$2.50^{FGH} \pm 0.02$	$0.81^{EF} \pm 0.01$	$1.67^{FG} \pm 0.02$	$0.81^{M} \pm 0.006$	$0.87^{IJK} \pm 0.02$	$65.61^{ABC} \pm 0.29$	$27.84^{DE} \pm 0.82$	$72.59^{ABC} \pm 0.37$	$49.60^{A} \pm 0.20$	$80.71^{BC} \pm 0.53$
	Urea	$2.52^{FGH} \pm 0.02$	$0.71^{H} \pm 0.004$	$1.81^{EFG} \pm 0.02$	$1.29^{H} \pm 0.01$	$0.52^{JKL} \pm 0.02$	$65.30^{ABC} \pm 0.30$	$36.40^{B} \pm 0.38$	$70.61^{BCD} \pm 0.33$	$20.51^{G} \pm 0.16$	$88.51^{BC} \pm 0.43$
	Wood ash	$2.42^{GH} \pm 0.03$	$0.81^{EF} \pm 0.01$	$1.62^{FG} \pm 0.03$	$1.23^{I} \pm 0.001$	$0.38^{KLM} \pm 0.02$	$66.69^{AB} \pm 0.44$	$27.73^{DE} \pm 0.89$	$73.83^{AB} \pm 0.43$	$24.11^{F} \pm 0.28$	$91.60^{ABC} \pm 0.52$
	GNC	$2.29^{H} \pm 0.03$	$0.82^{DE} \pm 0.02$	$1.46^{H} \pm 0.03$	$1.23^{I} \pm 0.01$	$0.24^{M} \pm 0.03$	$68.51^{A} \pm 0.37$	$26.49^{EF} \pm 1.55$	$76.20^{A} \pm 0.49$	$24.34^{F} \pm 0.24$	$94.75^{A} \pm 0.59$

Table 13 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.72^{A} ± 0.09	0.85^{a} ± 0.01	2.87^{a} ± 0.08	1.31^{a} ± 0.01	1.56^{a} ± 0.07	48.72^{d} ± 1.28	24.13^{d} ± 1.28	53.29^{d} ± 1.34	19.13^{d} ± 0.92	65.43^{d} ± 1.64
Chopped	3.39^{b} ± 0.08	0.83^{a} ± 0.01	2.57^{b} ± 0.08	1.27^{b} ± 0.02	1.29^{b} ± 0.07	53.26^{c} ± 1.07	26.20^{c} ± 0.37	58.26^{c} ± 1.27	21.45^{c} ± 1.41	71.36^{c} ± 1.63
Chopped sun dried	3.12^{c} ± 0.07	0.76^{b} ± 0.01	2.36^{c} ± 0.06	1.24^{b} ± 0.02	0.12^{d} ± 0.05	57.02^{b} ± 0.90	32.43^{a} ± 0.68	61.57^{b} ± 0.98	23.37^{b} ± 1.10	75.18^{b} ± 1.17
Chopped sun dried and grinded	2.44^{d} ± 0.02	0.79^{b} ± 0.01	1.65^{d} ± 0.02	1.15^{c} ± 0.02	0.50^{c} ± 0.03	64.43^{a} ± 0.22	29.71^{b} ± 0.63	73.13^{a} ± 0.30	28.78^{a} ± 1.32	89.02^{a} ± 0.62
Chemical Treatments	**Chemical Treatments**					**Chemical Treatments**				
0% chemical (water)	3.24^{B} ± 0.22	0.86^{a} ± 0.02	2.38^{c} ± 0.21	1.40^{a} ± 0.01	0.97^{b} ± 0.20	55.30^{a} ± 3.08	22.79^{b} ± 1.83	61.30^{b} ± 3.35	13.48^{d} ± 0.77	78.35^{a} ± 4.39
Pot. Perma.	2.78^{d} ± 0.06	0.78^{b} ± 0.01	1.99^{d} ± 0.06	1.09^{c} ± 0.02	0.91^{c} ± 0.05	61.76^{a} ± 0.87	30.05^{a} ± 0.87	67.60^{a} ± 0.94	32.94^{a} ± 1.32	79.95^{a} ± 1.11
Urea	3.07^{c} ± 0.07	0.79^{b} ± 0.01	2.28^{b} ± 0.07	1.37^{a} ± 0.01	0.91^{c} ± 0.06	57.73^{b} ± 1.02	29.57^{a} ± 1.25	62.90^{b} ± 1.11	15.48^{c} ± 0.34	79.81^{b} ± 1.44
Wood ash	3.41^{a} ± 0.09	0.84^{a} ± 0.007	2.57^{a} ± 0.09	1.27^{b} ± 0.009	1.30^{a} ± 0.09	52.98^{d} ± 1.29	24.98^{b} ± 0.65	58.15^{c} ± 1.45	21.30^{b} ± 0.54	71.27^{b} ± 1.96
GNC	3.47^{a} ± 0.09	0.85^{a} ± 0.007	2.62^{a} ± 0.008	1.37^{a} ± 0.007	1.25^{a} ± 0.08	52.17^{d} ± 1.21	23.73^{b} ± 0.67	57.43^{c} ± 1.37	13.73^{c} ± 0.49	72.28^{b} ± 1.73
Overall mean ± SE	3.17 ± 0.04	0.81 ± 0.005	2.36 ± 0.04	1.24 ± 0.77	1.12 ± 0.04	56.36 ± 0.60	28.12 ± 0.41	61.57^{b} ± 0.67	23.18 ± 0.60	75.24 ± 0.83
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

NB: Values bearing the different superscripts (A,B,C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *Q. leucotrichophora* leaves were 7.26 ±0.03, 1.12± 0.01, 6.15± 0.04, 1.62 ± 0.02 and 4.52 ± 0.06 , respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

only; whereas reduction of 61.76, 30.05, 67.60, 32.94 and 79.95 % due to PP ; 57.73, 29.57, 62.90, 15.48 and 79.81% due to urea; 52.98, 24.98, 58.15, 21.30 and 71.27% due to WA; and 52.17, 23.73, 57.43, 15.73 and 72.28% due to GNC, respectively. Maximum ($P<0.0001$) reduction in TP was due to PP, followed by urea, water, GNC and WA. Likewise reduction in NTP was maximum in PP, followed by urea, WA, GNC and water. TT reduction was also maximum due to PP, followed by urea, water, WA and GNC; reduction of CT was also maximum in PP treatment, followed by WA, GNC, urea and water. HT was also reduced to a maximum extent in PP treatments, followed by urea, water, GNC and WA, respectively. Altogether it was observed that values of % reduction of the phenols were maximum in PP treatments.

Interactions among 4 physical and 5 chemical treatments were also found to be highly significant ($P < 0.0001$). On comparison, among the 20 different treatment combinations, it was observed that TP was reduced to a maximum extent in 20th treatment (CSDG + GNC), difference being non significant between 16th to 20th treatments. NTP was reduced to the maximum extent in 2nd (Fresh+PP), followed by 13th and 12th treatments; differences anmong the three were not significant. Reduction of TT was maximum in 20th followed by, 19th and17th treatments, which were all within CSDG. CT was reduced to the maximum extent in 17th treatment (CSDG + PP); reduction of HT was maximum in 6th, 16th, 19th and 20th treatments, difference being non significant among the 4 groups/ treatments. Extent of reduction of polyphenols was at minimum in water treated (0.0 % chemical) fresh *Q. leucotrichophora* leaves.

4.1.3 Physical treatment of *F. roxburghii* leaves

Effect of physical treatments on different polyphenols of *F. roxburghii* leaves is presented in Table 14. Concentration of TP, NTP,

Table 14 : Effect of different physical treatments on different polyphenol contents (% on DMB) in *F. roxburghii* leaves

Physical Treatments	Total phenol	Non tannin	Total tannin	Condensed tannin	Hydrolysable tannin
Fresh	$6.27^{A} \pm 0.17$	$1.12^{B} \pm 0.02$	$5.15^{A} \pm 0.17$	$2.33^{A} \pm 0.01$	$2.82^{B} \pm 0.17$
Chopped	$5.18^{B} \pm 0.02$	$1.08^{B} \pm 0.01$	$4.10^{B} \pm 0.02$	$1.86^{B} \pm 0.01$	$2.24^{C} \pm 0.02$
Chopped sun dried	$5.40^{B} \pm 0.02$	$1.29^{A} \pm 0.01$	$4.11^{B} \pm 0.02$	$1.78^{C} \pm 0.003$	$2.32^{C} \pm 0.01$
Chopped sun dried grinded	$5.34^{B} \pm 0.01$	$0.38^{C} \pm 0.04$	$4.95^{A} \pm 0.05$	$1.62^{D} \pm 0.12$	$3.33^{A} \pm 0.05$
Overall mean ± SE	5.55 ± 0.12	0.97 ± 0.09	4.58 ± 0.13	1.90± 0.07	2.68 ± 0.12
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Per cent reduction on physical treatment				
Chopped	17.35^{A} ± 0.38	3.81^{b} ± 0.47	20.29^{a} ± 0.37	20.03^{a} ± 0.06	20.51^{a} ± 0.73
Chopped sun dried	13.90^{b} ± 0.37	-15.32^{c} ± 0.64	20.26^{a} ± 0.46	23.51^{b} ± 0.23	17.57^{a} ± 0.75
Chopped sun dried ground	14.83^{b} ± 0.23	65.63^{a} ± 0.68	3.79^{b} ± 1.01	30.27^{a} ± 0.65	-18.09^{b} ± 1.60
Overall mean ± SE	15.36 ± 0.47	18.04 ± 10.48	14.78 ± 2.37	24.60 ± 1.30	6.66 ± 5.32
P Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means bearing different superscripts with in a column (A, B, C etc,) differ significantly (P < 0.0001).

TT, CT and HT (% DM basis) of fresh *Ficus roxburghii* leaves were 6.27, 1.12, 5.15, 2.33 and 2.82, respectively; in chopped leaves these polyphenols were 5.18, 1.08, 4.10, 1.86 and 2.24 %; in CSD leaves 5.40, 1.29, 4.11, 1.78 and 2.32 %; where as in CSDG leaves these were 5.34, 0.38, 4.95, 1.62 and 3.33 %, respectively.

All the polyphenols were reduced ($P < 0.0001$) in all the physical treatments (chopping, CSD and CSDG). However, the extent of reduction differed from one polyphenols to other and from one treatment to other physical treatment. TP was reduced to the maximum extent (17.35 %) due to chopping, followed by the other two treatments, CSD and CSDG, difference between which were not significant. The NTP and CT were reduced to the maximum extent due to CSDG; whereas the extent of reduction in TT and HT was maximum due to chopping and followed by CSDG, differences between the treatments chopping and CSD being non significant.

4.1.2 Effect of physical along with different chemical treatments on polyphenol content of *F. roxburghii* leaves

4.1.2.1 Effect of different physical treatments followed by PP treatments on polyphenol contents of *F. roxburghii* leaves

Polyphenol contents of physic-chemical (PP) treated *F. roxburghii* leaves have been presented in Table 15. When both the treatments were done together, it was observed that overall reduction in TP, NTP, TT, CT and HT was 64.07 (18.12-90.79), 24.95 (-40.35 to 93.55), 72.67 (26.03-92.47), 59.53 (7.49-96.07) and 83.35 (41.37-120.62) %, respectively. Reduction of these polyphenols due to physical treatments ranged from 46.04 to 84.37, 31.59 to 82.26, 59.52 to 84.82, 30.78 to 92.00 and 78.39 to 91.09 %, respectively; and due to PP (including water) treatments, level of reduction varied from 52.79 to 70.49, 17.96 to 27.67, 60.37 to 79.80, 46.31 to 64.75 and 71.98 to 93.34 %, respectively. Reduction was maximum due to physical

Table 15 : Effect of different physical and chemical (potassium permanganate) treatments on phenolic contents of *F. roxburghii* leaves

Physical Treatment	Chemical Treatment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannin	Total tannin	Condensed tannin	Hydrolysable tannin	Total phenol	Non tannin	Total tannin	Condensed tannin	Hydrolysable tannin
Fresh	0.0%PP (Water)	$5.13^{A} \pm 0.02$	$1.32^{D} \pm 0.01$	$3.81^{A} \pm 0.02$	$2.16^{A} \pm 0.01$	$1.65^{A} \pm 0.02$	$18.12^{M} \pm 0.46$	$-18.25^{Q} \pm 0.59$	$26.03^{J} \pm 0.43$	$7.47^{N} \pm 0.54$	$41.37^{I} \pm 0.84$
	0.1%PP	$2.93^{D} \pm 0.03$	$1.33^{D} \pm 0.01$	$1.60^{D} \pm 0.03$	$1.34^{EF} \pm 0.03$	$0.26^{F} \pm 0.02$	$53.19^{J} \pm 0.55$	$-18.98^{Q} \pm 0.70$	$68.90^{F} \pm 0.60$	$42.58^{IJ} \pm 1.17$	$90.62^{D} \pm 0.95$
	0.2%PP	$2.94^{D} \pm 0.01$	$1.32^{D} \pm 0.009$	$1.62^{D} \pm 0.02$	$1.31^{F} \pm 0.04$	$0.31^{EF} \pm 0.03$	$53.09^{J} \pm 0.23$	$-18.10^{Q} \pm 0.86$	$68.58^{F} \pm 0.42$	$43.90^{I} \pm 1.69$	$88.96^{DE} \pm 1.18$
	0.4%PP	$2.52^{G} \pm 0.02$	$1.21^{E} \pm 0.004$	$1.31^{E} \pm 0.02$	$1.10^{G} \pm 0.01$	$0.21^{F} \pm 0.02$	$59.76^{G} \pm 0.26$	$-8.47^{F} \pm 0.41$	$74.60^{E} \pm 0.33$	$52.84^{H} \pm 0.49$	$92.59^{D} \pm 0.56$
Chopped	0.0%PP (Water)	$2.72^{F} \pm 0.02$	$1.40^{C} \pm 0.01$	$1.32^{E} \pm 0.02$	$1.90^{B} \pm 0.01$	$-0.58^{J} \pm 0.02$	$56.65^{H} \pm 0.29$	$-25.01^{H} \pm 0.69$	$74.41^{E} \pm 0.39$	$18.48^{M} \pm 0.22$	$120.62^{A} \pm 0.87$
	0.1%PP	$2.81^{E} \pm 0.02$	$1.48^{B} \pm 0.01$	$1.33^{E} \pm 0.02$	$1.53^{CD} \pm 0.01$	$-0.21^{H} \pm 0.02$	$55.23^{I} \pm 0.33$	$-32.19^{I} \pm 0.59$	$74.24^{E} \pm 0.41$	$34.13^{KL} \pm 0.26$	$107.38^{B} \pm 0.75$
	0.2%PP	$3.54^{C} \pm 0.01$	$1.44^{BC} \pm 0.03$	$2.09^{C} \pm 0.02$	$1.44^{DE} \pm 0.007$	$0.66^{C} \pm 0.02$	$43.58^{K} \pm 0.23$	$-28.81^{HI} \pm 2.76$	$59.33^{G} \pm 0.37$	$38.23^{JK} \pm 0.30$	$76.75^{G} \pm 0.81$
	0.4%PP	$4.29^{B} \pm 0.04$	$1.57^{A} \pm 0.07$	$2.72^{B} \pm 0.06$	$1.58^{C} \pm 0.01$	$1.14^{B} \pm 0.06$	$31.60^{L} \pm 0.57$	$-40.35^{J} \pm 6.33$	$47.25^{H} \pm 1.19$	$32.28^{L} \pm 0.25$	$59.62^{H} \pm 2.08$
Chopped sun dried	0.0%PP (Water)	$2.30^{H} \pm 0.03$	$0.63^{F} \pm 0.006$	$1.68^{D} \pm 0.04$	$0.64^{H} \pm 0.001$	$1.03^{B} \pm 0.04$	$63.24^{F} \pm 0.54$	$44.02^{E} \pm 0.57$	$67.42^{F} \pm 0.71$	$72.35^{G} \pm 0.05$	$63.35^{H} \pm 1.30$
	0.1%PP	$1.04^{K} \pm 0.02$	$0.35^{G} \pm 0.002$	$0.69^{G} \pm 0.02$	$0.40^{JK} \pm 0.002$	$0.29^{EF} \pm 0.02$	$83.39^{C} \pm 0.20$	$68.31^{D} \pm 0.24$	$86.67^{C} \pm 0.33$	$82.95^{DE} \pm 0.06$	$89.74^{DE} \pm 0.64$
	0.2%PP	$1.17^{J} \pm 0.02$	$0.28^{H} \pm 0.01$	$0.89^{F} \pm 0.02$	$0.43^{IJ} \pm 0.13$	$0.46^{D} \pm 0.11$	$81.36^{D} \pm 0.36$	$75.37^{C} \pm 0.59$	$82.66^{D} \pm 0.45$	$81.47^{EF} \pm 5.76$	$83.65^{F} \pm 4.04$
	0.4%PP	$0.73^{L} \pm 0.01$	$0.31^{GH} \pm 0.02$	$0.42^{I} \pm 0.03$	$0.52^{I} \pm 0.002$	$-0.09^{G} \pm 0.03$	$88.36^{B} \pm 0.22$	$72.64^{CD} \pm 1.81$	$91.78^{A} \pm 0.60$	$77.82^{F} \pm 0.08$	$103.30^{C} \pm 1.07$
Chopped sun dried and grinded	0.0%PP (Water)	$1.68^{I} \pm 0.01$	$0.32^{GH} \pm 0.01$	$1.36^{E} \pm 0.02$	$0.30^{KL} \pm 0.01$	$1.05^{B} \pm 0.02$	$73.15^{E} \pm 0.18$	$71.06^{CD} \pm 1.33$	$73.61^{E} \pm 0.38$	$86.94^{CD} \pm 0.22$	$62.60^{H} \pm 0.81$
	0.1%PP	$0.62^{M} \pm 0.01$	$0.07^{J} \pm 0.01$	$0.55^{H} \pm 0.12$	$0.14^{MN} \pm 0.002$	$0.41^{DE} \pm 0.02$	$90.14^{A} \pm 0.15$	$93.55^{A} \pm 0.77$	$89.39^{B} \pm 0.34$	$93.96^{AB} \pm 0.13$	$85.62^{EF} \pm 0.72$
	0.2%PP	$1.04^{K} \pm 0.009$	$0.21^{I} \pm 0.02$	$0.83^{F} \pm 0.01$	$0.21^{LM} \pm 0.001$	$0.62^{C} \pm 0.01$	$83.38^{C} \pm 0.15$	$81.33^{B} \pm 0.74$	$83.83^{D} \pm 0.26$	$91.02^{BC} \pm 0.04$	$77.88^{G} \pm 0.46$
	0.4%PP	$0.58^{M} \pm 0.01$	$0.19^{I} \pm 0.01$	$0.39^{I} \pm 0.01$	$0.09^{N} \pm 0.004$	$0.30^{EF} \pm 0.004$	$90.79^{A} \pm 0.22$	$83.09^{B} \pm 0.70$	$92.47^{A} \pm 0.13$	$96.07^{A} \pm 0.10$	$89.49^{DE} \pm 0.17$

Table 15 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.38^{A} ± 0.26	1.30^{b} ± 0.01	2.08^{a} ± 0.26	1.47^{b} ± 0.10	0.61^{a} ± 0.16	46.04^{d} ± 4.22	-15.95^{c} ± 1.16	59.52^{d} ± 5.04	36.70^{c} ± 4.50	78.39^{c} ± 5.54
Chopped	3.34^{b} ± 0.16	1.47^{a} ± 0.02	1.86^{b} ± 0.15	1.61^{a} ± 0.04	0.25^{c} ± 0.18	46.77^{c} ± 2.62	-31.59^{d} ± 2.14	63.81^{c} ± 2.92	30.78^{d} ± 1.92	91.09^{a} ± 6.26
Chopped sun dried	1.31^{c} ± 0.15	0.39^{c} ± 0.04	0.92^{c} ± 0.12	0.50^{c} ± 0.04	0.42^{b} ± 0.11	79.09^{b} ± 2.46	65.09^{b} ± 3.24	82.13^{b} ± 2.36	78.65^{b} ± 1.67	85.01^{b} ± 3.84
Chopped sun dried and grinded	0.98^{d} ± 0.11	0.20^{d} ± 0.02	0.78^{d} ± 0.10	0.19^{d} ± 0.02	0.60^{a} ± 0.08	84.37^{a} ± 1.83	82.26^{a} ± 2.10	84.82^{a} ± 1.86	92.00^{a} ± 0.89	78.90^{c} ± 2.67
Potassium permanganate	**Potassium permanganate Treatments**					**Potassium permanganate Treatments**				
0.0%PP (Water)	2.96^{A} ± 0.34	0.92^{a} ± 0.12	2.04^{a} ± 0.27	1.25^{a} ± 0.20	0.79^{a} ± 0.21	52.79^{d} ± 5.39	17.96^{b} ± 10.54	60.37^{d} ± 5.17	46.31^{b} ± 8.77	71.98^{d} ± 7.61
0.1%PP	1.85^{d} ± 0.27	0.81^{b} ± 0.16	1.04^{d} ± 0.11	0.85^{b} ± 0.15	0.19^{d} ± 0.06	70.49^{a} ± 4.25	27.67^{a} ± 14.00	79.80^{a} ± 2.20	63.41^{a} ± 6.60	93.34^{a} ± 2.18
0.2%PP	2.17^{b} ± 0.28	0.81^{b} ± 0.15	1.36^{b} ± 0.14	0.85^{b} ± 0.14	0.51^{b} ± 0.04	65.35^{b} ± 4.48	27.45^{a} ± 13.21	73.60^{c} ± 2.64	63.66^{a} ± 6.07	81.81^{c} ± 1.59
0.4%PP	2.03^{c} ± 0.39	0.82^{b} ± 0.15	1.21^{c} ± 0.24	0.82^{b} ± 0.15	0.39^{c} ± 0.12	67.63^{c} ± 6.23	26.73^{a} ± 13.64	76.52^{b} ± 4.75	64.75^{a} ± 6.26	86.25^{b} ± 4.22
Overall mean ± SE	2.25± 0.17	0.84^{b} ± 0.07	1.41 ± 0.11	0.94^{b} ± 0.08	0.47 ± 0.07	64.07 ± 2.65	24.95 ± 6.32	72.67 ± 2.12	59.53 ± 3.55	83.35 ± 2.43
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A, B, C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *F. roxburghii* leaves were 6.27 ± 0.17, 1.12± 0.02, 5.15± 0.17, 2.33 ± 0.01 and 2.82 ± 0.17, respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

treatment CSDG followed by CSD and chopping for TP, NTP, TT and CT; whereas for HT maximum reduction was due to chopping, followed by CSD and CSDG.

Interactions between physical and PP treatments were also found to be highly significant ($P < 0.0001$). On comparison, among the 16 groups of different treatment combinations, it was observed that the reduction of TP, and CT was significantly higher in 16th (CSDG + 0.4 % PP) and 14th (CSDG + 0.1 % PP) treatment; NTP was reduced at maximum level in 14th; TT in 12th (CSD + 0.4 % PP) and HT was in 5th (chopped + water) treatments.

4.1.2.2 Effect of different physical treatments followed by urea treatment on polyphenol contents of *F. roxburghii* leaves

Concentrations of different polyphenols in *F. roxburghii* leaves subjected through different physico-chemical (urea) treatments are presented in Table 16.

Overall reduction of polyphenols, in TP, NTP, TT, CT and HT was 62.65 (18.12-90.40), 29.49 (-25.01 to 85.66), 69.87 (26.03-96.76), 59.59 (7.47-96.72) and 78.36 941.37-120.62) %, respectively. Reduction was maximum for HT, followed by TT, TP, CT and NTP respectively.

Reductions of these phenols (TP, NTP, TT, CT and HT) due to physical treatments ranged from 42.55 to 81.50, -11.88 to 78.07, 54.39 to 85.30, 32.85 to 90.51 and 72.20 to 91.08 %, respectively. Extent of reduction was maximum due to CSDG followed by CSD for all the polyphenols, except HT which was maximum due to CSD. Reduction of these polyphenols due to chemical (urea) treatments (including water), varied from was 52.79 to 69.81, 17.96 to 38.50, 60.37 to 76.62, 46.31 to 65.35 and 71.98 to 85.93 % of TP, NTP, TT,

Table 16 : Effect of different physical and chemical (urea) treatments on phenolic contents of *F. roxburghii* leaves

Physical Treatment	Chemical Treatment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0% Urea (Water)	$5.13^{A} \pm 0.02$	$1.32^{B} \pm 0.01$	$3.81^{A} \pm 0.02$	$2.16^{A} \pm 0.01$	$1.65^{A} \pm 0.02$	$18.12^{N} \pm 0.46$	$-18.25^{K} \pm 0.59$	$26.03^{N} \pm 0.43$	$7.47^{P} \pm 0.54$	$41.37^{L} \pm 0.84$
	0.5% Urea	$3.74^{C} \pm 0.03$	$1.16^{D} \pm 0.01$	$2.58^{C} \pm 0.02$	$1.60^{C} \pm 0.002$	$0.98^{E} \pm 0.02$	$40.43^{L} \pm 0.41$	$-3.26^{J} \pm 0.47$	$49.93^{L} \pm 0.44$	$31.29^{N} \pm 0.12$	$65.33^{H} \pm 0.73$
	1.5% Urea	$3.00^{E} \pm 0.03$	$1.23^{C} \pm 0.006$	$1.77^{F} \pm 0.03$	$1.12^{H} \pm 0.02$	$0.65^{G} \pm 0.01$	$52.14^{J} \pm 0.45$	$-10.07^{J} \pm 0.54$	$65.67^{E} \pm 0.50$	$51.87^{I} \pm 0.80$	$77.08^{F} \pm 0.45$
	2.5% Urea	$2.54^{G} \pm 0.04$	$1.30^{B} \pm 0.01$	$1.24^{I} \pm 0.04$	$1.38^{E} \pm 0.12$	$-0.14^{K} \pm 0.05$	$59.52^{H} \pm 0.64$	$-15.52^{K} \pm 0.64$	$75.94^{F} \pm 0.73$	$40.76^{L} \pm 0.69$	$105.00^{B} \pm 1.85$
Chopped	0.0% Urea (Water)	$2.72^{F} \pm 0.02$	$1.40^{A} \pm 0.01$	$1.32^{H} \pm 0.02$	$1.90^{B} \pm 0.01$	$-0.58^{L} \pm 0.02$	$56.65^{F} \pm 0.29$	$-25.01^{L} \pm 0.69$	$74.41^{G} \pm 0.39$	$18.48^{O} \pm 0.22$	$120.62^{A} \pm 0.87$
	0.5% Urea	$4.36^{B} \pm 0.01$	$1.29^{B} \pm 0.003$	$3.07^{B} \pm 0.01$	$1.54^{D} \pm 0.003$	$1.53^{B} \pm 0.01$	$30.47^{M} \pm 0.19$	$-15.22^{K} \pm 0.31$	$40.40^{M} \pm 0.21$	$33.98^{M} \pm 0.16$	$45.71^{K} \pm 0.49$
	1.5% Urea	$3.23^{D} \pm 0.01$	$1.24^{C} \pm 0.04$	$2.00^{E} \pm 0.03$	$1.26^{F} \pm 0.002$	$0.74^{F} \pm 0.04$	$48.42^{K} \pm 0.16$	$-10.54^{J} \pm 3.67$	$61.24^{J} \pm 0.68$	$46.07^{K} \pm 0.09$	$73.77^{G} \pm 1.31$
	2.5% Urea	$3.03^{E} \pm 0.01$	$0.67^{E} \pm 0.004$	$2.36^{D} \pm 0.01$	$1.21^{G} \pm 0.007$	$1.16^{C} \pm 0.01$	$51.61^{J} \pm 0.18$	$40.26^{H} \pm 0.43$	$54.08^{K} \pm 0.20$	$48.27^{J} \pm 0.03$	$58.89^{J} \pm 0.36$
Chopped sun dried	0.0% Urea (Water)	$2.30^{H} \pm 0.03$	$0.63^{F} \pm 0.006$	$1.68^{G} \pm 0.04$	$0.64^{J} \pm 0.001$	$1.03^{DE} \pm 0.04$	$63.24^{G} \pm 0.54$	$44.02^{G} \pm 0.57$	$67.42^{H} \pm 0.71$	$72.35^{G} \pm 0.05$	$63.35^{HI} \pm 1.30$
	0.5% Urea	$0.60^{N} \pm 0.005$	$0.44^{H} \pm 0.02$	$0.17^{N} \pm 0.12$	$0.28^{M} \pm 0.002$	$-0.11^{K} \pm 0.02$	$90.40^{A} \pm 0.09$	$61.13^{E} \pm 1.35$	$96.76^{A} \pm 0.31$	$88.05^{D} \pm 0.09$	$103.96^{B} \pm 0.62$
	1.5% Urea	$1.28^{J} \pm 0.01$	$0.48^{G} \pm 0.002$	$0.80^{K} \pm 0.01$	$0.69^{I} \pm 0.01$	$0.11^{I} \pm 0.01$	$79.63^{E} \pm 0.19$	$57.27^{F} \pm 0.24$	$84.49^{D} \pm 0.27$	$70.32^{H} \pm 0.07$	$96.20^{D} \pm 0.46$
	2.5% Urea	$0.89^{L} \pm 0.01$	$0.50^{G} \pm 0.004$	$0.38^{M} \pm 0.01$	$0.41^{K} \pm 0.01$	$-0.02^{J} \pm 0.01$	$85.87^{C} \pm 0.15$	$55.20^{F} \pm 0.36$	$92.53^{B} \pm 0.25$	$82.53^{F} \pm 0.05$	$100.80^{C} \pm 0.43$
Chopped sun dried and grinded	0.0% Urea (Water)	$1.68^{I} \pm 0.01$	$0.32^{I} \pm 0.01$	$1.36^{H} \pm 0.02$	$0.30^{L} \pm 0.01$	$1.05^{D} \pm 0.02$	$73.15^{F} \pm 0.18$	$71.06^{D} \pm 1.33$	$73.61^{G} \pm 0.38$	$86.94^{E} \pm 0.22$	$62.60^{I} \pm 0.81$
	0.5% Urea	$0.72^{M} \pm 0.01$	$0.21^{K} \pm 0.006$	$0.51^{L} \pm 0.01$	$0.15^{P} \pm 0.001$	$0.36^{H} \pm 0.01$	$88.55^{B} \pm 0.13$	$81.08^{B} \pm 0.40$	$90.18^{C} \pm 0.23$	$93.72^{A} \pm 0.02$	$87.26^{E} \pm 0.42$
	1.5% Urea	$1.13^{K} \pm 0.01$	$0.16^{L} \pm 0.01$	$0.97^{J} \pm 0.02$	$0.20^{O} \pm 0.001$	$0.77^{F} \pm 0.02$	$82.03^{D} \pm 0.20$	$85.66^{A} \pm 0.60$	$81.24^{E} \pm 0.37$	$91.51^{B} \pm 0.02$	$72.76^{G} \pm 0.68$
	2.5% Urea	$1.11^{K} \pm 0.005$	$0.29^{J} \pm 0.02$	$0.83^{K} \pm 0.01$	$0.24^{N} \pm 0.001$	$0.59^{G} \pm 0.10$	$82.25^{D} \pm 0.10$	$74.50^{C} \pm 1.46$	$83.94^{D} \pm 0.23$	$89.86^{C} \pm 0.07$	$79.04^{F} \pm 0.42$

Table 16 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	$3.60^{A} \pm 0.25$	$1.25^{a} \pm 0.02$	$2.35^{a} \pm 0.25$	$1.55^{a} \pm 0.10$	$0.78^{a} \pm 0.17$	$42.55^{d} \pm 4.07$	$-11.88^{c} \pm 1.52$	$54.39^{d} \pm 4.86$	$32.85^{c} \pm 4.23$	$72.20^{d} \pm 5.93$
Chopped	$3.34^{b} \pm 0.16$	$1.15^{b} \pm 0.07$	$2.19^{b} \pm 0.16$	$1.47^{b} \pm 0.07$	$0.71^{b} \pm 0.21$	$46.79^{c} \pm 2.55$	$-2.63^{b} \pm 6.59$	$57.53^{c} \pm 3.18$	$36.70^{b} \pm 3.06$	$74.75^{c} \pm 7.31$
Chopped sun dried	$1.27^{c} \pm 0.17$	$0.51^{b} \pm 0.02$	$0.76^{c} \pm 0.15$	$0.51^{c} \pm 0.04$	$0.25^{c} \pm 0.12$	$79.78^{b} \pm 2.66$	$54.41^{b} \pm 1.68$	$85.30^{b} \pm 2.90$	$78.31^{a} \pm 1.88$	$91.08^{b} \pm 4.21$
Chopped sun dried and grinded	$1.16^{d} \pm 0.09$	$0.25^{c} \pm 0.12$	$0.91^{d} \pm 0.08$	$0.22^{c} \pm 0.01$	$0.69^{d} \pm 0.07$	$81.50^{a} \pm 1.42$	$78.07^{a} \pm 1.54$	$82.24^{a} \pm 1.54$	$90.51^{a} \pm 0.64$	$75.41^{a} \pm 2.34$
Urea	**Urea Treatments**					**Urea Treatments**				
0.0% Urea (Water)	$2.96^{A} \pm 0.34$	$0.92^{a} \pm 0.12$	$2.04^{a} \pm 0.27$	$1.25^{a} \pm 0.20$	$0.79^{a} \pm 0.21$	$52.79^{d} \pm 5.39$	$17.96^{d} \pm 10.54$	$60.37^{d} \pm 5.17$	$46.31^{d} \pm 8.77$	$71.98^{b} \pm 7.61$
0.5% Urea	$2.35^{b} \pm 0.44$	$0.77^{b} \pm 0.12$	$1.58^{b} \pm 0.33$	$0.89^{b} \pm 0.18$	$0.69^{b} \pm 0.16$	$62.46^{c} \pm 7.04$	$30.93^{c} \pm 10.59$	$69.32^{c} \pm 6.33$	$61.76^{c} \pm 7.54$	$75.56^{a} \pm 5.69$
1.5% Urea	$2.16^{c} \pm 0.25$	$0.78^{c} \pm 0.12$	$1.38^{c} \pm 0.13$	$0.82^{c} \pm 0.11$	$0.57^{c} \pm 0.07$	$65.56^{b} \pm 3.97$	$30.58^{b} \pm 10.90$	$73.16^{a} \pm 2.56$	$64.94^{b} \pm 4.59$	$79.95^{b} \pm 2.48$
2.5% Urea	$1.89^{d} \pm 0.24$	$0.69^{d} \pm 0.10$	$1.20^{d} \pm 0.19$	$0.81^{d} \pm 0.13$	$0.40^{b} \pm 0.14$	$69.81^{a} \pm 3.77$	$38.50^{a} \pm 8.71$	$76.62^{b} \pm 3.69$	$65.35^{a} \pm 5.47$	$85.93^{c} \pm 4.79$
Overall mean ± SE	2.34 ± 0.17	0.79 ± 0.06	1.55 ± 0.12	0.94 ± 0.08	0.61 ± 0.08	62.65 ± 3.66	29.49 ± 5.07	69.87 ± 2.40	59.59 ± 3.46	78.36 ± 2.75
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *F. roxburghii* leaves were 6.27 ± 0.17, 1.12± 0.02, 5.15± 0.17, 2.33 ± 0.01 and 2.82 ± 0.17, respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

CT and HT, respectively. Reduction was maximum in case of 2.5% urea treatment for TP, NTP and CT; where as it was maximum in 1.5 % urea treatment for TT and for HT it was due to 0.5 % urea treatments.

Interaction between physical treatments and urea treatments were also found to be highly significant ($P < 0.0001$). Maximum reduction for TP and TT was in 10^{th} treatment (CSD + 0.5 % urea); whereas the reduction was maximum in 15^{th} (CSDG + 1.5 % urea) treatment for NTP; in 14^{th} (CSDG + 0.5 % urea) for CT treatment and in 5^{th} (chopped+water) treatment for HT.

4.1.2.3 Effect of different physical treatments followed by wood ash (WA) treatment on polyphenol contents of *F. roxburghii* leaves

Polyphenol contents of physico-chemically (WA) treated *F. roxburghii* leaves have been presented in Table 17. Overall reduction of polyphenols in *F. roxburghii* leaves was 57.17 (18.12-87.55), 29.95 (-61.75 to 85.75), 63.09 (26.03-88.62), 55.77 (7.47-96.72) and 69.13 (-13.64 to 140.02) % for TP, NTP, TT, CT and HT, respectively. Reduction of respective polyphenols due to physical treatments ranged from 28.93 to 83.08, - 44.13 to 79.98, 31.73 to 83.75, 26.75 to 93.09 and 32.68 to 85.80 %, respectively; and reduction of these polyphenols (TP, NTP, TT, CT and HT) due to WA (including water) treatments varied from 52.79 to 62.85, 17.96 to 37.71, 57.58 to 68.58, 46.31 to 61.92 and 56.11 to 74.37 %, respectively. Extent of overall reduction was maximum for HT, followed by TT, TP, CT and NTP, respectively. Out of four physical treatments, the reduction of TP, NTP, TT and CT was maximum due to CSDG; whereas, HT was reduced at maximum in fresh leaves treated only with water. On observing of the effect of chemical (WA) treatments, it was revealed that 5% WA was effective in reducing the TP, TT and CT at maximum level; whereas the reduction of NTP was maximum at 2.5 to 5% WA, difference among the

Table 17 : Effect of different physical and wood ash (WA) treatments on phenolic contents of *F. roxburghii* leaves

Physical Treatment	Chemical Treatment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0% WA (Water)	$5.13^{B} \pm 0.02$	$1.32^{E} \pm 0.01$	$3.81^{C} \pm 0.02$	$2.16^{A} \pm 0.01$	$1.65^{D} \pm 0.02$	$18.12^{J} \pm 0.46$	$-18.25^{H} \pm 0.59$	$26.03^{I} \pm 0.43$	$7.47^{K} \pm 0.54$	$41.37^{H} \pm 0.84$
	2.5% WA	$4.00^{D} \pm 0.01$	$1.57^{C} \pm 0.01$	$2.43^{E} \pm 0.01$	$1.30^{F} \pm 0.02$	$1.13^{E} \pm 0.02$	$36.26^{H} \pm 0.30$	$-40.05^{J} \pm 0.57$	$52.85^{D} \pm 0.26$	$44.21^{F} \pm 0.78$	$59.99^{G} \pm 0.76$
	5% WA	$3.12^{E} \pm 0.06$	$1.75^{B} \pm 0.01$	$1.37^{G} \pm 0.06$	$1.42^{E} \pm 0.003$	$-0.05^{I} \pm 0.07$	$50.23^{G} \pm 1.01$	$-56.45^{K} \pm 1.05$	$73.43^{E} \pm 1.25$	$39.07^{G} \pm 0.16$	$101.81^{C} \pm 2.36$
	10% WA	$2.68^{F} \pm 0.20$	$1.81^{A} \pm 0.01$	$0.87^{H} \pm 0.01$	$2.00^{B} \pm 0.08$	$-1.13^{K} \pm 0.09$	$57.23^{F} \pm 0.31$	$-61.75^{L} \pm 1.09$	$83.11^{C} \pm 0.21$	$14.23^{J} \pm 3.61$	$140.02^{A} \pm 3.09$
Chopped	0.0% WA (Water)	$2.72^{F} \pm 0.02$	$1.40^{D} \pm 0.01$	$1.32^{G} \pm 0.02$	$1.90^{C} \pm 0.01$	$-0.58^{J} \pm 0.02$	$56.65^{F} \pm 0.29$	$-25.01^{I} \pm 0.69$	$74.41^{E} \pm 0.39$	$18.48^{I} \pm 0.22$	$120.62^{B} \pm 0.87$
	2.5% WA	$5.14^{B} \pm 0.01$	$0.71^{G} \pm 0.006$	$4.43^{B} \pm 0.01$	$1.60^{D} \pm 0.004$	$2.83^{B} \pm 0.01$	$18.02^{J} \pm 0.15$	$36.88^{F} \pm 0.59$	$13.92^{J} \pm 0.12$	$31.20^{H} \pm 0.18$	$-0.36^{J} \pm 0.24$
	5% WA	$4.13^{C} \pm 0.02$	$0.64^{H} \pm 0.01$	$3.49^{D} \pm 0.02$	$1.35^{F} \pm 0.003$	$2.14^{C} \pm 0.03$	$34.07^{I} \pm 0.42$	$42.71^{E} \pm 0.52$	$32.18^{H} \pm 0.45$	$41.96^{F} \pm 0.13$	$24.10^{I} \pm 0.91$
	10% WA	$5.83^{A} \pm 0.03$	$1.01^{F} \pm 0.01$	$4.82^{A} \pm 0.03$	$1.61^{D} \pm 0.003$	$3.20^{A} \pm 0.03$	$6.98^{K} \pm 0.48$	$9.53^{G} \pm 0.53$	$6.42^{K} \pm 0.68$	$30.71^{H} \pm 0.16$	$-13.64^{K} \pm 1.14$
Chopped sun dried	0.0% WA (Water)	$2.30^{G} \pm 0.03$	$0.63^{H} \pm 0.006$	$1.68^{F} \pm 0.04$	$0.64^{G} \pm 0.001$	$1.03^{E} \pm 0.04$	$63.24^{E} \pm 0.54$	$44.02^{E} \pm 0.57$	$67.42^{F} \pm 0.71$	$72.35^{E} \pm 0.05$	$63.35^{G} \pm 1.30$
	2.5% WA	$1.35^{I} \pm 0.02$	$0.30^{IJ} \pm 0.002$	$1.06^{H} \pm 0.22$	$0.70^{G} \pm 0.002$	$0.36^{H} \pm 0.02$	$78.44^{C} \pm 0.26$	$73.54^{CD} \pm 0.20$	$79.50^{D} \pm 0.36$	$70.02^{E} \pm 0.08$	$87.34^{D} \pm 0.71$
	5% WA	$1.32^{I} \pm 0.01$	$0.29^{J} \pm 0.02$	$1.03^{H} \pm 0.01$	$0.70^{G} \pm 0.004$	$0.33^{H} \pm 0.01$	$78.92^{C} \pm 0.22$	$74.13^{C} \pm 1.44$	$79.96^{D} \pm 0.22$	$69.94^{E} \pm 0.16$	$88.24^{D} \pm 0.50$
	10% WA	$0.99^{J} \pm 0.01$	$0.22^{K} \pm 0.02$	$0.77^{J} \pm 0.02$	$0.46^{H} \pm 0.001$	$0.31^{H} \pm 0.02$	$84.22^{B} \pm 0.10$	$79.99^{B} \pm 1.58$	$85.14^{B} \pm 0.36$	$80.28^{D} \pm 0.05$	$89.16^{D} \pm 0.69$
Chopped sun dried and grinded	0.0% WA (Water)	$1.68^{H} \pm 0.01$	$0.32^{I} \pm 0.01$	$1.36^{G} \pm 0.02$	$0.30^{I} \pm 0.01$	$1.05^{E} \pm 0.02$	$73.15^{D} \pm 0.18$	$71.06^{D} \pm 1.33$	$73.61^{E} \pm 0.38$	$86.94^{C} \pm 0.22$	$62.60^{G} \pm 0.81$
	2.5% WA	$1.04^{J} \pm 0.01$	$0.22^{K} \pm 0.02$	$0.82^{IJ} \pm 0.03$	$0.19^{J} \pm 0.001$	$0.64^{F} \pm 0.03$	$83.39^{B} \pm 0.16$	$80.49^{B} \pm 1.83$	$84.03^{BC} \pm 0.51$	$91.99^{B} \pm 0.01$	$77.45^{F} \pm 0.93$
	5% WA	$0.74^{K} \pm 0.01$	$0.16^{L} \pm 0.01$	$0.58^{K} \pm 0.02$	$0.08^{K} \pm 0.001$	$0.50^{G} \pm 0.02$	$88.21^{A} \pm 0.14$	$85.77^{A} \pm 1.24$	$88.74^{A} \pm 0.42$	$96.72^{A} \pm 0.04$	$82.15^{E} \pm 0.79$
	10% WA	$0.78^{K} \pm 0.01$	$0.19^{K} \pm 0.01$	$0.59^{K} \pm 0.01$	$0.08^{K} \pm 0.001$	$0.51^{G} \pm 0.01$	$87.55^{A} \pm 0.16$	$82.60^{B} \pm 0.52$	$88.62^{A} \pm 0.25$	$96.72^{A} \pm 0.05$	$89.93^{E} \pm 0.49$

Table 17 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.73[B] ± 0.24	1.61[a] ± 0.05	2.12[b] ± 0.29	1.72[a] ± 0.10	0.40[d] ± 0.30	40.46[c] ± 3.87	-44.13[d] ± 4.39	58.86[c] ± 5.66	26.25[d] ± 4.14	85.80[a] ± 9.90
Chopped	4.46[a] ± 0.30	0.94[b] ± 0.08	3.52[a] ± 0.35	1.62[b] ± 0.05	1.90[a] ± 0.39	28.93[d] ± 4.83	16.03[e] ± 6.92	31.73[d] ± 6.81	30.59[c] ± 2.15	32.68[d] ± 13.57
Chopped sun dried	1.49[c] ± 0.13	0.36[c] ± 0.04	1.13[c] ± 0.09	0.63[c] ± 0.03	0.51[c] ± 0.08	76.20[b] ± 2.02	67.92[b] ± 3.65	78.01[b] ± 1.69	73.15[b] ± 1.09	82.02[b] ± 2.81
Chopped sun dried and grinded	1.06[d] ± 0.10	0.22[d] ± 0.02	0.84[d] ± 0.08	0.16[d] ± 0.02	0.68[b] ± 0.06	83.08[a] ± 1.56	79.98[a] ± 1.53	83.75[a] ± 1.60	93.09[a] ± 1.05	76.03[c] ± 2.09
Wood ash	**wood ash Treatments**					**wood ash Treatments**				
0.0% wood ash (Water)	2.96[A] ± 0.34	0.92[a] ± 0.12	2.04[b] ± 0.27	1.25[a] ± 0.20	0.79[b] ± 0.21	52.79[d] ± 2.39	17.96[b] ± 10.54	60.37[c] ± 5.17	46.31[d] ± 8.77	71.98 [b] ± 7.61
2.5% wood ash	2.88[b] ± 0.45	0.70[c] ± 0.14	2.18[a] ± 0.37	0.95[c] ± 0.14	1.24[a] ± 0.25	54.03[c] ± 7.15	37.71[a] ± 12.37	57.58[d] ± 7.20	59.35[b] ± 6.06	56.11[c] ± 8.79
5% wood ash	2.33[d] ± 0.35	0.71[c] ± 0.16	1.62[d] ± 0.29	0.89[d] ± 0.14	0.73[c] ± 0.22	62.85[a] ± 5.62	36.64[a] ± 14.46	68.58[a] ± 5.61	61.92[a] ± 6.05	74.08[a] ± 7.70
10% wood ash	2.57[c] ± 0.52	0.81[b] ± 0.17	1.76[c] ± 0.46	1.04[b] ± 0.21	0.73[c] ± 0.40	58.99[b] ± 8.33	27.59[b] ± 15.33	65.82[b] ± 8.87	55.49[c] ± 8.82	74.37[a] ± 14.36
Overall mean ± SE	2.69 ± 0.21	0.78 ± 0.07	1.90 ± 0.17	1.03 ± 0.08	0.87 ± 0.14	57.17 ± 3.32	29.95 ± 6.57	63.09 ± 3.40	55.77 ± 3.76	69.13 ± 4.98
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh F. roxburghii leaves were 6.27 ± 0.17, 1.12± 0.02, 5.15± 0.17, 2.33 ± 0.01 and 2.82 ± 0.17, respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

treatments being non significant. HT was reduced at maximum level in 5 to 10 % WA treatments; differences between 5 % and 10% became non significant.

Interactions between physical and WA treatments were also found to be very significant (P<0.0001). On comparison among the 16 respective groups of different treatment combinations (Physical × WA), it was revealed that TP, TT and CT was reduced at maximum level in 15th (CSDG + 5% WA) and 16th (CSDG + 10 % WA) treatments, difference among the treatments being non significant; whereas maximum reduction of NTP was in 15th (CSDG + 5% WA), and HT was in 4th treatment (Fresh + 10 % WA).

4.1.2.4 Effect of different physical treatments followed by GNC treatment of *F. roxburghii* leaves

Polyphenol contents of physical followed by GNC treated *F. roxburghii* leaves have been presented in Table 18. Overall reduction of TP, NTP, TT, CT and HT was 58.22 (18.12-86.63), 27.67 (-71.96 to 89.16), 64.87 (26.03-88.53), 58.35 (7.47-89.55) and 70.26 (41.37-120.62) %, respectively. Reduction of respective polyphenols due to physical treatments ranged from 32.99 to 80.58, -49.66 to 80.21, 46.97 to 80.66, 28.30 to 88.67 and 62.39 to 81.73 %. Whereas, reduction due to WA (including water) treatments, it varied from 52.79 to 61.83, 17.96 to 37.23, 60.37 to 68.49, 46.31 to 63.27 and 64.50 to 71.98 %, respectively. Physical treatment, CSDG, reduced the TP, NTP and CT at maximum level; however, reduction of TT was maximum in both the physical treatments, CSD and CSDG, values being comparable between the groups. HT was reduced at 81.73% (maximum) in CSD.

All the chemical treatment (including water) was effective in reducing the polyphenol content of *F. roxburghii* leaves, but maximum

Table 18 : **Effect of different physical and chemical [ground nut cake (GNC)] treatments on phenolic contents of *F. roxburghii* leaves**

Physical Treatment	Chemical Tretment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
Fresh	0.0% GNC (Water)	$5.13^{A} \pm 0.02$	$1.32^{E} \pm 0.01$	$3.81^{A} \pm 0.02$	$2.16^{A} \pm 0.01$	$1.65^{B} \pm 0.02$	$18.12^{M} \pm 0.46$	$-18.25^{J} \pm 0.59$	$26.03^{L} \pm 0.43$	$7.47^{L} \pm 0.54$	$41.37^{J} \pm 0.84$
	1.7%GNC	$4.62^{B} \pm 0.01$	$1.93^{A} \pm 0.01$	$2.70^{E} \pm 0.02$	$1.12^{Q} \pm 0.01$	$1.57^{C} \pm 0.02$	$26.29^{L} \pm 0.23$	$-71.96^{M} \pm 0.85$	$47.66^{H} \pm 0.40$	$51.84^{F} \pm 0.27$	$44.20^{I} \pm 0.73$
	3.4%GNC	$3.62^{E} \pm 0.01$	$1.76^{B} \pm 0.02$	$1.86^{F} \pm 0.01$	$1.31^{F} \pm 0.02$	$0.55^{H} \pm 0.01$	$42.34^{I} \pm 0.18$	$-56.75^{L} \pm 1.66$	$63.89^{Q} \pm 0.25$	$43.76^{Q} \pm 0.77$	$80.52^{D} \pm 0.46$
	7.5%GNC	$3.44^{F} \pm 0.01$	$1.70^{C} \pm 0.02$	$1.74^{Q} \pm 0.03$	$1.32^{F} \pm 0.03$	$0.41^{I} \pm 0.06$	$45.21^{H} \pm 0.23$	$-51.67^{K} \pm 1.87$	$66.27^{F} \pm 0.60$	$43.15^{Q} \pm 1.35$	$85.38^{C} \pm 2.15$
Chopped	0.0%GNC (Water)	$2.72^{Q} \pm 0.02$	$1.40^{D} \pm 0.01$	$1.32^{H} \pm 0.02$	$1.90^{B} \pm 0.01$	$-0.58^{K} \pm 0.02$	$56.65^{Q} \pm 0.29$	$-25.01^{J} \pm 0.69$	$74.41^{E} \pm 0.39$	$18.48^{K} \pm 0.22$	$120.62^{A} \pm 0.87$
	1.7%GNC	$4.19^{C} \pm 0.01$	$0.85^{Q} \pm 0.006$	$3.35^{B} \pm 0.01$	$1.63^{D} \pm 0.002$	$1.72^{AB} \pm 0.01$	$33.13^{K} \pm 0.23$	$24.43^{Q} \pm 0.56$	$35.02^{K} \pm 0.25$	$30.20^{I} \pm 0.10$	$39.01^{JK} \pm 0.41$
	3.4%GNC	$4.03^{D} \pm 0.03$	$1.03^{F} \pm 0.01$	$3.00^{D} \pm 0.03$	$1.67^{C} \pm 0.005$	$1.32^{D} \pm 0.03$	$35.76^{J} \pm 0.44$	$7.79^{H} \pm 0.49$	$41.84^{I} \pm 0.52$	$28.19^{J} \pm 0.22$	$53.11^{H} \pm 1.00$
	7.5%GNC	$4.00^{D} \pm 0.02$	$0.73^{H} \pm 0.004$	$3.27^{C} \pm 0.02$	$1.48^{E} \pm 0.003$	$1.78^{A} \pm 0.02$	$36.20^{J} \pm 0.35$	$34.38^{F} \pm 0.43$	$36.59^{J} \pm 0.41$	$36.33^{H} \pm 0.12$	$36.81^{K} \pm 0.81$
Chopped sun dried	0.0%GNC (Water)	$2.30^{H} \pm 0.03$	$0.63^{I} \pm 0.006$	$1.68^{Q} \pm 0.04$	$0.64^{H} \pm 0.001$	$1.03^{E} \pm 0.04$	$63.24^{F} \pm 0.54$	$44.02^{E} \pm 0.57$	$67.42^{F} \pm 0.71$	$72.35^{E} \pm 0.05$	$63.35^{Q} \pm 1.30$
	1.7%GNC	$1.12^{K} \pm 0.008$	$0.22^{KL} \pm 0.03$	$0.90^{J} \pm 0.03$	$0.52^{I} \pm 0.002$	$0.38^{IJ} \pm 0.03$	$82.08^{C} \pm 0.14$	$80.07^{BC} \pm 2.49$	$82.52^{C} \pm 0.64$	$77.84^{D} \pm 0.08$	$86.38^{BC} \pm 1.19$
	3.4%GNC	$0.94^{L} \pm 0.12$	$0.25^{K} \pm 0.01$	$0.69^{K} \pm 0.01$	$0.35^{J} \pm 0.001$	$0.35^{IJ} \pm 0.01$	$85.01^{B} \pm 0.27$	$77.83^{C} \pm 1.25$	$86.57^{B} \pm 0.21$	$85.16^{C} \pm 0.05$	$87.73^{BC} \pm 0.35$
	7.5%GNC	$0.93^{L} \pm 0.02$	$0.26^{K} \pm 0.03$	$0.67^{K} \pm 0.04$	$0.37^{J} \pm 0.001$	$0.30^{J} \pm 0.04$	$85.24^{B} \pm 0.32$	$77.04^{C} \pm 2.29$	$87.02^{B} \pm 0.82$	$84.10^{C} \pm 0.07$	$89.44^{B} \pm 1.53$
Chopped sun dried and grinded	0.0%GNC (Water)	$1.68^{I} \pm 0.01$	$0.32^{J} \pm 0.01$	$1.36^{H} \pm 0.02$	$0.30^{K} \pm 0.01$	$1.05^{E} \pm 0.02$	$73.15^{E} \pm 0.18$	$71.06^{D} \pm 1.33$	$73.61^{E} \pm 0.38$	$86.94^{B} \pm 0.22$	$62.60^{Q} \pm 0.81$
	1.7%GNC	$0.84^{M} \pm 0.01$	$0.25^{K} \pm 0.02$	$0.59^{L} \pm 0.02$	$0.26^{L} \pm 0.001$	$0.33^{IJ} \pm 0.02$	$86.63^{A} \pm 0.11$	$77.86^{C} \pm 1.95$	$88.53^{A} \pm 0.38$	$88.70^{A} \pm 0.05$	$88.39^{BC} \pm 0.67$
	3.4%GNC	$1.14^{K} \pm 0.01$	$0.19^{L} \pm 0.002$	$0.94^{J} \pm 0.01$	$0.24^{L} \pm 0.01$	$0.70^{G} \pm 0.01$	$81.88^{C} \pm 0.11$	$82.77^{B} \pm 0.24$	$81.68^{C} \pm 0.18$	$89.55^{A} \pm 0.02$	$75.18^{E} \pm 0.31$
	7.5%GNC	$1.21^{J} \pm 0.01$	$0.12^{M} \pm 0.01$	$1.09^{I} \pm 0.02$	$0.24^{L} \pm 0.01$	$0.85^{F} \pm 0.01$	$80.67^{D} \pm 0.16$	$89.16^{A} \pm 0.58$	$78.82^{D} \pm 0.30$	$89.49^{A} \pm 0.04$	$70.01^{F} \pm 0.53$

Table 18 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	4.20^{A} ± 0.18	1.68^{a} ± 0.06	2.53^{b} ± 0.21	1.48^{b} ± 0.10	1.05^{a} ± 0.15	32.99^{d} ± 2.90	-49.66^{d} ± 5.10	50.96^{b} ± 4.16	36.55^{c} ± 4.44	62.87^{c} ± 5.24
Chopped	3.73^{b}± 0.15	1.00^{b} ± 0.07	2.73^{a} ± 0.21	1.67^{a} ± 0.04	1.06^{a} ± 0.25	40.43^{c} ± 2.44	10.40^{c} ± 5.83	46.97^{c} ± 4.15	28.30^{d} ± 1.66	62.39^{c} ± 8.84
Chopped sun dried	1.32^{c} ± 0.15	0.34^{c} ± 0.04	0.98^{c} ± 0.11	0.47^{c} ± 0.03	0.52^{c} ± 0.08	78.89^{b} ± 2.36	69.74^{b} ± 3.93	80.88^{a} ± 2.08	79.87^{b} ± 1.33	81.73^{a} ± 2.80
Chopped sun dried and grinded	1.22^{d} ± 0.08	0.22^{d} ± 0.02	1.00^{c} ± 0.07	0.26^{d} ± 0.006	0.73^{b} ± 0.07	80.58^{a} ± 1.25	80.21^{a} ± 1.80	80.66^{a} ± 1.40	88.67^{a} ± 0.28	79.05^{b} ± 2.45
Ground Nut Cake	**Ground Nut Cake Treatments**					**Ground Nut Cake Treatments**				
0.0%GNC (Water)	2.96^{A} ± 0.34	0.92^{a} ± 0.12	2.04^{a} ± 0.27	1.25^{a} ± 0.20	0.79^{a} ± 0.21	52.79^{d} ± 5.39	17.96^{c} ± 10.54	60.37^{d} ± 5.17	46.31^{c} ± 8.77	71.98^{b} ± 7.61
1.7%GNC	2.69^{b} ± 0.44	0.81^{b} ± 0.18	1.88^{b} ± 0.30	0.88^{b} ± 0.14	1.00^{a} ± 0.17	57.03^{c} ± 7.09	27.60^{b} ± 15.93	63.43^{c} ± 5.85	62.15^{b} ± 5.89	64.50^{d} ± 5.94
3.4%GNC	2.43^{c} ± 0.36	0.81^{b} ± 0.17	1.62^{d} ± 0.23	0.89^{b} ± 0.16	0.73^{d} ± 0.09	61.24^{b} ± 5.77	27.91^{b} ± 14.77	68.49^{a} ± 4.53	61.66^{b} ± 6.80	74.14^{a} ± 3.35
7.5%GNC	2.39^{d} ± 0.35	0.70^{c} ± 0.16	1.69^{c} ± 0.25	0.86^{c} ± 0.14	0.83^{b} ± 0.15	61.83^{a} ± 5.53	37.23^{a} ± 14.27	67.18^{b} ± 4.95	63.27^{a}± 6.13	70.41^{c} ± 5.38
Overall mean ± SE	2.62 ± 0.19	0.81 ± 0.08	1.81 ± 0.13	0.97 ± 0.08	0.84 ± 0.08	58.22 ± 2.96	27.67 ± 6.90	64.87 ± 2.54	58.35 ± 3.52	70.26 ± 2.85
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A,B,C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *F. roxburghii* leaves were 6.27 ± 0.17, 1.12± 0.02, 5.15± 0.17, 2.33 ± 0.01 and 2.82 ± 0.17, respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

reduction (P < 0.0001) in TP, NTP and CT contents was due to 7.5 % GNC treatments; whereas, TT and HT was reduced at maximum level even at 3.4 % GNC treatment.

Interactions between physical and GNC treatments were also found to be highly significant (P<0.0001). On comparison among the 16 different treatments combination (Physical × GNC), it was revealed that TP and TT was reduced at maximum level in 14th treatment (CSDG + 1.7 % GNC); whereas NTP was in 16th (CSDG+7.5%GNC); CT was in 14th (CSDG+1.7%GNC), 15th (CSDG+3.4%GNC) and 16th (CSDG+7.5%GNC) treatments and HT was in 5th treatments (Chopped + Water).

4.1.2.5 Effect of different physical treatments followed by chemical treatments on different polyphenol content of *F. roxburghii* leaves (*comparison between the chemicals*)

Comparative values on the effect of four physical treatments followed by the treatments with water, PP, urea, WA and GNC has been presented in Table 19. Overall reduction in TP, NTP, TT, CT and HT due to all such physico- chemical treatments was 62.31 (18.12-88.10), 30.34 (-60.13 to 85.99), 69.27 (26.03-88.56), 61.08 (7.47-95.14) and 76.03 (41.37-120.62) %, respectively. Reduction due to physical treatments ranged from 37.05 to 84.51, -33.21 to 82.22, 44.38 to 85.01, 34.62 to 92.01 and 52.44 to 89.95 % , respectively, for fresh (water), chopped , CSD and CSDG. Out of four physical treatments reduction of TP, NTP, TT and CT was maximum due to CSDG. Extent of reduction of TT between the treatments CSD and CSDG was comparable. Reduction of HT was maximum in CSD treatment. Values of reduction of these polyphenols among chemicals (water, PP, urea, WA and GNC) ranged from 52.79 to 65.94, 17.96 to 33.95, 60.37 to 76.64, 46.31 to 65.94 and 78.98 to 87.13 %, respectively. Reduction of TP was maximum in PP and urea treatments,

Table 19 : Effect of different physical and chemical treatments on phenolic contents of *F. roxburghii* leaves

Physical Treatment	Chemical Treatment	Poly phenols (%)					Reduction on treatment (%)				
		Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins	Total phenol	Non tannins	Total tannins	Condensed tannins	Hydrolysable tannins
		Chemical Treatments					Chemical Treatments				
Fresh	0.0% Chemical (Water)	5.13^{A} ± 0.03	1.32^{CD} ± 0.01	3.81^{D} ± 0.02	2.06^{A} ± 0.01	1.65^{D} ± 0.02	18.12^{J} ± 0.46	-18.25^{GH} ± 0.59	26.03^{H} ± 0.43	7.47^{K} ± 0.54	41.37^{F} ± 0.87
	Pot. Permn.	2.80^{F} ± 0.06	1.29^{D} ± 0.02	1.51^{IKL} ± 0.04	1.25^{D} ± 0.04	0.26^{KF} ± 0.02	55.35^{B} ± 0.96	-15.18^{G} ± 1.48	70.69^{CD} ± 0.87	46.44^{F} ± 1.51	90.72^{BC} ± 0.66
	Urea	3.09^{EF} ± 0.15	1.23^{D} ± 0.02	1.86^{EF} ± 0.17	1.37^{D} ± 0.06	0.49^{GH} ± 0.14	50.70^{EF} ± 2.39	-9.76^{G} ± 1.59	63.85^{DE} ± 3.24	41.31^{F} ± 2.56	82.47^{CD} ± 5.05
	Wood ash	3.27^{DE} ± 0.17	1.71^{A} ± 0.03	1.56^{JKL} ± 0.20	1.57^{C} ± 1.00	-0.02^{K} ± 0.28	47.91^{FG} ± 2.65	-52.75^{J} ± 2.83	69.80^{CD} ± 3.82	32.51^{G} ± 4.10	100.61^{B} ± 9.93
	GNC	3.89^{BC} ± 0.16	1.79^{A} ± 0.03	2.10^{B} ± 0.13	1.25^{D} ± 0.03	0.85^{CD} ± 0.16	37.95^{IH} ± 2.51	-60.13^{J} ± 2.72	59.27^{G} ± 2.50	46.25^{F} ± 1.29	70.03^{DE} ± 5.58
Chopped	0.0% Chemical (Water)	2.72^{F} ± 0.02	1.40^{BC} ± 0.01	1.32^{KL} ± 0.02	1.90^{B} ± 0.01	-0.58^{K} ± 0.02	56.65^{B} ± 0.29	-25.09^{IK} ± 0.69	74.41^{C} ± 0.39	18.48^{H} ± 0.22	120.62^{A} ± 0.87
	Pot. Permn.	3.54^{CD} ± 0.18	1.50^{B} ± 0.03	2.05^{B} ± 0.17	1.52^{C} ± 0.02	0.53^{GH} ± 0.17	43.47^{GH} ± 2.92	-33.78^{IJ} ± 2.55	60.27^{G} ± 3.35	34.88^{G} ± 0.76	81.25^{CD} ± 6.00
	Urea	3.54^{CD} ± 0.18	1.07^{E} ± 0.09	2.48^{D} ± 0.13	1.33^{D} ± 0.04	1.14^{C} ± 0.10	43.50^{GH} ± 2.81	4.83^{F} ± 7.66	51.91^{F} ± 2.62	42.77^{F} ± 0.89	59.46^{E} ± 3.48
	Wood ash	5.04^{A} ± 0.21	0.79^{F} ± 0.05	4.25^{A} ± 0.17	1.52^{C} ± 0.04	2.72^{A} ± 0.13	19.69^{J} ± 3.36	29.71^{H} ± 4.37	17.51^{I} ± 3.27	34.62^{G} ± 1.57	3.37^{G} ± 4.73
	GNC	4.07^{B} ± 0.03	0.87^{F} ± 0.04	3.20^{C} ± 0.05	1.59^{C} ± 0.02	1.61^{B} ± 0.06	35.03^{I} ± 0.45	22.20^{H} ± 3.32	37.82^{H} ± 0.90	31.58^{G} ± 0.05	42.98^{F} ± 2.22
Chopped sun dried	0.0% Chemical (Water)	2.30^{G} ± 0.03	0.63^{G} ± 0.01	1.68^{JKL} ± 0.04	0.64^{H} ± 0.001	1.03^{C} ± 0.04	63.24^{D} ± 0.54	44.02^{E} ± 0.57	67.42^{CD} ± 0.71	72.35^{B} ± 0.05	63.35^{E} ± 1.30
	Pot. Permn.	0.98^{IJ} ± 0.06	0.31^{I} ± 0.01	0.67^{MN} ± 0.06	0.45^{I} ± 0.04	0.22^{KF} ± 0.08	84.37^{AB} ± 0.90	72.11^{B} ± 1.05	87.04^{AB} ± 1.15	80.75^{D} ± 1.85	92.23^{BC} ± 2.79
	Urea	0.92^{IJ} ± 0.08	0.47^{H} ± 0.01	0.45^{I} ± 0.08	0.46^{I} ± 0.05	-0.01^{K} ± 0.03	85.30^{AB} ± 1.33	57.87^{C} ± 0.86	91.26^{A} ± 1.54	80.30^{D} ± 2.23	100.32^{B} ± 1.00
	Wood ash	1.22^{I} ± 0.05	0.27^{IJ} ± 0.01	0.95^{M} ± 0.04	0.62^{H} ± 0.03	0.33^{KF} ± 0.01	80.53^{B} ± 0.80	75.89^{AB} ± 1.09	81.53^{B} ± 0.79	73.41^{E} ± 1.46	88.24^{BC} ± 0.40
	GNC	1.00^{IJ} ± 0.03	0.24^{IJ} ± 0.01	0.75^{MN} ± 0.04	0.41^{IK} ± 0.02	0.34^{KF} ± 0.02	84.11^{AB} ± 0.45	78.32^{AB} ± 1.15	85.37^{AB} ± 0.69	82.37^{CD} ± 0.97	87.85^{BC} ± 0.70
Chopped sun dried and grinded	0.0% Chemical (Water)	1.68^{H} ± 0.01	0.32^{I} ± 0.01	1.36^{KL} ± 0.02	0.30^{KH} ± 0.01	1.05^{C} ± 0.02	73.15^{C} ± 0.18	71.06^{B} ± 1.33	73.61^{C} ± 0.38	86.94^{BC} ± 0.22	62.60^{E} ± 0.81
	Pot. Permn.	0.75^{IJ} ± 0.06	0.16^{I} ± 0.02	0.59^{MN} ± 0.06	0.13^{I} ± 0.01	0.44^{KH} ± 0.04	88.10^{A} ± 1.01	85.99^{A} ± 1.67	88.56^{AB} ± 1.09	93.68^{A} ± 0.63	84.33^{CD} ± 1.48
	Urea	0.99^{IJ} ± 0.06	0.22^{IJ} ± 0.02	0.77^{MN} ± 0.06	0.19^{KH} ± 0.01	0.57^{GH} ± 0.05	84.28^{AB} ± 0.92	80.41^{AB} ± 1.47	85.12^{AB} ± 1.14	91.69^{AB} ± 0.48	79.69^{CD} ± 1.81
	Wood ash	0.85^{IJ} ± 0.04	0.19^{IJ} ± 0.01	0.66^{MN} ± 0.04	0.11^{I} ± 0.02	0.55^{GH} ± 0.02	86.38^{AB} ± 0.65	82.95^{A} ± 0.95	87.13^{AB} ± 0.70	95.14^{A} ± 0.67	80.51^{CD} ± 0.76
	GNC	1.06^{IJ} ± 0.05	0.19^{IJ} ± 0.02	0.87^{MN} ± 0.06	0.25^{KH} ± 0.01	0.62^{GH} ± 0.07	83.06^{AB} ± 0.78	83.26^{A} ± 1.53	83.01^{B} ± 1.24	89.24^{AB} ± 0.12	77.86^{CD} ± 2.35

Table 19 (Contd.)

Physical Treatments	Physical Treatments					Physical Treatments				
Fresh	3.41[B] ± 0.11	1.49[A] ± 0.04	1.91[b] ± 0.11	1.42[b] ± 0.04	0.49[b] ± 0.10	45.68[A] ± 1.72	-33.21[A] ± 3.21	62.83[b] ± 2.05	39.00[A] ± 1.89	82.53[b] ± 3.58
Chopped	3.95[A] ± 0.12	1.08[b] ± 0.05	2.86[A] ± 0.14	1.52[A] ± 0.02	1.34[A] ± 0.14	37.05[B] ± 1.93	3.37[A] ± 4.12	44.38[A] ± 2.77	34.62[B] ± 1.06	52.44[A] ± 5.10
Chopped sun dried	1.13[C] ± 0.06	0.35[B] ± 0.02	0.78[C] ± 0.05	0.50[C] ± 0.02	0.28[C] ± 0.04	82.01[b] ± 0.90	68.97[b] ± 1.54	84.85[A] ± 0.98	78.68[b] ± 0.92	**89.95[A] ± 1.44**
Chopped sun dried and grinded	0.97[C] ± 0.04	0.20[C] ± 0.01	0.77[C] ± 0.04	0.19[B] ± 0.01	0.59[b] ± 0.03	84.51[A] ± 0.65	82.22[A] ± 0.83	85.01[A] ± 0.72	92.01[A] ± 0.43	79.21[b] ± 1.06
Chemical Treatments	Chemical Treatments					Chemical Treatments				
0% chemical (water)	2.96[A] ± 0.34	0.91[A] ± 0.12	2.04[A] ± 0.27	1.25[A] ± 0.20	0.79[A] ± 0.21	52.79[A] ± 5.39	17.96[A] ± 10.54	60.37[A] ± 5.17	46.31[A] ± 8.77	71.98[A] ± 7.61
Pot. Permanganate	2.02[A] ± 0.18	0.81[b] ± 0.08	1.20[A] ± 0.10	0.84[A] ± 0.08	0.36[A] ± 0.05	67.82[A] ± 2.88	27.28[b] ± 7.69	76.64[A] ± 1.95	65.94[A] ± 3.57	87.13[A] ± 1.77
Urea	2.14[A] ± 0.18	0.75[A] ± 0.06	1.39[A] ± 0.13	0.84[A] ± 0.08	0.55[b] ± 0.07	65.94[A] ± 2.94	33.34[A] ± 5.74	73.03[b] ± 2.57	64.02[A] ± 3.40	80.48[b] ± 2.63
Wood ash	2.59[A] ± 0.25	0.74[A] ± 0.09	1.85[b] ± 0.22	0.96[b] ± 0.09	0.90[A] ± 0.17	58.63[b] ± 4.06	33.95[A] ± 7.99	63.99[A] ± 4.21	58.92[b] ± 4.03	68.18[A] ± 6.16
GNC	2.51[b] ± 0.22	0.77[A] ± 0.10	1.73[b] ± 0.15	0.88[C] ± 0.08	0.85[A] ± 0.08	60.03[b] ± 3.50	30.91[b] ± 8.50	66.36[A] ± 2.92	62.36[A] ± 3.55	69.68[A] ± 2.89
Overall mean ± SE	2.36 ± 0.10	0.78 ± 0.04	1.58 ± 0.08	0.91 ± 0.04	0.68 ± 0.05	62.31 ± 1.63	30.34 ± 3.56	69.27 ± 1.49	61.08 ± 1.82	76.03 ± 1.89
PhTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PhTreat*ChTreat	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values bearing the different superscripts (A, B, C etc) differ significantly.

Total phenol, non-tannin phenol, total tannin phenol, condensed tannin and the hydrolysable tannin content (% DM basis) of fresh *F.roxburghii* leaves were 6.27 ± 0.17, 1.12± 0.02, 5.15± 0.17, 2.33 ± 0.01 and 2.82 ± 0.17, respectively.

Ph Treat: Physical treatment; Ch Treat: Chemical treatment

NTP in urea and WA treatment, CT in PP, urea and GNC treatments, difference among the groups being comparable. Reduction of TT and HT was also maximum in PP treatments.

PHASE II

4.2 STUDIES ON THE EFFECT OF DIFFERENT PHYSICAL ALONG WITH UREA TREATMENTS OF *F. ROXBURGHII* LEAVES ON *IN VITRO* DM AND OM DEGRADABILITY, AND DE AND ME VALUES

TP (Total Phenols) and TT (Total tannins) content of fresh *Q. leucotricophora* leaves was 7.26 and 6.15 percent respectively (Table 8), where as in fresh *F. roxburghii* leaves, these polyphenols were 6.27 and 5.15% respectively (Table 14). Moreover, out of all treatment combinations, reduction of TT in *F. roxburghii* was maximum (96.76%) through 0.5% urea treated CSD leaves. F. roxburghii leaves contained lower TT as compared to *Q. leucotrichophora* and also the urea treatment was most effective in reducing TT and also economical and user friendly, urea treatment of *F. roxburgii* leaves was selected for *in vitro* studies to estimate the *in vitro* DMD%, DOM%, ME and DE values.

4.2.1 Chemical composition of processed *F. roxburghii* (tremal) leaves

Chemical composition of different types of processed tremal leaves with respect to OM, CP, EE, TCHO (Total carbohydrates), Total ash, Ca and P has been presented in Table 20.

OM, CP, EE, TCHO and ash contents of tremal hay was 87.43, 13.92, 4.18, 69.33 and 12.57% respectively, where as the concentration of these proximate priciples in urea treated CSD tremal hay was 85.38, 14.12, 3.99, 67.27 and 14.62 in 0.5% treatment (UT); 85.13, 14.20, 4.01, 66.92 and 14.87 in 1.5% UT and 84.45, 14.23, 4.40, 65.82 and 15.55 in 2.5% UT. Fibre fraction of untreated tremal

Table 20 : Proximate and mineral composition of detannified *F. roxburghii* (tremal) leaves (on % DM basis)

Attributes	Control (UTH)	Group 1 (CSD 0.5% UTTH)	Group 2 (CSD 1.5% UTTH)	Group 3 (CSD 2.5% UTTH)
Proximate Composition				
OM	87.43	85.38	85.13	84.45
CP	13.92	14.12	14.20	14.23
EE	4.18	3.99	4.01	4.40
TCHO	69.33	67.27	66.92	65.82
Ash	12.57	14.62	14.87	15.55
Fibre fractions				
NDF	61.88	60.00	58.31	57.99
ADF	57.08	56.56	56.27	53.71
Minerals				
Calcium	2.59	2.41	2.39	2.33
Phosphorous	0.27	0.22	0.24	0.22

[Untreated *Tremal* hay (UTH), chopped sundried 0.5% Urea treated *Tremal* hay (CSD 0.5% UTTH), chopped sundried 1.5% Urea treated *Tremal* hay (CSD 0.5% UTTH) and chopped sundried 2.5% Urea treated *Tremal* hay (CSD 0.5% UTTH) represents control, 1, 2 and 3 groups respectively].

Table 21 : Effect of detannified tremal leaves on *In vitro* digestibility and gas production parameters

Parameters	Control (UTH)	Group 1 (CSD 0.5% UTTH)	Group 2 (CSD 1.5% UTTH)	Group 3 (CSD 2.5% UTTH)	Overall mean ± SE	P value
Net gas (ml) / 200mg sample	22.17^{B} ± 0. 60	26.33^{a} ± 0.67	14.33^{c} ± 0.44	16.00^{c} ± 0.58	19.71 ± 1.47	< 0.0001
Ammonia N mg / 30ml	3.41^{d} ± 0.04	3.52^{c} ± 0.04	4.06^{b} ± 0.01	4.78^{a} ± 0.02	3.94 ± 0.16	< 0.0001
Methane %	13.37^{b} ± 2.89	21.50^{a} ± 0.72	22.41^{a} ± 0.85	25.62^{a} ± 1.48	20.72 ± 1.55	0.0056
Methane (ml)/ 200mg sample	2.98^{b} ± 0.70	5.65^{a} ± 0.06	3.21^{b} ± 0.15	4.08^{b} ± 0.10	3.98 ± 0.35	0.003
Methane (ml)/g of substrate	14.90^{b} ± 3.48	28.26^{a} ± 0.28	16.05^{b} ± 0.75	20.42^{b} ± 0.51	19.91 ± 1.76	0.003
Protozoa($\times 10^{4}$) / ml of sample	2.07^{a} ± 0.20	0.89^{b} ± 0.13	0.70^{b} ± 0.16	0.89^{b} ± 0.06	1.14 ± 0.18	0.0006
IVDMTD %	70.69^{a} ± 0.37	69.24^{a} ± 0.51	63.52^{b} ± 0.93	58.58^{c} ± 1.34	65.51 ± 1.50	< 0.0001

Values bearing the different superscripts (A, B, C etc) differ significantly.

hay was 61.88 and 57.08 for NDF and ADF; whereas these were 60.0 and 56.56% in 0.5% urea treated CSDTH; 58.31 and 56.27 in 1.5% urea treated CSDTH; and 57.99 and 53.71in 2.5% urea treated CSDTH. Calcium and P content of untreated TH was 2.59 and 0.27%; whereas concentration of these minerals was gradually reduced to 2.41 and 0.22% in 0.5% urea treated CSDTH, 2.39 and 0.24% in 1.5% urea treated CSDTH, and 2.33 and 0.22% in 2.5% urea treated CSDTH.

4.2.2 Effect of processing of tremal leaves on methane and ammonia production and on *in vitro* degradability

Effect of processing tremal leaves on total gas, methane and ammonia production, and on *in vitro* degradability has been presented in Table 21. Total (net) gas production was significantly (P <0.0001) more (26.33ml) in 0.5% urea treated tremal hay (TH); followed by untreated TH, 2.5% urea treated CSDTH and 1.5% urea treated CSDTH; differences between the 1.5% and 2.5% urea treatment being non significant. However, NH_3-N (mg/30ml) was obviously more (P <0.0001) in 2.5% urea treated CSDTH followed by 1.5% and 0.5% urea treated CSDTH and was minimum (lowest) (P <0.0001) in untreated TH (control).

Methane percentage in total gas was, however, significantly higher (P=0.003) in 2.5% urea treated CSDTH, followed by 1.5 and 0.5% urea treated CSDTH, differences being non significant between the urea treated TH. It was significantly (P=0.003) less in control, untreated TH. Net methane production (ml/ 200mg of substrate) was higher (P = 0.003) in 0.5% urea treated CSDTH, followed by 2.5% and 1.5% urea treated CSDTH, and was lowest in control; difference between control, 1.5 and 2.5% urea treated CSDTH was not significant. Protozoal count was significantly (P<0.0006) higher in control hay than that of urea treated hay. Difference in protozoal counts among the urea treated TH became non significant. It was revealed that the *in vitro* true degradability of

Table 22 : Molar proportion of volatile fatty acids of processed tremal leaves

Parameters	Control (UTH)	Group 1 (CSD 0.5% UTTH)	Group 2 (CSD 1.5% UTTH)	Group 3 (CSD 2.5% UTTH)	Overall mean ± SE	P value
Acetic Acid	$70.66^{b} \pm 1.78$	$77.27^{a} \pm 2.09$	$74.55^{ab} \pm 1.07$	$71.21^{b} \pm 0.78$	73.42 ± 1.04	0.049
Propionic Acid	21.79 ± 2.29	21.93 ± 2.09	19.31 ± 0.88	21.60 ± 0.43	21.16 ± 0.77	0.645
Butyric Acid	7.55 ± 0.67	7.50 ± 0.01	6.14 ± 0.41	7.19 ± 0.42	5.42 ± 0.84	0.649
A:P Ratio	3.34 ± 0.48	3.52 ± 0.48	3.88 ± 0.22	3.30 ± 0.10	3.54 ± 0.17	0.658

Table 23 : Digestible organic matter (DOM), metabolisable energy and digestible energy (DE) of detannified tremal leaves

Parameters	Control (UTH)	Group 1 (CSD 0.5% UTTH)	Group 2 (CSD 1.5% UTTH)	Group 3 (CSD 2.5% UTTH)	Overall mean ± SE	P value
DOM %	$51.91^{B} \pm 0.53$	$54.30^{a} \pm 0.59$	$43.61^{c} \pm 0.31$	$46.92^{c} \pm 0.51$	48.69 ± 1.39	< 0.0001
ME(MJ/kg)*	$6.06^{b} \pm 0.08$	$6.63^{a} \pm 0.09$	$5.01^{c} \pm 0.06$	$5.24^{c} \pm 0.08$	5.74 ± 0.20	< 0.0001
DE (Mcal/kg)*	$1.77^{b} \pm 0.02$	$1.93^{a} \pm 0.03$	$1.46^{c} \pm 0.02$	$1.53^{c} \pm 0.02$	1.67 ± 0.06	< 0.0001
ME(MJ/kg)+	$5.28^{b} \pm 0.07$	$5.78^{a} \pm 0.08$	$4.33^{c} \pm 0.05$	$4.54^{c} \pm 0.07$	4.98 ± 0.18	< 0.0001
DE (Mcal/kg)+	$1.54^{b} \pm 0.02$	$1.68^{a} \pm 0.02$	$1.26^{c} \pm 0.02$	$1.32^{c} \pm 0.02$	1.45 ± 1.05	< 0.0001

Values bearing the different superscripts (A, B, C etc) differ significantly.

* Calculated by formula of Steingass and Menke (1986)

+ Calculated by formula of Krishmoortthy *et al* (1995)

DM (IVDMTD %) was significantly higher (P<0.0001) in control hay, followed by 0.5, 1.5 and 2.5% urea treated CSDTH. Differences between control hay and 0.5% urea treated TH was not significant.

4.2.3 Effect of processing of tremal leaves on VFAs

Molar proportion of VFAs of processed tremal leaves has been presented in Table 22. Overall proportion of acetic, propionic and butyric acid was 73.42, 21.16 and 5.42%, respectively. On comparison between the processed leaves, it was revealed that, the proportion of acetic acid was higher (P<0.05) in 0.5% urea treated CSDTH, followed by 1.5 and 2.5% urea treated TH; acetic acid was minimum in untreated TH (control). However, the difference between UTH, 1.5 and 2.5% urea treated CSDTH were nonsignificant; differences between 0.5 and 1.5% urea treated CSDTH were also found to be non significant. No significant differences were also observed for propionic acid and butyric acid, and also for A: P ratio among the processed TH.

4.2.4 DE and ME value for processed tremal hay

Digestible OM, DE and ME value of processed TH has been presented in Table 23. DOM(%), DE and ME values were significantly higher in 0.5% urea treated CSDTH, followed by un- treated TH (control), 1.5 and 2.5% urea treated CSDTH; difference between the 1.5 and 2.5% urea treatment was found to be non significant. Respective values for DOM% were 51.91, 54.30, 43.61 and 46.92% in untreated TH (control), 0.5, 1.5 and 2.5% urea treated CSDTH.

PHASE III

4.3 STUDIES ON THE PERFORMANCE OF GADDI GOATS FED ON BIUL AND TREMAL HAY BASED DIETS

4.3.1 Chemical composition of feeds

Proximate composition, fibre fraction, polyphenols, Ca, and P content of fresh leaves, overnight water soaked leaves and dried leaves have been presented in Table 24.

Table 24 : Chemical compositions of tree leave and concentrate mixture offered during metabolism trial (on % DM basis)

Attributes	Biul hay	Tremal hay	Chopped sun dried and 0.5% urea treated tremal hay	Concentrate Mixture
Proximate Composition				
OM	87.86±0.43	88.10±0.49	88.71±0.20	91.03±0.20
CP	19.44±0.17	12.81±0.17	15.01±0.41	23.35±0.49
EE	4.10±0.07	3.37±0.08	3.64±0.11	1.91±0.11
TCHO	64.33±0.65	71.92±0.72	70.07±0.40	65.77±0.29
Cell wall constituents				
NDF	39.83±0.61	60.89±0.57	61.25±0.65	18.07±0.12
ADF	33.62±0.34	55.04±1.23	54.52±1.18	12.83±0.14
Cellulose	24.40±0.40	37.73±0.30	37.09±0.34	8.92±0.18
Hemi cellulose	6.22±0.91	5.85±0.72	6.73±0.61	5.24±0.18
Acid detergent Lignin	9.22±0.08	17.34±1.03	17.43±0.90	3.90±0.13
Minerals				
Ash	12.14±0.43	11.90±0.49	11.29±0.20	8.97±0.20
Calcium	3.24±0.09	2.57±0.03	2.48±0.06	1.68±0.19
Phosphorous	0.26±0.01	0.24±0.01	0.24±0.01	0.81±0.03

OM, CP, EE, TCHO and total ash content of biul hay was 87.86, 19.44, 4.10, 64.33 and 12.4%, respectively; in TH these were 88.1, 12.81. 3.37, 71.92 and 14.90% respectively; and in urea treated chaffed TH 88.71, 15.01, 3.64, 70.07 and 11.29%, respectively. In concentrate mixture, these proximate compositions were 91.03, 23.35, 1.91, 65.77 and 8.97%, respectively.

Among the fibre fractions, NDF, ADF, cellulose and hemicelluloses and ADL content was 39.83, 33.62, 24.40, 6.22 and 9.22% in biul hay; 60.89, 55.04, 37.73, 5.85 and 17.34 in untreated TH; 61.25, 54.52, 37.09, 6.73 and 17.43% in 0.5% urea treated CSDTH; and 18.07, 12.83, 8.92, 5.24 and 3.90% respectively, in concentrate mixture. Ca and P content was 3.24 and 0.26 in biul hay, 2.57 and 0.24% in untreated TH, 2.48 and 0.24% in 0.5% urea treated CSDTH and 1.68 and 0.81 percent in concentrate mixture respectively.

Polyphenols; TT, CT and HT content was 0.39, 0.29 and 0.01% in biul; 5.17, 2.38, and 2.79 in untreated TH; 0.04, 0.03 and 0.01 in 0.5% urea treated CSDTH.

4.3.2 Performance of growing gaddi goats during initial phase of adaptation

Overall performance of goats with respect of body weight, feed intake, feed: gain etc has been presented in Table 27. Feeding trial was initiated at 18.82 ± 1.08 kg body weight. The body weight of gaddi goats went on reducing till 21st day feeding trial (Table 26); the attainment of body weight at 3rd week in respective groups was 18.35, 18.08 and 18.47 kg in control (Biul) (Gr 1), tremal hay (Gr 2) and 0.5 % urea TCSDTH (Gr 3). Live weights, reduction in live weights (- 0.77, - 0.84, and -0.34), feed: gain (-0.21, -16.10 and -36.56) or DM: gain (-0.20, -15.69 and -35.42), did not differ significantly among the groups. Total feed/ DMI (kg/d) was significantly ($P < 0.02$) higher in control

Table 25 : Weekly changes in total DMI (kg/d)

Week	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal hay)	Overall mean ± SE	P Value
1st week	$0.634^{B} \pm 0.21$	$0.684^{a} \pm 0.10$	$0.641^{ab} \pm 0.90$	0.653 ± 0.58	0.064
2nd week	$0.667^{a} \pm 0.16$	$0.666^{a} \pm 0.07$	$0.606^{b} \pm 0.01$	0.646 ± 0.06	0.008
3rd week	$0.689^{a} \pm 0.80$	$0.667^{b} \pm 0.16$	$0.645^{ab} \pm 0.72$	0.667 ± 0.80	0.099
4th week	$0.699^{a} \pm 0.20$	$0.676^{b} \pm 0.01$	$0.692^{c} \pm 0.20$	0.689 ± 0.30	< 0.0001
5th week	0.720 ± 0.07	0.711 ± 0.01	0.725 ± 0.60	0.718 ± 0.04	0.358
6th week	$0.726^{b} \pm 0.04$	$0.721^{b} \pm 0.30$	$0.740^{a} \pm 0.30$	0.729 ± 0.36	0.008
7th week	$0.785^{a} \pm 0.30$	$0.773^{b} \pm 0.30$	$0.780^{ab} \pm 0.004$	0.779 ± 0.28	0.086
8th week	0.792 ± 0.20	0.792 ± 0.30	0.790 ± 0.004	0.791± 0.02	0.813
9th week	0.772 ± 0.18	0.785 ± 0.11	0.745 ± 0.001	0.767 ± 0.08	0.121
10th week	0.777 ± 0.60	0.773 ± 0.14	0.767 ± 0.15	0.772 ± 0.78	0.852
11th week	$0.765^{a} \pm 0.30$	$0.696^{b} \pm 0.80$	$0.693^{b} \pm 0.08$	0.718 ± 0.11	< 0.0001
12th week	$0.826^{a} \pm 0.80$	$0.699^{b} \pm 0.30$	$0.768^{c} \pm 0.002$	0.764 ± 0.16	< 0.0001
13th week	$0.834^{a} \pm 0.70$	$0.722^{b} \pm 0.90$	$0.796^{c} \pm 0.005$	0.784 ± 0.15	< 0.0001

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 26 : Weekly changes in body weight (kg)

Week	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD trema hayl)	Overall mean ± SE	P value
0th week	18.72 ± 2.25	18.91 ± 1.90	18.81 ± 2.04	**18.82 ± 1.08**	0.998
1st week	17.87 ± 1.98	18.70 ± 2.03	18.23 ± 2.00	**18.26 ± 1.05**	0.958
2nd week	18.00 ± 2.24	18.18 ± 1.90	18.00 ± 1.99	**18.05 ± 1.07**	0.998
3rd week	18.35 ± 2.30	18.07 ± 1.91	18.47 ± 2.01	**18.30 ± 1.08**	0.991
4th week	19.25 ± 2.14	19.17 ± 1.87	18.83 ± 2.02	**19.08 ± 1.05**	0.988
5th week	19.48 ± 2.13	19.40 ± 1.90	18.93 ± 1.98	**19.27 ± 1.05**	0.979
6th week	19.73 ± 2.15	19.74 ± 1.88	19.23 ± 1.94	**19.58 ± 1.04**	0.979
7th week	19.94 ± 2.14	19.81 ± 1.87	19.41 ± 1.93	**19.72 ± 1.03**	0.981
8th week	20.16 ± 2.15	20.05 ± 1.87	19.42 ± 1.68	**19.87 ± 1.00**	0.958
9th week	20.52 ± 2.17	20.24 ± 1.87	19.58 ± 1.66	**20.11 ± 1.00**	0.938
10th week	20.79 ± 2.23	20.48 ± 1.87	19.83 ± 1.67	**20.36 ± 1.02**	0.938
11th week	21.06 ± 2.29	20.58 ± 1.84	19.95 ± 1.65	**20.53 ± 1.03**	0.923
12th week	21.27 ± 2.34	20.64 ± 1.82	20.00 ± 1.64	**20.63 ± 1.04**	0.901
13th week	21.55 ± 2.40	20.76 ± 1.76	20.08± 1.66	**20.80 ± 1.05**	0.872

Table 27 : Performance of growing goats during adaptation period of 3 weeks .

Parameters	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
Initial body wt.(kg)	18.72 ± 2.26	18.92 ± 1.91	18.81 ± 2.04	**18.82 ± 1.08**	0.998
Final body wt.(kg)	18.35 ± 2.30	18.08 ± 1.92	18.47 ± 2.01	**18.30 ± 1.09**	0.991
Total body wt. gain (kg)	-0.37 ± 0.23	-0.84 ± 0.09	-0.34 ± 0.16	**-0.52 ± 0.11**	0.118
Average daily gain(g)	-33.57 ± 17.47	-44.36 ± 4.27	-25.50 ± 11.15	**-35.14 ± 6.72**	0.629
Total feed intake (kg/d)	0.70^{A} ± 0.01	0.69^{a} ± 0.09	0.65^{b} ± 0.01	**0.68 ± 0.01**	0.020
Total DMI (kg/d)	0.67^{a} ± 0.01	0.67^{a} ± 0.01	0.63^{b} ± 0.01	**0.66 ± 0.07**	0.022
Feed : Gain Ratio	-0.21 ± 14.67	-16.10 ± 2.00	-36.56 ± 12.19	**-17.62 ± 7.32**	0.120
DMI : Gain Ratio	-0.20 ± 14.11	-15.69 ± 1.95	-35.42 ± 11.81	**-17.10 ± 7.07**	0.118
CPI : Gain Ratio	0.01 ± 0.27	-0.20 ± 0.02	-0.55 ± 0.18	**-0.25 ± 0.12**	0.176

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 28 : Performance of growing goats during feeding trial after adaptation period (4th to 13th week; 70days)

Parameters	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
Initial body wt.(kg)	18.35 ± 2.30	18.08 ± 1.91	18.47 ± 2.09	**18.30 ± 1.09**	0.991
Final body wt.(kg)	21.55 ± 2.41	20.76 ± 1.76	20.33 ± 1.90	**20.88 ± 1.08**	0.913
Total body wt. gain (kg)	3.20^{A} ± 0.31	2.69^{a} ± 0.20	1.86^{b} ± 0.12	**2.58 ± 0.20**	0.007
Average daily gain(g)	43.48^{a} ± 4.57	32.99^{b} ± 1.35	27.49^{b} ± 0.54	**34.66 ± 2.48**	0.009
Total feed intake (kg/d)	0.80^{a} ± 0.01	0.76^{c} ± 0.01	0.77^{b} ± 0.01	**0.78 ± 0.01**	< 0.0001
Total DMI (kg/d)	0.77^{a} ± 0.01	0.74^{c} ± 0.01	0.75^{b} ± 0.01	**0.75 ± 0.01**	< 0.0001
Feed : Gain Ratio	19.02^{b} ± 1.94	23.00^{b} ± 0.87	28.27^{a} ± 0.94	**23.43 ± 1.34**	0.003
DMI : Gain Ratio	18.30^{b} ± 1.87	22.42^{b} ± 0.85	27.39^{a} ± 0.91	**22.70 ± 1.31**	0.003
CPI : Gain Ratio	0.36^{ab} ± 0.04	0.28^{b} ± 0.01	0.43^{a} ± 0.01	**0.36 ± 0.02**	0.006

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

(0.70) kg followed by the goats fed on TH based diets (Gr 2) and 0.5 % urea treated CSDTH (Gr 3). DMI / feed intake between the goats in control and Gr II was comparable (similar). Urea treatment could not increase the palatability of TH in any of the three weeks (Table 25). CPI: gain were 0.01, -0.02 and -0.55 in control, tremal hay (Gr 2) and 0.5 % urea TCSDTH (Gr3) respectively, differences between the values in respective groups being non significant.

4.3.3 Performance of growing gaddi goats during feeding trial after adaptation period

Data on performance of goats have been presented in Table 25, 26 and 28. Weekly body weight of the goats went on increasing from 3rd week onwards (Table 25) in group 1 (control) and 3 (0.5 % urea treated CSDTH); and in 2nd group body weight started to increase from 4th week onwards. As the feeding trial progressed, the live weight increased with the increase of age and at the end of feeding trial (at 13th week) the goats in respective groups attained body weight of 21.55, 20.76 and 20.08 kg in control (biul hay), Gr 2 (TH) and Gr 3 (0.5 % urea treated CSDTH), respectively. Values of body weights did not differ significantly in any of the weeks. However, feed intake of goats in respective groups differ significantly in 4th, 6th, 11th, 12th and 13th weeks (Table 26). During feeding trial in most of the weeks feed intake was higher in goats fed on biul hay based diets and feed intake was less on feeding urea treated hay (Gr 3). Overall performance has been presented in Table 28. At the end of feeding trial at 13th week attainment of body weight was 21.55, 20.76 and 20.33 kg in gr 1, gr 2 and gr. 3. Total gain in body weight was significantly higher ($P < 0.007$) in Gr 1 (3.20 kg) and 2 (2.69 kg) than that of the goats in group 3 (1.86 kg). However, average daily gain was comparable between the goats in group 2 (32.99 g) and 3 (27.49). It was significantly higher (P, 0.0001) in goats fed on hay

based diets (Gr 1), followed by the goats in Gr 2 fed TH and 3 fed urea treated TH. Total DMI of the goats in respective groups was 0.77, 0.74 and 0.75 kg in groups 1, 2 and 3. The values differ significantly (P < 0. 0001) among the groups.

Feed: gain and DMI: gain was significantly lower (P < 0.003) in control (19.02), followed by TH fed group (23.00) and was higher (P < 0.003) in goats (28.27) fed urea treated TH (Gr 3). DMI: gain ratio also followed the same trend. Feed/ DMI: gain ratio between the goats of control and tremal hay fed group did not differ significantly. However, the CPI: gain ratio differed significantly (P< 0.006) between Gr 2 (0.28) and 3 (0.43); but difference between Gr 1 (0.36) and 2; and between Gr 2 and 3 were not significant.

4.3.4 Digestibility of nutrients and nutritive value of the diets of goats

Digestibility of nutrients has been presented in Table 29. Digestibility of DM of biul hay (Gr 1), TH (Gr 2) and urea treated CSDTH (Gr 3) based dites was 69.76, 63.76 and 68.56 %; it was 78.39, 73.30 and 77.18 for OM; 68.03, 65.14 and 67.35 for CP; 81.17, 86.18 , 89.16 for EE; and 75.87, 72.31 and 75.48 for TCHO in Gr 1, 2 and 3, respectively. Values of DM and OM digestibilities of diets were significantly (P < 0.01) higher in group 1 and 3 than that in group 2, values of DM and OM digestibilities of biul and urea treated CSDT hay based diets were comparable. Digestibilities of CP, EE and TCHO of respective diets did not differ significantly.

However, NDF digestibility was significantly (P < 0.01) higher in biul hay based diets (64.61%) followed by the diets based on urea treated CSDTH (63.99) and was lower (P < 0.01) in TH based diets (62.60).

Table 29 : Digestibility (%) of different nutrients and fibre fractions during metabolism trial

Parameters	Group1 (Bisl)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
DM	69.76^{A} ± 1.01	63.76^{b} ± 1.55	68.56^{a} ± 0.66	**67.36 ± 0.98**	0.011
OM	78.39^{a} ± 1.01	73.30^{b} ± 1.09	77.18^{a} ± 0.71	**76.29 ± 0.82**	0.011
CP	68.03 ± 0.74	65.14 ± 3.35	67.35 ± 0.53	**66.84 ± 1.57**	0.771
EE	81.17 ± 2.59	86.18 ± 3.55	89.16 ± 4.13	**85.50 ± 2.70**	0.079
TC	75.87^{a} ± 1.12	72.31^{b} ± 1.17	75.48^{ab} ± 0.88	**74.56 ± 0.73**	0.081
NDF	64.61^{a} ± 4.39	62.60^{c} ± 1.66	63.99^{b} ± 0.70	**92.99 ± 2.38**	0.010
ADF	61.14 ± 1.06	60.74 ± 1.49	61.70 ± 0.95	**61.79 ± 1.36**	0.090
Cellulose	46.17 ± 2.59	45.12 ± 3.55	47.07 ± 4.13	**44.12 ± 2.36**	0.029
Hemicellulose	71.29 ± 1.79	67.47 ± 1.21	69.52 ± 0.51	**69.43 ± 0.82**	0.163

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 30 : Average body weight and daily intake of various nutrients during metabolism trial under different treatments

Parameters	Group1 (Bisl)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
Body weight (kg)	20.76 ± 2.15	20.56 ± 1.74	19.89 ± 1.58	**20.40 ± 0.97**	0.941
Body size (kg $W^{0.75}$)	9.70 ± 0.76	9.63 ± 0.62	9.40 ± 0.56	**9.58 ± 0.34**	0.945
Dry matter intake (g/d)	703.52 ± 48.72	725.25 ± 51.39	729.47 ± 35.08	**719.41 ± 24.07**	0.912
Dry matter intake (g/kg $W^{0.75}$/d)	72.83 ± 2.07	75.26 ± 1.94	77.76 ± 1.57	**75.28 ± 1.15**	0.231
Organic matter intake (g/d)	486.69 ± 30.22	470.15 ± 27.90	501.59 ± 19.21	**486.18 ± 14.23**	0.706
Organic matter intake (g/kg $W^{0.75}$/d)	50.47^{AB} ± 1.62	48.86^{b} ± 0.73	53.57^{a} ± 1.31	**50.97 ± 0.89**	0.025
CP intake g/d	138.43^{a} ± 9.65	98.77^{b} ± 7.11	120.40^{ab} ± 5.70	**119.20 ± 6.32**	0.016
CP intake g/d/kg $W^{0.75}$	14.33^{a} ± 0.37	10.24^{c} ± 0.21	12.84^{b} ± 0.25	**12.47 ± 0.53**	< 0.0001
EE intake g/d	11.17^{a} ± 0.74	6.06^{b} ± 0.95	9.87^{a} ± 1.05	**9.04 ± 0.81**	0.009
EE intake g/d/kg $W^{0.75}$	1.17^{a} ± 0.09	0.62^{b} ± 0.07	1.05^{a} ± 0.08	**0.95 ± 0.08**	0.003
Total carbohydrate intake g/d	340.64 ± 25.29	377.02 ± 21.58	382.81 ± 1.12	**366.57 ± 12.20**	0.351
Total carbohydrate intake g/d/kg $W^{0.75}$	35.22^{b} ± 0.90	39.20^{a} ± 0.59	40.81^{a} ± 1.12	**38.41 ± 0.85**	0.005
TDN intake g/d	463.34 ± 33.18	452.47 ± 28.00	483.12 ± 19.53	**466.31 ± 14.84**	0.734
TDN intake g/d/kg $W^{0.75}$	47.93^{b} ± 1.20	46.99^{b} ± 0.44	51.56^{a} ± 1.10	**48.83 ± 0.78**	0.021

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Digestibility of ADF, cellulose and hemicelluloses did not differ among the groups, and it ranged from 60.74 to 61.70, 45.12 to 47.07 and 69.52 to 71.29%, respectively.

Nutritive values of the diets in respective group have been presented in Table 30. Urea treatment of CSDTH increased (P< 0.015) TDN value of diet in group 3 (66.30) than that of the diet based on untreated TH in group 2 (62.53). However, TDN value of biul hay based control diet in group 1 (65.83%) did not differ significantly for that of urea treated CSDTH in Gr 3.

DCP values of respective diets in Gr 1, 2 and 3 were 13.26, 8.28 and 10.54% respectively. It was significantly (P< 0.02) higher in biul hay based diet, followed by urea treated CSDTH and was lowest in Gr 2 of diet based on tremal hay (TH).

4.3.5 Nutrient intake and plane of nutrition of goats during metabolism trials

Body weight and DMI (g/d, g/kg $W^{0.75}$ and % body weight) of goats during metabolism trial did not differ significantly between the groups. Body weight ranges between 19.89 to 20.76 kg and DMI ranges from 704 to 729 g/d; 72.83 to 77.76 g/$W^{0.75}$/d; and 3.43 to 3.70%; TDN intake (g/d) also did not differ between the groups, it ranged from 452 to 483 g/d; but on expression of its intake per kg metabolic body size, it was significantly (P< 0.021) higher in Gr 3 (51.56) than that in group 1 (47.93) and 2 (46.99) differences being non significant between the other two groups.

CP and DCP intake (g/d, g/kg $W^{0.75}$/d) of goats differ significantly between the groups. CP and DCP intake (g/d) was higher (P< 0.016) in Gr 1(138 and 94 g/d) followed by the group 3 (120 and 77 g/d) and was lowest in group 2 (99 and 60 g/d); differences between group 1

and 3 being nonsignificant. CP and DCP intake (g/kg $W^{0.75}$/d), was highest (P < 0.0001) in group 1 (14.33, 9.64), followed by group 3 (12.84, 8.20) and was lowest in group 2 (10.24, 6.22).

4.3.6 Nitrogen balance of goats during metabolism trial

Nitrogen balance of goats fed on different diets has been presented in Table 32.

N- Intake (g/d) of goats was 22.15, 15.80 and 19.16 in group 1, 2, and 3, respectively. It was significantly higher (P< 0.016) in group 1 and 3 than that in group 2. Outgo of N (g/d) was 6.97, 5.52 and 6.28 through faeces and 3.99, 2.38 and 1.96 g/d through urine in groups 1, 2 and 3, differences among the groups being non significant. However, the balance of N was significantly higher (P< 0.001) in group 1 (11.19 g/d) followed by group 3 (11.02 g/d) and was lowest in group 2 (7.90 g/d). Values of N- balances as % intake and absorbed ranged between 50.00 to 57.22 and 96.28 to 97.83%; the difference between the groups being non significant. N- balance when expressed as gain per kg metabolic size was comparable between Gr 1 (1.15) and 3 (1.17), but lower (P< 0.005) in group 2 (0.82).

4.3.7 Calcium and phosphorous balances of goats during metabolic trial

Data on Ca and P intake, out go and balance have been presented in Table 33.

Ca intake (g/d) and balance (g/d) was slightly higher (P < 0.044) in goats fed biul hay based diets; differences between the goats fed on TH and urea treated CSDTH based diets being non significant. Values of Ca outgo through faeces (g/d) and urine (g/d) did not differ significantly between the groups.

Table 31 : Nutritive value of diet (%, DM basis) during metabolism trial

Parameters	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
TDN	$65.83^{A} \pm 0.25$	$62.53^{b} \pm 1.20$	$66.30^{a} \pm 0.57$	**64.89 ± 0.65**	0.015
DCP	$13.26^{a} \pm 0.73$	$8.28^{c} \pm 0.43$	$10.54^{b} \pm 0.08$	**10.69 ± 0.66**	0.020

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 32 : Average plane of nutrition during metabolism trial

Parameters	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
Body weight (kg)	20.76 ± 2.15	20.56 ± 1.74	19.89 ± 1.58	**20.40 ± 0.97**	0.941
Body size (kg $W^{0.75}$)	9.70 ± 0.76	9.63 ± 0.62	9.40 ± 0.56	**9.58 ± 0.34**	0.945
Dry matter intake (g/d)	703.52 ± 48.72	725.25 ± 51.39	729.47 ± 35.08	**719.41 ± 24.07**	0.912
Dry matter intake (g/kg $W^{0.75}$/d)	72.83 ± 2.07	75.26 ± 1.94	77.76 ± 1.57	**75.28 ± 1.15**	0.231
DMI % of body wt.	3.43 ± 0.17	3.55 ± 0.12	3.70 ± 0.13	**3.56 ± 0.08**	0.467
CP intake g/d	$138.43^{A} \pm 9.65$	$98.77^{b} \pm 7.11$	$120.40^{ab} \pm 5.70$	**119.20 ± 6.32**	0.016
CP intake g/d/kg $W^{0.75}$	$14.33^{a} \pm 0.37$	$10.24^{c} \pm 0.21$	$12.84^{b} \pm 0.25$	**12.47 ± 0.53**	< 0.0001
DCP intake g/d	$93.99^{a} \pm 10.79$	$59.96^{b} \pm 5.03$	$76.99^{ab} \pm 4.17$	**76.98 ± 5.66**	0.028
DCP intake g/d/kg $W^{0.75}$	$9.64^{a} \pm 0.52$	$6.22^{c} \pm 0.27$	$8.20^{b} \pm 0.18$	**8.02 ± 0.46**	0.0003
TDN intake g/d	463.34 ± 33.12	452.47 ± 28.00	483.12 ± 19.53	**466.31 ± 14.84**	0.734
TDN intake g/d/kg $W^{0.75}$	$47.93^{b} \pm 1.20$	$46.99^{b} \pm 0.44$	$51.56^{a} \pm 1.10$	**48.83 ± 0.78**	0.021

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 33 : Nitrogen balance during metabolism trial

Parameters	Group1	Group2	Group3	Overall mean ± SE	P value
Nitrogen intake(g/d)	$22.15^{A} \pm 1.54$	$15.80^{b} \pm 1.14$	$19.26^{ab} \pm 0.91$	**19.07 ± 1.01**	0.016
Faecal nitrogen (g/d)	6.97 ± 0.70	5.52 ± 0.71	6.28 ± 0.24	**6.26 ± 0.36**	0.272
Urinary nitrogen (g/d)	$3.99^{a} \pm 0.09$	$2.38^{b} \pm 0.08$	$1.96^{ab} \pm 0.03$	**2.80 ± 0.05**	0.056
Nitrogen balance (g/d)	$11.19^{a} \pm 1.69$	$7.90^{c} \pm 4.10$	$11.02^{b} \pm 2.92$	**10.01 ± 2.69**	< 0.0001
N-balance (as % of intake)	50.52 ± 3.54	50.00 ± 3.24	57.22 ± 0.43	**52.49 ± 1.50**	0.751
N-balance (as % of absorbed)	$96.28^{b} \pm 0.46$	$96.85^{ab} \pm 0.52$	$97.83^{a} \pm 0.24$	**96.99 ± 0.29**	0.079
N-balance (g/kg $W^{0.75}$)	$1.15^{a} \pm 0.08$	$0.82^{b} \pm 0.04$	$1.17^{a} \pm 0.03$	**1.04 ± 0.07**	0.0005
Values bearing the different superscripts (A, B, C etc) in a row differ significantly.					

Table 34 : Calcium and Phosphorous balance during metabolism trial

Parameters	Group1 (Biul)	Group2 (Tremal hay)	Group3 (0.5 % urea treated CSD tremal)	Overall mean ± SE	P value
Calcium balance					
Calcium intake (g/d)	$22.97^{A} \pm 1.62$	$18.37^{b} \pm 1.33$	$17.91^{b} \pm 0.89$	**19.75 ± 0.97**	0.044
Faecal calcium outgo (g/d)	16.45 ± 1.76	14.13 ± 1.22	13.27 ± 0.91	**14.62 ± 0.81**	0.276
Urinary calcium outgo (g/d)	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	**0.06 ± 0.01**	0.526
Calcium balance (g/d)	$6.47^{a} \pm 1.21$	$4.18^{b} \pm 1.45$	$4.57^{b} \pm 2.68$	**5.07 ± 1.98**	0.032
Calcium balance (as % of intake)	28.68 ± 3.32	22.97 ± 1.13	25.72 ± 1.71	**25.79 ± 1.37**	0.253
Calcium balance (as % of absorbed)	99.22 ± 0.17	98.62 ± 0.28	98.58 ± 0.18	**98.81 ± 0.14**	0.118
Calcium balance (g/kg $W^{0.75}$)	$0.69^{a} \pm 0.10$	$0.44^{b} \pm 0.12$	$0.49^{ab} \pm 0.03$	**0.54 ± 0.05**	0.046
Phosphorous balance					
Phosphorous intake (g/d)	2.39 ± 0.13	2.36 ± 0.13	2.16 ± 0.08	**2.30 ± 0.07**	0.370
Faecal phosphorous outgo (g/d)	1.01 ± 0.06	0.90 ± 0.08	0.88 ± 0.04	**0.93 ± 0.04**	0.347
Urinary phosphorous outgo (g/d)	0.21 ± 0.10	0.29 ± 0.14	0.30 ± 0.06	**0.27 ± 0.06**	0.821
Phosphorous balance (g/d)	1.163 ± 1.69	1.165 ± 0.35	0.979 ± 0.98	**1.10 ± 0.89**	0.751
Phosphorous balance (as % of intake)	48.02 ± 4.68	49.43 ± 1.37	45.20 ± 1.68	**47.55 ± 1.64**	0.609
Phosphorous balance (as % of absorbed)	84.55 ± 7.74	82.51 ± 6.42	76.74 ± 3.56	**81.26 ± 3.37**	0.662
Phosphorous balance (g/kg $W^{0.75}$)	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	**0.11 ± 0.01**	0.662

Values bearing the different superscripts (A, B, C etc) in a row differ significantly.

Table 35 : Effect of different treatments on blood parameters of goats

Period	Group			Mean ± SE	P value
	I	II	III		
Glucose (mg/dl)					
0	29.34 ± 1.24	27.84 ± 2.03	29.68 ± 0.76	28.95 ± 0.79	0.646
90	29.87 ± 1.08	27.06 ± 1.74	28.96 ± 1.02	28.63 ± 0.77	0.352
Total Protein (g/dl)					
0	7.83 ± 0.13	7.68 ± 0.19	7.80 ± 0.11	7.77 ± 0.08	0.748
90	8.00 ± 0.17	7.83 ± 0.14	7.85 ± 0.16	7.89 ± 0.08	0.697
Albumin(g/dl)					
0	3.18 ± 0.06	3.25 ± 0.06	3.20 ± 0.09	3.21 ± 0.04	0.772
90	3.13 ± 0.03	3.15 ± 0.06	3.13 ± 0.09	3.13 ± 0.03	0.950
Globulin(g/dl)					
0	4.65 ± 0.16	4.43 ± 0.14	4.60 ± 0.18	4.56 ± 0.09	0.596
90	4.88 ± 0.17	4.68 ± 0.10	4.73 ± 0.23	4.76 ± 0.09	0.703
A:G Ratio					
0	0.69 ± 0.04	0.74 ± 0.02	0.70 ± 0.05	0.71 ± 0.02	0.617
90	0.64 ± 0.02	0.67 ± 0.02	0.69 ± 0.05	0.66 ± 0.02	0.785
Urea (mg/dl)					
0	45.88 ± 0.85	45.65 ± 0.33	45.83 ± 0.42	45.78 ± 0.30	0.959
90	46.18 ± 0.62	46.00 ± 0.39	46.18 ± 0.50	46.12 ± 0.27	0.962
Blood Urea Nitrogen (mg/dl)					
0	21.42 ± 0.40	21.32 ± 0.15	21.40 ± 0.20	21.38 ± 0.14	0.959
90	21.56 ± 0.29	21.48 ± 0.18	21.56 ± 0.24	21.54 ± 0.13	0.962
Creatinine (mg/dl)					
0	0.92 ± 0.02	0.89 ± 0.03	0.92 ± 0.03	0.91± 0.01	0.814
90	0.93 ± 0.03	0.97 ± 0.04	0.96 ± 0.03	0.95 ± 0.02	0.703
Total Bilirubin (mg/dl)					
0	0.04 ± 0.02	0.05 ± 0.02	0.05 ± 0.06	0.05 ± 0.01	0.967
90	0.06 ± 0.01	0.06 ± 0.05	0.05± 0.01	0.05 ± 0.01	0.976
Total Cholesterol (mg/dl)					
0	71.78^{ab} ± 3.37	67.11^{b} ± 5.45	81.84^{a} ± 2.34	73.58 ± 2.77	0.051
90	78.01 ± 7.39	78.01 ± 7.54	87.04 ± 4.36	81.02 ± 3.67	0.557
Triglyceride (mg/dl)					
0	24.85 ± 0.90	25.25 ± 0.45	24.53 ± 0.19	24.88 ± 0.32	0.695
90	24.98 ± 0.56	24.25 ± 0.13	25.26 ± 3.30	24.83 ± 0.23	0.209

Table 36 : Effect of treatments on serum enzyme profile of goats

Period	Group			Mean ± SE	P value
	I	II	III		
AST (IU/L)					
0	67.91 ± 2.04	65.29 ± 1.84	69.58 ± 1.26	67.59 ± 1.06	0.266
90	71.45 ± 1.55	68.55 ± 2.07	72.14 ± 1.00	70.71± 0.96	0.293
ALT (IU/L)					
0	39.33 ± 1.69	36.65 ± 3.12	40.25 ± 2.65	38.74 ± 1.41	0.604
90	42.46 ± 3.16	40.08 ± 3.07	42.03 ± 3.40	41.52 ± 1.71	0.859
ALP (IU/L)					
0	71.00 ± 1.60	69.08 ± 1.90	69.40 ± 1.83	69.82 ± 0.97	0.724
90	71.58 ± 1.44	69.25 ± 2.08	70.23 ± 2.05	70.35 ± 1.02	0.690

Table 37 : Effect of treatments on serum mineral profile of goats

Period	Group			Mean ± SE	P value
	I	II	III		
Calcium (mg/dl)					
0	10.50 ± 0.41	9.68 ± 0.29	9.98 ± 0.35	10.05 ± 0.29	0.296
90	10.28 ± 0.23	9.78 ± 0.14	10.08 ± 0.25	10.04± 0.13	0.291
Phosphorous (mg/dl)					
0	5.73^{b} ± 0.16	6.54^{a} ± 0.16	6.22^{ab} ± 0.34	6.16 ± 0.16	0.053
90	5.89 ± 0.16	6.66 ± 0.18	6.24 ± 0.36	6.26 ± 0.16	0.153

Ca balance (g/d and g/kgW$^{0.75}$/d) was higher (P < 0.05) (6.47 and 0.69) in group 1, followed by in group 3 (4.5 g and 0.49) and group 2 (4.18 and 0.44); differences between the last two groups being non significant. Ca balances as % intake or absorbed did not differ between the groups.

P intake, outgo through faeces and urine, and balances did not differ between the groups. Intake of P (g/d) in respective groups was 2.39, 2.36 and 2.16 in group 1, 2 and 3 and balances (g/d) were 1.16, 1.17 and 0.98 in group 1, 2 and 3.

4.3.8 Effect of tremal hay based diets on blood parameters

Blood parameters [viz. glucose, total protein, albumin, globulin, A: G ratio, urea, blood urea nitrogen (BUN), creatinine, total bilirubin, total cholesterol, triglycerides, serum enzyme and serum mineral profile] estimated in the serum has been presented in Table 34. It was observed that all the parameters were within normal range in all the goats feed on respective diets.

Differences between the treatments

Values of respective parameters neither differ among the groups, nor were different between the 0 (initial) and 90 days of feeding trial.

Effect of respective diets on serum enzyme profile has been presented in the Table 35. The SGOT, SGPT and ALP values were within the normal range and were comparable among the groups.

The serum Ca and P values have been presented in the Table 37. The values of serum Ca and P neither differ between the 0 day (initial) and 90 day (final) of feeding trial, nor were different among the goats in different groups.

Discussion

Discussion

PHASE I

Studies on the processing of *Ficus roxburghii* (tremal) and *Quercus leucotrichophora* (Bun) leaves through physical and chemical treatments

5.1.1 Chemical composition of tree leaves

CP, EE, NDF, ADF, cellulose, ash and Ca content of *F. roxburghii* leaves is more than that of *Q. leucotrichophora* leaves; whereas the OM, total carbohydrates, hemicelluloses and P content is more in *Q. leucotrichophora* leaves (Table 7).

Values of CP, EE, Ca and P content of *F. roxburghii* tree leaves were comparable to those of Devarajan (1999). CP contents (14.10 and 13.80%) comparable to the present value (13.86%) was also reported by Thornea *et al.* (1999) and Sahoo *et al.* (2010). Reported CP values of these leaves ranged from 10.90 (Khanal *et al.,* 2001) to 17.80% (Prakash *et al.,* 2009). However, the NDF and ADF values were higher than that of previously reported values (Thorney *et al.,* 1999; Khanal and Subba, 2001; Sharma, 2002; Khanal and Upreti, 2008; Das *et al.,* 2008). Ca and P contents of *F. roxburghii* leaves are comparable to the reported values of Osti *et al.* (2006) and Prakash *et al.* (2009). It seems that the *F. roxburghii* leaves used in the present experiments in 1st phase during the winter months were relatively more mature.

Values of CP, EE, NDF, total ash, Ca and P content of *Q. leucotrichophora* tree leaves are comparable to that of Upreti and Shrestha (2006). Values reported by Upreti and Shrestha (2006) ranged from 81.79 to 97.42, 8.40 to 15.60, 1.40 to 3.50, 46.14 to 68.02, 2.58 to 18.21 and 0.10 to 0.30% for OM, CP, EE, NDF, total ash, Ca and P, respectively. Proximate chemical compositions of *Q. leucotrichophora* leaves reported by other workers (Devarajan, 1999; Sharma, 2002 and Singh *et al.,* 2005) though not similar to the present values, but are also within the ranges reported by Upreti and Shrestha (2006).

Total tannin content of *Q. leucotrichophora* and *F. roxburghii* was 6.15 (Table 8) and 5.15 (Table 14), respectively. Lower level of total tannins along with higher CP and Ca % in *F. roxburghii leaves*, is indicative of better feeding value of *F. roxburghii leaves* than that of *Q. leucotrichophora* leaves. Moreover, feeding of oak leaves may induce toxicity due to its higher level of HT (Sharma, 2002). HT content of oak and tremal leaves in the present studies is 4.52 (Table 8) and 2.82% (Table 14), respectively. Though the concentrations of proximate major nutrients in both the tree leaves are adequate but, it may not be used even as maintenance forage in ruminants due to presence of such anti-nutritional factors.

5.1.2 Physical treatment of *Q. leucotrichophora* leaves

All the physical treatments (chopping, CSD and CSDG) were effective in reducing all the polyphenols (Table 8). Percentage reduction was maximum of HT (47.72%) followed by TT (38.89%), TP (33.80%), CT (13.90%) and NTP (6.40%). It is revealed that the reduction of polyphenols is proportional to the particle size of the leaves. Chopping facilitated the oxidative enzymes. Grinding increases the surface area, which in turn accelerated the oxidation and de-naturation of the poly phenols. Moreover, it was revealed that the degree of susceptibility to

oxidative enzyme varies from one polyphenol to other. It seems that the degree of susceptibility of HT to oxidative enzyme is relatively more than the other polyphenols (Haslam,1966; Bagheripour *et al.*, 2008). Effect of physical treatment on reduction of polyphenols, however, reported to be variable from one feed to other, and also from one polyphenols to other. Makkar and Singh (1993) did not observe any effect on TP and CT on drying of mature oak leaves. However, Makkar and Singh (1991) reported that drying was effective for the feed stuffs having high moisture content. Bensalem *et al.* (1999) reported that sun drying was more effective in reducing CT in acacia foliage than shade drying.

5.1.3 Effects of physical treatments followed by different chemical treatments on polyphenols content of *Q. leucotrichophora* leaves

5.1.3.1 Effect of different physical treatments followed by potassium permanganate treatments

It was revealed that when both the treatments (physical and PP) were done one after another, there was significant ($P<0.0001$) effect in reducing the polyphenols in *Q. leucotrichophora* leaves (Table 9). Overall reduction of polyphenols only through physical treatment was 33.80, 6.40, 38.89, 13.90 and 47.72% for TP, NTP, TT, CT and HT, respectively (Table 8); whereas, extent of reduction of these polyphenols was further increased to 61.76, 30.05, 67.60, 32.94 and 79.95%, respectively, when both the treatments were done one after another. It seems that with the increase in surface area through physical treatment, contact between phenol oxidase and polyphenols increases which in turn increases the oxidation of polyphenols (Bensalem *et al.,* 2005). $KMnO_4$ being a strong oxidizing agent further increases the oxidation of polyphenols to reduce the level of respective polyphenols at maximum possible extent. Extent of reduction of

polyphenols was further proportional with the concentraction of PP. However, as susceptibility of respective polyphenols to phenol oxidase and to that of PP varies with the nature of polyphenols and accordingly the extent of their reduction vary between the polyphenols. Makkar and Singh (1992) also reported the reduction of tannins level by about 95% through PP (0.03M) treatment.

5.1.4 Effect of different physical treatments followed by urea treatment on polyphenol content of *Q. leucotrichophora* leaves

Overall reduction of polyphenols only through physical treatment was 33.80, 6.40, 38.89, 13.90 and 47.72% for TP, NTP, TT, CT and HT, respectively (Table 8); whereas, extent of reduction of these polyphenols was further increased to 57.73, 29.72, 62.90, 15.48 and 79.81%, respectively, when physical treatments were followed by urea treatment (Table 10).

It was revealed that the extent of reduction increased as the concentration of urea increased. The decrease in tannin content with urea treatment was also reported by previous workers (Price *et al.,* 1979; Makkar, 2003). Surface area of leaf material increases on grinding of which in turn increases the oxidation of polyphenols in presence of urea. Evolution of ammonia from urea increases the pH of the treatment medium. Tannins are easily oxidized at alkaline pH to quinines, which may promote covalent bonds to other compounds/ polymer residue (Rawel *et al.*, 2000).

5.1.5 Effect of physical treatments followed by wood ash treatment on polyphenol content of *Q. leucotrichophora* leaves

Physical treatment followed by wood ash treatment had significant effect on reduction of all the polyphenols of *Q. leucotrichophora* leaves (Table 11). TP% was reduced upto 33.8% on

physical treatment (PT) whereas extent of reduction was further increased upto 52.98 % on physical treatment followed by wood ash treatment, likewise NTP was reduced from 6.4 % (on PT) to 24.98% on PT followed by wood ash (WA) treatment. Rest of the polyphenols, TT, CT and HT reduced upto 38.89, 13.90 and 47.72% on PT, but extent of reduction further increased upto 58.15.90, 21.30 and 71.27% when the treatments were done one after another. However, maximum reduction was of HT (i.e upto 93.62%) followed by TT (i.e 75.72%) on 10% WA treatment of CSDG oak leaves. Extent of reduction was increased obviously due to reduction of partical size on grinding of leaves along with the effect of increased pH of treatment medium on increasing the concentration of WA. Chopping/grinding of leaves is expected to increase the availability of tannins to the enzyme (phenol oxidases) (Makkar and Singh 1992; Ben Salem, *et al* 2005). Alkaline pH due to WA hydrolyses the tannins into simpler phenols which are leached out of the physically processed leaves (Makkar Singh, 1992). Thus, it seems that increased rate of oxidation of tannins are responsible for the reduction of phenols in treated leaves. Higher the level of WA, lower the proportion of TP and CT in acacia foliage was also noted by Bensalem *et al.* (2005).

5.1.6 Effect of different physical treatments followed by GNC treatment on different polyphenol contents of *Q. leucotrichophora* leaves

Like other treatments, when physical treatment followed by GNC treatment was done, reduction of polyphenols was increased further (Table 12). Overall reduction of respective polyphenols only due to physical treatment was 33.88, 6.40, 38.89, 13.90 and 47.72% for TP, NTP, TT, CT and HT, respectively. Whereas, when physical treatment followed by GNC treatment was done overall reduction of these polyphenols was increased upto 52.17, 23.73, 57.43, 1.73

and 72.28 %, respectively. Possible reasons for reduction of these polyphenols due to physical treatments have been discussed in earlier section. Over and above, GNC might have played an important role in inactivating the tannins/different polyphenols on further complexation between tannins and proteins, polymerization and oxidation.

As in case of other physico-chemical treatments, it has been also observed that the extent of reduction was maximum with CSDG oak leaves treatment with 3.4 to 7.5% GNC. GNC treatment reduces the assayable tannins of oak leaves to the maximum extent on grinding of leaves. Reduction of polyphenols (TP, NTP, CT and HT) increased even upto 69.96, 32.63, 76.80, 25.08 and 95.29%, on GNC treatment CSDG oak leaves.

5.1.7 Comparative studies on the effect of different physical treatment followed by the treatment with chemicals /WA / GNC on polyphenol content of *Q. leucotrichophora* leaves

Potassium permagnate being one of the most potent oxidizing agents had reduced the polyphenols to maximum level, followed by urea being a source of alkaline NH_3 (Table 13). WA and GNC treatment also reduced the polyphenols relatively at lower degree. Alkaline pH of the suspension of WA/ GNC accelerates the hydrolysis of tannins into simpler phenolics which are then leached out of the leaves. Phenol oxidase of plant origin (or added oxidizing chemicals) further oxidize the tannins into quinones, leading to destruction of active phenolic groups of tannins (Field *et al.,* 1990; Makkar and Singh, 1992).

5.1.8 Effect of physical treatment on different polyphenol content in *F. roxburghii* leaves

All the physical treatment was effective in reducing all the polyphenols (Table 14). However, present reduction varies from

one polyphenols to other. Maximum overall reduction was of CT (i.e. 24.60%) followed by NTP (18.04%), TP (15.36%), TT (14.78%) and was lowest of HT (6.66%). Whereas in case of *Q. leucotrichophora* maximum reduction on physical treatment was of HT (47.72%) and lowest was of NTP (6.40%). This was obviously due to the difference in the nature of polyphenols between the plant species. Both the nature and quantity of polyphenols differ greatly between plant species (Synge, 1975).

Chopping significantly reduces all the polyphenols; chopping followed by sun drying further reduces CT and HT. Whereas, NTP was increased ($P<0.0001$) on sun drying of chopped leaves, but initial reduction of TT and HT of chopped leaves was again increased on sun drying and grinding. It seems that rate of oxidation of polyphenol differs from one polyphenol to other; which in turn changes the proportion of polyphenols on DM basis. Moreover, there was respiratory loss of OM/sugars till the plant cells were dried. Among the polyphenols, the percentage of NTP in fresh *F. roxburghii* leaves was lowest and the quantitative proportion was only 1.12%, which increased to 1.29% on chopping, followed by sun drying. This increase was probably due to reduction of DM/ soluble sugars due to respiratory loss (Mullen and Koller, 1988). The percentage increase or decrease of respective polyphenols, in respective physical processing of leaves may be due to differences in the rate of losses between polyphenols and soluble sugars/ other OM/DM. Rate of losses of OM either as polyphenols or other soluble sugars/DM may vary between the three processes of losses. i.e., 1. Due to oxidation of polyphenols; 2. Due to respiration till drying and 3. Due to physical processing (Mullen and Koller, 1988; Makkar, 2003; Bensalem *et al.*, 2005; Vitti *et al.,* 2005).

5.1.9 Effect of different physical treatments followed by potassium permanganate treatment on polypohenol content of *F. roxburghii* leaves

Extent of overall reduction of TP, NTP, TT, CT and HT through physical treatment was 15.36, 18.04, 14.78, 24.60 and 6.66% respectively (Table 14). Extent of reduction of these poplyphenols was further increased up to 64.07, 24.95, 72.67, 59.53 and 83.35%, respectively (Table 15), when physical treatment followed by potassium permanganate (PP) treatment was done. Both the treatments had its effect in reducing the polyphenols. Potassium permanganate being a strong oxidizing agent had an additive effect in reducing the polyphenol over and above that of the physical treatment. Moreover, there were oxidative losses due to phenol oxidase and physical processing.

With the decrease in particle size on grinding of dry leaves, extent of reduction of most of the polyphenols increases. Grinding increases the exposure of polyphenols to PP, which in turn increases the rate of oxidation of polyphenols along with the other organic chemicals/compounds of leaf. Rate of decrease of polyphenols varies from one polyphenol to other polyphenols; this is due to relative loss of other organic matter/chemicals of *F. roxburghii* leaves. Detannification through PP treatment has also been reported by Makkar and Singh (1992).

5.1.10 Effect of different physical treatments followed by urea treatment on polypohenol content of *F. roxburghii* leaves

Effect of physical treatment along with urea on reduction of polyphenol content differs from one leaf to other. In case of *Q. leucotricophora* leaves, extent of reduction of almost all the polyphenols were increased with the decrease in particle size along with the increase in the level of urea. But in case of *F. roxburghii*

leaves, NTP content increases in urea/ water treated green fresh/ chopped leaves (Table 16). This might be due to the higher rate of respiratory losses of soluble carbohydrates of green leaves (Mullen and Koller 1988), in relation to the least concentration (1.12%) of NTP in fresh tremal leaves. However, other polyphenols (TP, TT, CT & HT) are as usually reduced with the sun drying and grinding, followed by urea treatment. As the concentration of urea increases, TP, TT, CT and HT content decreases. This might be due to transformation of complex polyphenols to simpler polyphenols due to alkaline hydrolysis, followed by leaching out of simpler polyphenols and final oxidation of it (Makkar, 2003, Bensalem *et al.*, 2005, Vitti *et al.,* 2005).

5.1.11 Effect of different physical treatments along with wood ash (WA) on the polyphenol content of *F. roxburghii* leaves

Physical treatment followed by wood ash treatment had significant effect on reduction of all the polyphenols of *F. roxburghii* leaves (Table 17). TP% was reduced upto 15.36% on physical treatment (PT) whereas extent of reduction was further increased upto 57.17% on physical treatment followed by wood ash treatment, likewise NTP was reduced from 18.04% (on PT) to 29.95% on PT followed by wood ash (WA) treatment. Rest of the polyphenols; TT, CT and HT reduced upto 14.48, 24.60 and 6.66 % on PT, but extent of reduction was further increased upto 63.09, 55.77 and 69.13% when both the treatment was done one after another. However, maximum reduction was of CT (i.e upto 96.72%) followed by TT (i.e 88.62-88.74%), TP (87.55-88.21%) on 5-10% WA treatment of CSDG tremal leaves. Extent of reduction was increased obviously due to reduction of partical size on grinding of leaves along with the effect of increased pH of treatment medium on increasing the concentration of WA. Chopping/grinding of leaves is expected to increase the availability of tannins to the enzyme (phenol oxidases) (Makkar and Singh, 1992; Bensalem *et al.*,

2005). Alkaline pH due to WA hydrolyses the tannins into simpler phenols which are leached out of the physically processed leaves (Makkar and Singh, 1992). Thus, it seems that increased rate of oxidation of tannins are responsible for the reduction of phenols in treated leaves. Higher the level of WA, lower the proportion of TP and CT in acacia foliage was also noted by Bensalem *et al.* (2005).

Trend of changes in the concentration of polyphenols in tremal leaves treated physioco- chemically with wood ash (WA) is almost similar with that of urea treatment. WA being alkaline in nature (having pH of 10 - 11), though weaker than urea, might have similar effect of alkaline hydrolysis of complex polyphenols to simpler ones, followed by oxidation of leached out simpler phenols. However, oxidation of polyphenols with phenol oxidases of tremal leaves was common phenomenon in all treatments.

5.1.12 Effect of different physical treatments followed by GNC treatment on different polyphenol contents of *F. roxburghii* leaves

Both the treatments, physical (Table 14) and physical followed by GNC (Table 18), have their effect in reducing all the polyphenols in *F. roxburghii* leaves. However, like other physiochemical treatments, GNC treatment of physically treated tremal leaves had further reduced the polyphenol contents. However, NTP content was increased from 1.12% in fresh tremal leaves to 1.32 to 1.93% through water/GNC treatment.

Simple water soaking of fresh and chopped tremal leaves also increased the NTP content up to 1.32 and 1.40%. Such increase in NTP in green fresh tremal leaves might be due to particular/specific nature of NTP which is less prone to oxidation unlike other polyphenols; moreover, there might be respiratory and other losses of soluble sugars of green tremal leaves. Such losses of DM/OM (water-soluble solids)

either through respiration of leaves or DM loss through water soaking, might have ultimately increased the proportion of NTP in green/ fresh treated tremal leaves. Respiratory losses of soluble sugars were also reported by Mullen and Koller (1988). Physical treatments, sun drying and grinding, however, reduces the NTP content of tremal leaves from 1.12% to 0.38%, further to 0.32% on water soaking of CSDG tremal leaves; and to 0.12 to 0.25% on GNC treated CSDG tremal leaves.

5.1.13 Comparative studies on the effect of different physical treatment followed by the treatment with chemicals /WA / GNC on polyphenol content of *F. roxburghii* leaves

Overall, on comparison between the chemicals (Table 19), it was observed that for reduction of TP, potassium permanganate (PP) and urea were most effective to the extent of 66 – 68% ($P<0.0001$), followed by GNC and WA to the extent of 59 – 60%, and water to the extent of 53%, respectively. For reduction of NTP, urea and WA was most effective to the extent of 33-34%; followed by GNC and PP to the extent of 27 – 31%; and water to the extent of 18%. For TT and HT, most effective was PP to the extent of 77 and 87%; followed by urea to the extent of 73 and 80%; GNC and WA to the extent of 66 and 70%, respectively. For reduction of CT, treatment with PP, urea and GNC was found to be most effective to the extent of 62 – 66%, differences being non significant among the three chemicals; followed by WA to the extent of 59% and water to the extent of 46%, respectively.

On comparison of extent of reduction in TT among different interactive processing; it was observed that urea treatment of CSD tremal leaves was most effective (91.26%) ($P<0.0001$); however, differences among PP, urea and GNC treated CSD and CSDG tremal leaves and WA treated CSDG tremal leaves were non-significant.

Possible mechanism of action/ reasons of reduction of different polyphenols on treatment with different chemicals have already been

discussed in the previous session, along with the remarks of previous workers on the subject.

PHASE II

5.2 EFFECT OF DIFFERENT PHYSICAL TREATMENT FOLLOWED BY UREA TREATMENT OF *F. ROXBURGHII* LEAVES (HAY) ON *IN VITRO* DIGESTIBILITY, GAS PRODUCTION, TVFA, DE AND ME VALUES

DE and ME values of *F. roxburghii* leaves depend on number of factors viz, (i) digestibility of organic nutrients, (ii) gas production, (iii) TVFA production, (iv) plant secondary metabolites (tannins, saponins) content in leaves, (v) chemical composition and fibre fractions of leaves etc. Thus, effect of these factors on DE and ME values of the tremal leaves has been discussed in this section.

5.2.1 Chemical composition of processed F. roxburghii leaves

Almost all the organic nutrients (proximate composition) except CP and EE were reduced on urea treatment of tremal hay (Table 20). Ca and P contents of tremal hay were also in decreasing trend with the increase in the concentration of urea from 0.5% to 2.5% (w/w). Soluble nutrients including phytochemicals of hay might have been dissolved on overnight soaking of tremal hay in urea solution (Makkar, 2003; Makkar and Singh, 1993). Leaching out of organic chemicals along with the oxidative destruction has in turn increased the total ash content of tremal leaves, keeping the EE content almost comparable to that of control. Complex/compound fat might have been relatively resistant of being oxidized/dissolved/leached out of the leaf.

5.2.2 Effect of processed tremal hay on in vitro digestibility, DE and ME values

Net gas production (ml/200mg sample) was significantly ($P<0.0001$) more in 0.5% UTCSDTH (urea treated chaffed sundried

tremal hay), followed by UTH (untreated tremal hay/control) and 1.5 to 2.5% UTCSDTH (Table 21). Similarly methane production (ml/ 200mg sample) was significantly (P=0.003) higher in 0.5% UTCSDTH than that of other three groups including control hay. It seems that microbial fermentation under *in vitro* conditions was most favourable in presence of optimum ammonia nitrogen concentration in 0.5% UTCSDTH. This is reflected on higher methane (ml/20mg sample) (P=0.003) (Table 21) and acetic acid (P<0.05) production (Table 22), and DOM (%) on *in vitro* fermentation of 0.5% UTCSDTH (Table 23). Higher (P<0.0001) OM digestibility along with relatively favourable/ optimum NH_3-N concentration in turn increased (P<0.0001) the DE and ME values of 0.5% UTCSDTH (Table 23). However, IVDMD (%) was comparable between the control (UTH) and 0.5% UTCSDTH. This might be due to lowest (P<0.0001) ammonia N concentration (mg/ 30ml) and highest (P<0.0006) protozoal population in the control group (Fadel Elseed *et al.,* 2003; Fadel Elseed, 2005; Mohammadabadi *et al.*, 2010). *In vitro* DOM (%) and ME (MJ/kg) of *F. roxburghii* leaves reported by Singh and Makkar (2000) (i.e., 54.9% & 9.2%) was however higher than that of present values. Singh *et al* (2005) reported DMD of *F. roxburghii* leaves of 66.9%, which is lower than the present IVDMTD% of UTH (i.e., 70.69%).

PHASE III

5.3 CHEMICAL COMPOSITION OF THE FEED STUFFS OFFERED TO THE EXPERIMENTAL ANIMALS

Digestibility of OM and ash content of biul and tremal (treated/ untreated) hay was almost comparable (Table 24). But, CP and EE content of biul hay (19.44 & 4.10%) was higher than that of tremal hay (12.82 & 3.37%); however, on treatment with 0.5% urea, CP was increased up to 15.01%. As in the case of CP, the T-CHO and fibre fractions between biul and tremal leaf hay was different due to

differences in the genus of respective trees. NDF, ADF, cellulose and lignin content was lower in biul hay than that of tremal hay. Morphologically the biul leaf is thinner than that of tremal leaves. Moreover, the veins of the biul leaf are also thinner than that of the tremal leaves. The ash content of biul and tremal leaf hay though are comparable, the Ca and P content of biul hay was higher than that of tremal hay. Treated (0.5% UTCSDTH) and untreated tremal hay was comparable in almost all the proximate compositions except CP content. OM, CP, EE, NDF, ADF, ash, Ca and P content of buil is comparable to the reported values of Upreti and Shrestha (2006). The values of such proximate composition and fibre fraction of tremal leaves in the present studies are higher than that of Upreti and Shrestha (2006); however, CP content is comparable to that of Amatya (1990), Shrestha and Tiwari (1991), Singh *et al.*, (2005) and Dhyani *et al.,* 2011). Relatively higher fibre fractions including lignin content of *F. roxburghii* leaves in the present studies indicated that the tremal hay used in the *in vivo* trial was of much mature one.

Composition of the concentrate mixture was comparable with that of any standard concentrate mixture.

5.4 PERFORMANCE OF GADDI GOATS

Feed (DM) intake of goats fed on TH based diets was in reducing trend till 4th week, however in 0.5% UTCSDTH group, the reduction in DMI was only up to 2nd week (Table 25). But in control group, DMI was in increasing trend throughout the experimental period with the progress of the feeding trial. Goats in all the groups took three weeks adaptation time. During the adaptation period, the body weight was in reducing trend in all the groups, which in turn reduces the feed: gain ratio (Table 27). However, the bodyweight of goats in the respective groups remained comparable throughout the trial (Table 26). It seems

that the differences in the composition of hay did not have any significant impact on the overall growth performance of the *Gaddi* goats. Though there was a numerical difference of 0.79 between group I and II, and 0.68kg between group II and III at 13th week. But body weight attainment at 13th week of feeding was 21.55, 20.76 and 20.08 in groups I, II and III, respectively; however, the palatability of the diet varies among the groups ($P<0.02 – 0.0001$). DMI/ feed intake during adaptation period was significantly higher in control and UTH fed goats than that of the goats fed with diets containing 0.5% UTCSDTH. However, the body weight loss, feed:gain, DMI:gain and CPI:gain ratio did not differ among the groups during the adaptation period (Table 27). However, on overcoming the initial stress of changing the dietary constituents during the adaptation period, significant differences in the total body weight gain ($P<0.007$), ADG ($P<0.009$), total feed intake ($P<0.0001$), total DMI ($P<0.0001$), feed/DM: gain ratio ($P=0.0003$) and CPI:gain ratio ($P=0.006$) were there among the groups (Table 28).

The attainment of body weight at the end of feeding trial did not differ significantly among the groups. Effects of respective diets could not be reflected statistically due to the higher SE values of final body weight; whereas the SE were relatively less in total gain, ADG, feed/ DMI and feed/DMI/CPI: gain ratios; the mean values of such production parameters differed significantly ($P=0.007$ to <0.0001) among the groups. It seems that higher ($P<0.02$) TDN along with DCP values of biul hay based diets had significant positive effect in increasing the feed/DM intake and body weight gain of Gaddi goats in comparison to that of the diet based on tremal hay containing lower TDN and DCP values. However, feed/DM intake was significantly higher ($P<0.03$) in goats fed on control diet followed by 0.5% UTCSDTH and TH; the body weight gain with respect of CPI being most efficient

in goats fed on tremal hay followed by Biul hay (control) fed goats, values of CPI:gain being significantly higher in 0.5% UTCSDTH groups. Differences between Biul group and urea treated hay groups were non-significant. Overall performance with respect to body weight gain and feed/DM: gain was better in the control group, followed by group II and was lowest in goats fed with urea treated leaves in group III (Table 28). An ADG of 29.7 (g/d) [which is comparable to the ADG of goats in group II] and feed :gain of 10.3 in female growing Khari goats fed on *F. roxburghii* leaves had been reported by Khanal and Upreti (2008).

5.5 DIGESTIBILITY OF DIFFERENT NUTRIENTS AND FIBRE FRACTIONS

Digestibility of DM, OM and NDF was significantly ($P<0.01$) higher in goats fed Biul hay based diet, followed by the goats fed with 0.5% urea treated tremal hay based diets, differences being non significant between group I and III (Table 29). Digestibility of DM, OM and NDF was lower ($P<0.01$) in tremal hay based diets. Digestibilities of other proximate principles did not differ significantly among the groups. Higher CP along with lower level of fibre fraction in biul hay might have improved the digestibility of DM, OM and NDF in control group. Overnight urea treatment of tremal hay might have played a role in dissociation of lingo-cellulose/ hemicellulose bond, which in turn increases the digestibility of nutrients as comparable to that of in group I. Increased digestibility of DM and OM through urea treatment of roughages has also been reported by several workers (Prasad *et al.*, 1998; Vu *et al.,* 1999; Akter *et al.,* 2004).

5.6 PLANE OF NUTRITION

Body weight, DMI and OMI (g/d or g/kg $BW^{0.75}$) of goats during metabolism trial did not differ among the groups (Table 30 and 32).

However, as the CP and EE content of biul hay was more than that of tremal hay, the plane of nutrition with respect to CPI and EEI was significantly higher (P<0.0001–0.003) in goats fed control diet. Higher DCP% of the biul hay based di*et al*so contributed significantly in increasing the CPI of goats in group I. However, as the NDF and ADF content of tremal hay is more than biul hay, the total carbohydrates (g/kg $BW^{0.75}$) intake in goats fed tremal hay was increased (P<0.005), differences between group II and III being non significant. Similarly, though the TDN intake (g/d) did not differ significantly between the groups; as the values of body weight during metabolism trial was numerically lower in groups III, the TDN intake per kg metabolic body size (g/kg $BW^{0.75}$) became significantly higher (P<0.02) in group III, followed by group I and II, differences between I and II being non significant. Higher intake of ADF/total carbohydrate per kg metabolic body size along with nonsignificant differences in digestibility of ADF among the groups contributed significantly (P<0.02) in TDN intake per kg metabolic body size per day.

5.7 NUTRITIVE VALUES OF THE DIETS

TDN value of biul and urea (0.5%) treated CSDTH based diet was higher (P<0.02) than that of untreated tremal hay based diet, differences between the diet in groups I and III being non significant (Table 31). However, DCP (%) was higher (P<0.02) in group I, followed by group III and was lowest in group II. As the digestibility of DM, OM and NDF and TC was comparable between the diets in group I and III; moreover as the OM, total carbohydrates and NDF content was more in urea treated CSDTH than that of biul hay, the TDN value of urea treated tremal hay increased significantly higher than that of untreated tremal hay. Increase of energy value of urea treated hay was such that it became even comparable to that of biul hay based diet. Differences in the CP digestibility being non significant

between the group, higher CP content of biul make the DCP% significantly higher (P<0.02) in group I, followed by group III (due to additional urea) and group II. As the CP% was lowest in untreated tremal hay (group II), the DCP value obviously became lower in group II than that of other two groups.

5.8 NITROGEN BALANCE

Improvement of the nutritive value of tremal hay on urea treatment increased (P<0.0001– 0.0005) the overall nitrogen balance (g/d, g/kg $BW^{0.75}$) of goats in group III than that of goats in group II (Table 33). Biul having better TDN along with DCP, improved the nitrogen balance (g/d) to the maximum extent in goats in group I, followed by the goats in group III and II. As the N intake (g/d) was minimum in group II, the N balance was obviously minimum in the goats fed tremal hay based diets (group II). However, utilization of absorbed N (N balance as percentage absorbed) was comparable among the groups.

5.9 CALCIUM AND PHOSPHOROUS BALANCE DURING THE METABOLISM TRIAL

Ca and P balances were also positive in all the groups (Table 34). Positive balances of Ca, P and N indicated that the experimental goats were in growing stage. Ca balance (g/d) was significantly higher (P<0.03) in group I, followed by groups II and III, differences being comparable between the groups II and III. Ca balances per kg metabolic body size (g/kg $W^{0.75}$/d) was also in similar trend, significantly (P<0.05) higher in group I, followed by group III and II, differences being non significant between I and III, and II and III, respectively.

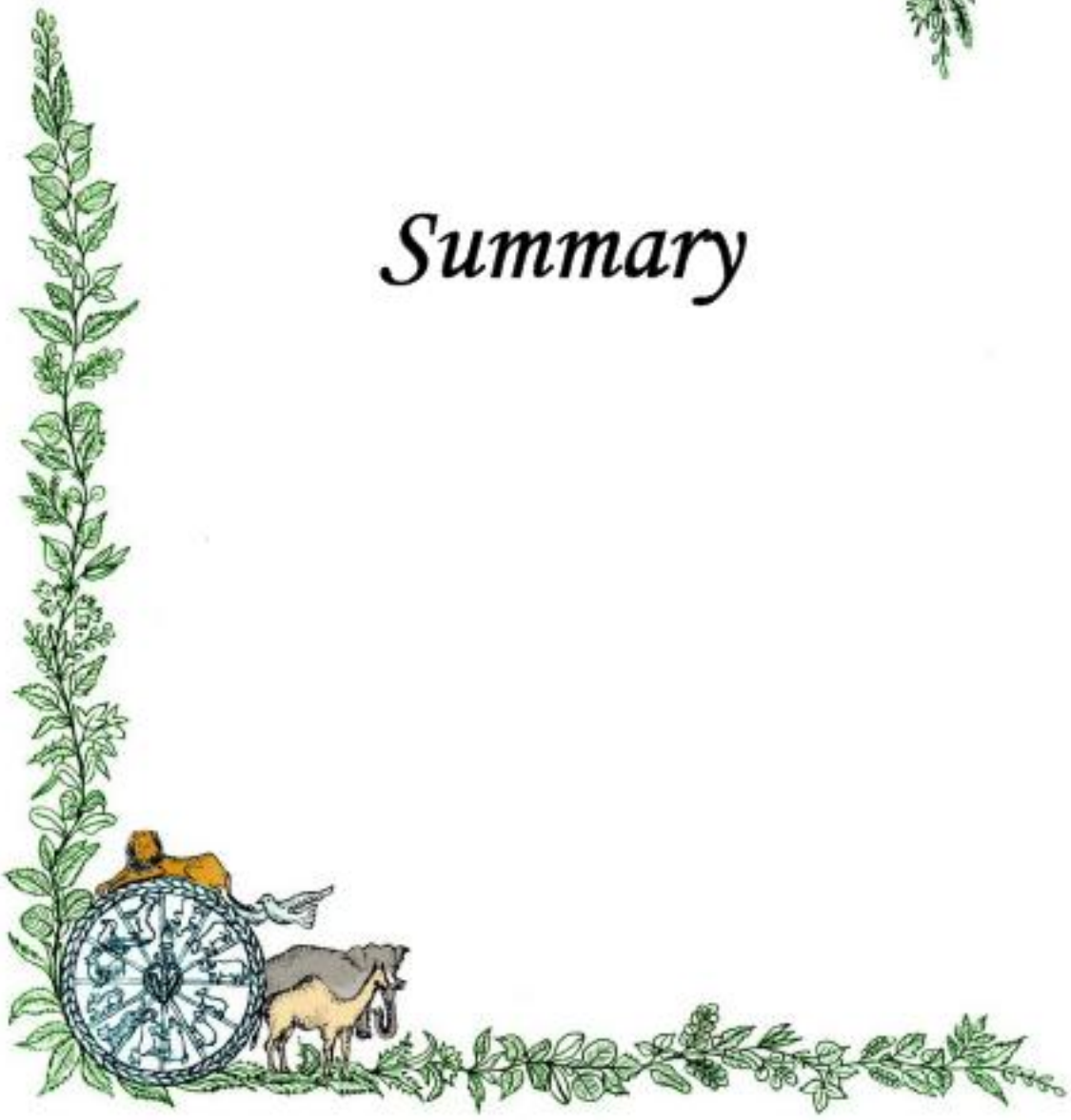
As the intake of P was relatively less than that of Ca, its balances were less in goats in all the groups. The differences among the groups were non-significant.

It seems that the differences in tannin and fibre content between the leaves had significantly affected the protein and Ca utilization of goats; but did not have any significant effect on the P utilization.

5.10 EFFECT OF DIFFERENT DIETS ON THE BLOOD BIOCHEMICAL PARAMETERS

Blood parameters did not differ among the groups (Table 35, 36 and 37). All the parameters were within the normal range, at the beginning and end of the trial. This indicates that tremal hay based diets were adopted by the goats very well. Tremal hay and 0.5% UTCSDTH did not have any adverse effects in maintaining different blood parameters within normal physiological level. Differences in Ca balances (Table 34) also did not have any significant differences in serum Ca and P profile of goats even after 90 days of feeding.

Summary



6 Summary

Ficus roxburghii (tremal) and *Quercus leucotrichophora* (bun) leaves though available in the North West Himalayan Region (NWHR) in adequate quantity, but, have unsatisfactory nutritive values due to higher level of tannins. At higher level, tannins reduce forage quality and adversely affect herbivore nutrition on reducing intake, digestibility of protein and other valuable nutrients. Efforts are made to bring the level of tannin at its optimum level, to overcome its anti-nutritional effects and to promote its beneficial effects in animal production and health. Use of tree leaves with optimum level of tannin is thus a promising area to develop an alternate diet for ruminants in hilly areas. *In vivo* studies on such detannified (tannin level optimised) leaves of *F. roxburghii* and *Q. leucotrichophora* leaves are very limited. Thus, the present study has been taken up with the objective to detannify these leaves through different physico-chemical treatments and to develop an alternate diet for growing gaddi goats fed on detannified leaves.

The study was carried out in three phases. **During first phase**, *Q. leucotricophora* and *F. roxburghii* leaves collected from the local forest area, were then randomly divided into four groups for physical treatments (viz., (i) Untreated leaves [UL, Control] (ii) Chopped leaves [CL] (iii) Chopped and sundried leaves [CSDL] (iv) Chopped, sundried and ground leaves [CSDGL]. Polyphenol contents of leaves in all the groups were estimated. Thereafter, on taking the representative

samples of leaves in each group, the leaves were further subjected to four chemical treatments [viz., (i) Potassium permanganate; 0.0, 0.1, 0.2 and 0.4% (w/v) (ii) Urea; 0.0, 0.5, 1.5 and 2.5 % (w/v) (iii) Pine wood ash; 0.0, 2.5, 5.0, 10.0 % (w/v) (iv) Ground nut cake; 0.0, 1.7, 3.4. 7.5 % (w/v)] to study the effect of these treatments on polyphenol content of both the leaves.

During second phase, *in vitro* degradability through IVGPT (*In vitro* gas production technique) was carried out selecting urea treated *F. roxburghii* leaves from first phase. Effect of different physical treatments followed by urea (0.0, 0.5, 1.5 and 2.5%) treatment of tremal leaves on *in vitro* degradability, gas, ammonia and methane production, DE and ME values were estimated. Untreated tremal hay was taken as control.

During third phase, *in vivo* feeding trial was conducted in growing gaddi goats (18.82±1.08kg**)**. The goats were divided into three groups of four animals each. The goats in groups 1(control), 2, and 3 were offered *ad lib Grewia optiva* leaves hay (control), *F. roxburghii* leaves hay and 0.5% urea treated chopped sundried (UTCSD) *F. roxburghii* leaves hay along with the supplementation of concentrates mixture for supporting 40g average daily gain (ADG). The animals were weighed at weekly intervals. Daily feed intake was recorded throughout the feeding trial (90 days). Blood biochemical changes (glucose, total protein, albumin, globulin, creatinine, urea, blood urea nitrogen, calcium, phosphorous, aspartate aminotransferase, alanine aminotransferase, total bilirubin, total cholesterol, alkaline phosphatase and triglyceride) were recorded at 0 and 90 days of feeding trial. Metabolism trial in each goat was conducted after 77 days of feeding trial to study the nutrient utilization of respective diets.

FIRST PHASE

During 1st phase the oranic matter, crude protein, ether extract, total carbohydrate, total ash, neutral detergent fibre, acid detergent fibre, cellulose, hemi cellulose, calcium and phosphorous contents of *Q. leucotrichophora* (% on DM basis) were 94.02, 10.53, 4.02, 79.47, 5.98, 48.89, 31.51, 20.54, 17.38, 1.89 and 0.27, respectively and of *F. roxburghii* (% on DM basis) were 92.38, 13.86, 4.34, 74.18, 7.62, 60.78, 52.72, 36.80, 8.06, 2.38 and 0.22, respectively.

Detannification of oak leaves

Total phenols (TP), non tannin phenols (NTP), total tannins (TT), condensed tannins (CT) and hydrolysable tannins (HT) content of fresh *Q. leucotrichophora* leaves were 7.26, 1.12, 6.15, 1.62 and 4.52%, respectively. All the physical treatments were effective ($P<0.0001$) in reducing all the polyphenols in oak leaves. Maximum reduction ($P < 0.0001$) of TP (40.76), NTP (8.18), TT (46.79), CT (14.83) and HT (58.13) was due to CSDG followed by chopped and CSD oak leaves.

On **physical and chemical** treatment (**PCT**) with **potassium permanganate (PP)**, these polyphenols were further reduced up to 61.76, 30.05, 67.60, 32.94 and 79.95%, respectively. The reduction of TP, TT and CT was maximum ($P< 0.0001$) due to CSDG and was lowest in fresh leaves treated only with water. Among the chemical treatments, 0.4 % PP reduced ($P<0.0001$) all the polyphenols to the maximum extent followed by 0.2 and 0.1 % PP, and was minimum through water treatment. Out of 16 groups of different treatment combinations (Physical × Chemical), reduction ($P < 0.0001$) of TP was maximum in 4th (fresh 0.4% PP), NTP in 12th (CSD 0.4 % PP), TT in 14th (CSDG 0.1% PP), CT in 16th (CSDG 0.4% PP) and HT in 5th (chopped with water) treatment.

On PCT **with urea**, the overall (mean) reduction of TP, NTP, TT, CT and HT were 57.73, 27.72, 62.90, 15.48 and 79.81%, respectively. Both physical and chemical (including water) treatments had their effect in reducing ($P<0.0001$) all the polyphenols, but among the physical treatments, reduction of most of the polyphenols was maximum ($P<0.0001$) due to CSDG. Among the chemical treatments, 2.5 % urea treatment reduced all the polyphenols to the maximum extent, followed by 1.5 % urea treatments. Among the treatment combinations (Physical × Chemical), maximum reduction was of TP and NTP in 12th (CSD 2.5 % urea); TT and HT in 5th (Chopped with water); CT in 16th (CSDG 2.5 % urea) treatment.

On PCT **with wood ash (WA),** the overall reduction of TP, NTP, TT, CT and HT was 52.98, 24.98, 58.15, 21.30 and 71.27%, respectively. Among the physical treatment reduction of all the polyphenols was maximum ($P < 0.0001$) due to CSDG. Among the chemical treatments, 10% wood ash reduced all the polyphenols (except HT) to the maximum extent, followed by 5 and 2.5 % WA treatments. HT was reduced at maximum level (78.35%) through water treatment, followed by 10, 5 and 2.5% WA treatments. Among the treatment combinations (Physical × Chemical), it was observed that the reduction of all the polyphenols was maximum in 16th treatment (CSDG 10% WA).

On PCT **with ground nut cake (GNC),** overall reduction of TP, NTP, TT, CT and HT were 52.17, 23.73, 57.43, 15.73 and 72.28%, respectively. Among the physical treatments the reduction was maximum ($P<0.0001$) in CSDG followed by CSD, chopped and fresh leaves treated with water (FLTW). However, the effect of GNC was variable between the phenols. The reduction of NTP and CT was maximum due to 7.5% GNC, followed by 3.4 and 1.7% GNC; On

comparison between 16 groups of different combinations (Physical × GNC), it was revealed that the reduction of all the polyphenols was maximum in 16th treatment (CSDG + 7.5% GNC).

On comparing between the different treatment combinations of **chemical treatments (water, PP, urea, WA and GNC) along with physical treatments**, maximum (%) reduction of TP was in 20th (CSDG + GNC) followed by 19th (CSDG + WA), 17th (CSDG + PP), 18th (CSDG + Urea) and 16th (CSDG + Water) treatments; NTP was in 2nd (Fresh + PP) followed by 12th (CSD + PP) and 13th(CSD + Urea); TT was in 20th followed by, 19th, 17th treatments; CT was in 17th, HT was in 20th, followed by 6th (Chopped + Water), 19th and 16th; differences being nonsignificant among the treatments within respective polyphenol. Effect of CSDG was maximum in reducing TP, TT, CT and HT, where as PP was maximum in reducing all the polyphenols of *Q. leucotrichophora* leaves.

Detannification of tremal leaves

The TP, NTP, TT, CT and HT contents (% DM basis) of fresh *Ficus roxburghii* leaves (FFRL) were 6.27, 1.12, 5.15, 2.33 and 2.82, respectively. These polyphenols were reduced ($P < 0.0001$) to 5.18, 1.08, 4.10, 1.86 and 2.24% on chopping; 5.40, 1.29, 4.11, 1.78 and 2.32 % in CSD; and 5.34, 0.38, 4.95, 1.62 and 3.33%, in CSDG leaves, respectively. Extent of reduction differed from one polyphenols to other and from one treatment to other physical treatment. TP was reduced to the maximum extent (17.35%) due to chopping, followed by the CSDG and CSD, difference between which was nonsignificant. The NTP and CT was reduced to the maximum extent due to CSDG; whereas the extent of reduction in TT and HT was maximum due to chopping and followed by CSD and CSDG, differences being non significant between chopping and CSD.

On **PCT with PP**, these polyphenols (TP, NTP, TT, CT and HT) were further reduced up to 64.07, 24.95, 72.67, 59.53and 83.35%, respectively. Reduction was maximum due to physical treatment CSDG followed by CSD and chopping for TP, NTP, TT and CT; whereas for HT maximum reduction was due to chopping, followed by CSD and CSDG. Among the chemical treatments, 0.1% PP was found to be optimum in reducing ($P< 0.0001$) all the polyphenols to the maximum extent. On comparison, between the 16 groups of different treatment combinations (Physical × Chemical), it was observed that the reduction of TP, and CT was significantly ($P < 0.0001$) higher in 16th (CSDG + 0.4 % PP) and 14th (CSDG + 0.1 % PP) treatment; NTP was reduced at maximum level in 14th; TT in 12th (CSD + 0.4 % PP) and HT was in 5th (chopped + water) treatments.

On PCT **with urea**, overall reduction of these polyphenols (TP, NTP, TT, CT and HT) was 62.65, 29.49, 69.87, 59.59 and 78.36%, respectively. Reductions of these phenols (TP, NTP, TT, CT and HT) among the physical treatments ranged from 42.55 to 81.50, -11.88 to 78.07, 54.39 to 85.30, 32.85 to 90.51 and 72.20 to 91.08 %, respectively. Among the physical treatments, reduction was maximum in CSDG followed by CSD for all the polyphenols, except HT which was maximum in CSD; and among the urea treatments, reduction was maximum in 2.5% urea treatment for TP, NTP and CT; where as it was maximum in 1.5% urea treatment for TT, and for HT it was maximum in 0.5% urea treatments. Among the 16 groups of different treatment combinations (Physical × Chemical), maximum reduction for TP and TT was in 10th (CSD + 0.5 % urea); for NTP in 15th (CSDG + 1.5 % urea); for CT in 14th (CSDG + 0.5 % urea) and for HT in 5th (chopped+water) treatment, respectively.

On PCT **with wood ash**, the overall reduction of polyphenols in *F. roxburghii* leaves was 57.17, 29.95, 63.09, 55.77 and 69.13% for

TP, NTP, TT, CT and HT, respectively. Out of four physical treatments, the reduction ($P<0.0001$) of TP, NTP, TT and CT was maximum in CSDG; whereas, HT was reduced at maximum in fresh leaves treated only with water. Among the WA treatments, 5% WA could reduce ($P< 0.0001$) TP, TT and CT at maximum level; whereas, maximum NTP was reduced ($P<0.0001$) in 2.5 to 5% WA, difference between the treatments being non-significant. HT was reduced at maximum level in 5 to 10% WA treatments; differences between the group being non-significant. Among the 16 different treatment combinations (Physical × Chemical), it was revealed that TP, TT and CT was reduced ($P<0.0001$) at maximum level in 15th (CSDG + 5% WA) and 16th (CSDG + 10 % WA) treatments, difference between the treatments being non significant; whereas maximum reduction ($P<0.0001$) of NTP was in 15th (CSDG + 5% WA), and HT was in 4th treatment (Fresh + 10 % WA).

On PCT **with GNC,** overall reduction of TP, NTP, TT, CT and HT were 58.22, 27.67, 64.87, 58.35 and 70.26%, respectively. Among the physical treatment, CSDG reduced the TP, NTP and CT at maximum extent; however, reduction of TT was maximum in CSD and CSDG, differences being comparable between the groups. HT was reduced at 81.73 % (maximum) in CSD. Among the chemical treatments (including water) maximum reduction ($P < 0.0001$) in TP, NTP and CT contents was due to 7.5% GNC treatments; whereas, TT and HT was reduced at maximum level even at 3.4% GNC treatment. On comparing between the 16 different treatments combinations (Physical × GNC), it was revealed that TP and TT was reduced at maximum level in 14th treatment (CSDG + 1.7% GNC); whereas NTP was in 16th (CSDG+7.5%GNC); CT was in 14th (CSDG+1.7%GNC), 15th (CSDG+3.4%GNC) and 16th (CSDG+7.5%GNC) treatments and HT was in 5th treatments (Chopped + Water).

On comparing among the 20 different treatment combinations of five **chemical treatments (water, PP, urea, WA and GNC) and four physical treatments**, maximum (%) reduction of TP was in 17th (CSDG + PP) followed by 19th (CSDG + WA), 13th (CSD + Urea), 12th(CSD + PP), 18th(CSDG + Urea), 15th(CSD + GNC) and 20th (CSDG + GNC) treatments; NTP was in 17th, 20th, 19th, 18th, 15th and 14th(CSD + WA); TT was in 13th, 17th, 19th, 12th, 18th and15th treatments; CT was in 19th, 17th, 18th and 20th, differences being nonsignificant between the treatments within respective polyphenol; reduction of HT was maximum in 6th(Chopped + Water) treatment. Physical treatment, CSDG; and chemical treatment, PP was most effective in reducing most of the polyphenols of *F. roxburghii* leaves.

SECOND PHASE

Looking into the lower level of TP and TT in fresh *F. roxburghii* leaves (6.27 & 5.15%) than that of fresh *Q. leucotricophora* (7.26 & 6.15%); moreover, out of all treatment combinations, reduction of TT was maximum (96.76%) through 0.5% urea treated CSD (CSD 0.5% UTTH) in *F. roxburghii* leaves. Therefore for *in vitro* studies, urea treatment of CSDTH (chopped sundried tremal hay) was selected.

OM, CP, EE, TCHO, NDF, ADF, ash, Ca and P contents were 87.43, 13.92, 4.18, 69.33, 61.88, 57.08, 12.57, 2.59 and 0.27 % in untreated tremal hay (UTH) (control); 85.38, 14.12, 3.99, 67.27, 60.0, 56.56, 14.62, 2.41 and 0.22% in CSD 0.5% urea treated tremal hay (UTTH); 85.13, 14.20, 4.01, 66.92, 58.31, 56.27, 14.87, 2.39 and 0.24% in 1.5% urea treated chopped sundried tremal hay (CSD 1.5% UTTH); and 84.45, 14.23, 4.40, 65.82, 57.99, 53.71, 15.55, 2.33 and 0.22% in 2.5% urea treated CSDTH (CSD 2.5% UTTH).

Total (net) gas production was significantly ($P<0.0001$) more (26.33ml) in CSD 0.5% UTTH; followed by UTH, CSD 2.5% UTTH

and CSD 1.5% UTTH; difference being non significant between the later two treatments. However, ammonia nitrogen (NH3-N) (mg/ 30ml) was obviously more (P <0.0001) in CSD 2.5% UTTH followed by 1.5 and 0.5% urea treated CSDTH and was lowest (P <0.0001) in UTH (control).

Methane percentage in total gas was higher (P=0.003) in CSD 2.5% UTTH, followed by CSD 1.5% UTTH and CSD 0.5% UTTH, though the differences being non significant between the different levels of UTTH. It was significantly (P=0.003) less in UTH (control). Net methane production (ml/ 200mg of substrate) was higher (P = 0.003) in CSD 0.5% UTTH, followed by 2.5% and 1.5% urea treated CSDTH, and was lowest in control; differences being nonsignificant between control, 1.5 and 2.5% urea treated CSDTH. Protozoal count was significantly (P<0.0006) higher in UTH than that of urea treated hays, differences being nonsignificant among them. *In vitro* true degradability of dry matter (IVDMTD %) was significantly higher (P<0.0001) in UTH, followed by 0.5%, 1.5 and 2.5% urea treated CSDTH. Difference between UTH and CSD 0.5% UTTH was not significant.

Overall proportion of acetic, propionic and butyric acid was 73.42, 21.16 and 5.42%, respectively. Proportion of acetic acid was higher (P<0.05) in CSD 0.5% UTTH, followed by 1.5% and 2.5% urea treated TH; and was minimum in UTH. Differences were nonsignificant among the groups for propionic acid, butyric acid and A: P ratios.

Digestible organic matter (DOM) (%), digestible energy (DE) and metabolisable (ME) values were significantly higher in CSD 0.5% UTTH, followed by UTH, 1.5 and 2.5% urea treated CSDTH; difference between the later two being nonsignificant. Respective values for DOM% were 51.91, 54.30, 43.61 and 46.92% in UTH, 0.5, 1.5 and 2.5% urea treated CSDTH.

THIRD PHASE

OM, CP, EE, TCHO, NDF, ADF, cellulose, hemicelluloses, ADL and total ash, Ca and P content were 87.86, 19.44, 4.10, 64.33, 39.83, 33.62, 24.40, 6.22, 9.22, 12.4, 3.24 and 0.26 % in of buil hay; 88.1, 12.81. 3.37, 71.92, 60.89, 55.04, 37.73, 5.85, 17.34, 14.90, 2.57 and 0.24% in UTH; 88.71, 15.01, 3.64, 70.07, 61.25, 54.52, 37.09, 6.73, 17.43, 11.29, 2.48 and 0.24% in CSD 0.5% UTTH; and 91.03, 23,35, 1.91, 65.77, 18.07, 12.83, 8.92, 5.24, 3.90, 8.97, 1.68 and 0.81% in concentrate mixture, respectively. TT, CT and HT content was 0.39, 0.29 and 0.01% in biul hay; 5.17, 2.38, and 2.79 in UTH; 0.04, 0.03 and 0.01 in CSD 0.5% UTTH.

Feeding trial in gaddi goats was initiated at 18.82 ± 1.08kg body weight. During adaptation period, body weight went on reducing till 21st day feeding trial, on attaining 18.35, 18.08 and 18.47kg in control (biul hay) (Gr 1), UTH (Gr 2) and CSD 0.5% UTTH (Gr 3), on loosing live weight, average daily gain (ADG) and ratio of dry matter intake (DMI): gain of - 0.37, - 0.84, and -0.34kg; -33.57, -44.36 and -25.50 g/d; and -0.20, -15.69 and -35.42, in respective group. Total feed/ DMI (kg/d) of goat was significantly ($P < 0.02$) higher in control (0.70kg) followed by UTH and CSD 0.5% UTTH, being comparable between control and Gr 2. Crude protein intake (CPI): gain was 0.01, -0.02 and -0.55 in control, Gr 2 and Gr3 respectively, differences between groups being nonsignificant.

Weekly body weight of the goats went on increasing from 3rd week in group 1 (control) and 3 (CSD 0.5% UTTH); and from 4th week onwards in 2nd group; and attained body weight of 21.55, 20.76 and 20.08kg at 13th week of feeding trial in Gr.1 (biul hay), Gr 2 (UTH) and Gr 3, respectively. Body weights between the groups did not differ significantly in any of the weeks. However, feed intakes of goats in

respective groups differ significantly in 4th, 6th, 11th, 12th and 13th weeks. Total body weight gain was highest (P < 0.007) in Gr 1 (3.20 kg) followed by Gr 2 (2.69kg) and lowest in Gr 3 (1.86 kg). However, ADG (g/d) was comparable between the goats in Gr 2 (32.99 g) and 3 (27.49), being significantly higher (P<0.0001) in Gr 1 (43.48). Total DMI (kg/d) of the goats was 0.77, 0.74 and 0.75 in group 1, 2 and 3; values differing significantly (P<0. 0001) between the groups. Feed: gain and DMI: gain was significantly lower (P<0.003) in Gr 1, followed by Gr 2, difference being nonsignificant; and was higher (P < 0.003) in Gr 3. However, the CPI: gain ratio was lowest (P=0.006) in Gr 2, followed by Gr 1 and was highest in Gr 3; difference being nonsignificant between Gr 1 and 2, and Gr. 2 and 3.

Digestibility was 69.76, 63.76 and 68.56% of DM; 78.39, 73.30 and 77.18% of OM; 68.03, 65.14 and 67.35% of CP; 81.17, 86.18 and 89.16% of EE; 75.87, 72.31 and 75.48% of TCHO in biul hay (Gr 1), UTH (Gr 2) and CSD 0.5% UTTH (Gr 3), respectively. Values of DM and OM digestibility being higher (P< 0.01) in groups 1 and 3 than that in group 2, values being comparable between biul hay and CSD 0.5% UTTH diets. Digestibilities of CP, EE and TCHO of respective diets did not differ significantly between the groups. However, NDF digestibility was significantly (P< 0.01) higher in Gr.1 (64.61%) followed by the Gr 3 (63.99) and was lowest (P < 0.01) in Gr 3 (62.60). Digestibility of ADF (60.74 to 61.70%), cellulose (45.12 to 47.07%) and hemicelluloses (69.52 to 71.29%) did not differ significantly among the groups.

Urea treatment of CSDTH increased (P< 0.015) TDN value of diet in group 3 (66.30) than that of the diet based on UTH in group 2 (62.53). However, TDN value of biul hay based diet in group 1 (65.83%) did not differ significantly from that of CSD 0.5% UTTH in Gr 3. DCP

was 13.26, 8.28 and 10.54 % in Gr 1, 2 and 3, respectively, being significantly (P< 0.02) higher in biul hay based diet, followed by CSD 0.5% UTTH and lowest in UTH based diet based.

Body weight (BW) of goats during metabolism trial ranged from 19.89 to 20.76 kg; DMI from 704 to 729 g/d, 72.83 to 77.76 g/$W^{0.75}$/ d and 3.43 to 3.70% BW; and total digestible nutrients (TDN) intake from 452 to 483 g/d; difference being nonsignificant among the groups. However, on expressing the TDN intake per kg metabolic body size, it became significantly (P< 0.021) higher in Gr 3 (51.56), followed by Gr 1 (47.93) and 2 (46.99); differences being non significant between Gr 1 and 2.

CP and digestible crude protein (DCP) intake was (g/d) was higher (P< 0.016) in Gr 1(138 and 94 g/d) followed by Gr 3 (120 and 77 g/d) and was lowest in Gr 2 (99 and 60 g/d); difference between Gr 1 and 3 being nonsignificant. CP and DCP intake (g/kg$W^{0.75}$/d), was highest (P< 0.0001) in Gr 1 (14.33, 9.64), followed by Gr 3 (12.84, 8.20) and was lowest in group 2 (10.24, 6.22).

N- intake (g/d) of goats was 22.15, 15.80 and 19.16 in Gr 1, 2, and 3; being significantly higher (P< 0.016) in Gr 1 and 3 than that in Gr 2. Similarly, N balance was higher (P< 0.001) in group 1 (11.19 g/ d) followed by Gr 3 (11.02 g/d) and was lowest in Gr 2 (7.90 g/d). Values of N- balances, as % intake and absorbed, ranged between 50.00 to 57.22 and 96.28 to 97.83%; the difference among the groups being non significant. N- balance when expressed as gain per kg metabolic size, it was comparable between Gr 1 (1.15) and 3 (1.17), but lower (P< 0.005) in group 2 (0.82).

Ca intake (g/d) and balance (g/d) was higher (P < 0.044) in goats fed biul hay based diets (Gr 1); differences between the goats fed on UTH and CSD 0.5% UTTH based diets being non significant. Ca

balance (g/d and g/kgW$^{0.75}$/d) was higher (P< 0.05) (6.47 and 0.69) in Gr 1, followed by in Gr 3 (4.5 g and 0.49) and 2 (4.18 and 0.44); differences between Gr 3 and 2 being non significant. Ca balances as % intake or absorbed did not differ between the groups.

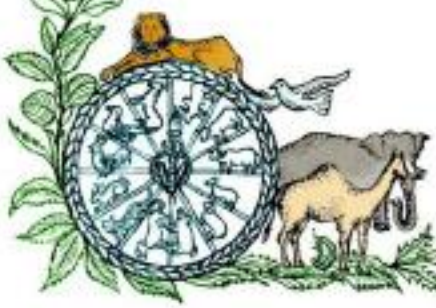
P intake, outgo through faeces and urine, and balances did not differ between the groups. Intake of P was 2.39, 2.36, 2.16 and balance (g/d) was 1.16, 1.17 and 0.98 in Gr 1, 2 and 3, differences between the groups being nonsignificant.

Blood parameters (viz. glucose, total protein, albumin, globulin, A: G ratio, urea, blood urea nitrogen (BUN), creatinine, total bilirubin, total cholesterol, triglycerides, serum enzyme and serum mineral profile) estimated in the serum were within normal range in all the goats fed on respective diets, without any significant differences among the groups.

On the basis of present study it is concluded that

- All the physical (Chopped, Chopped sun dried and Chopped Sun dried ground) and Chemical (Pottasium Permanganate, Urea, Wood ash and Ground Nut cake) treatments significantly reduced tannins of both *Ficus roxburghii* (tremal) and *Quercus leucotrichophora* (bun) leaves.
- Chopped sun dried *F. roxburghii* leaves can be safely fed to gaddi goats along with concentrates as an alternative basal diet.

Mini Abstract



7 Mini Abstract

An experiment was conducted to detannify the locally available *Ficus roxburghii* (tremal) and *Quercus leucotrichophora* (bun) leaves to develop an alternate diet for gaddi goats. Collected leaves were first subjected to four physical treatments [fresh; chopped; chopped & sun dried (CSD); CSD & grinded (CSDG)]. Physically treated leaves were further subjected to four chemical treatments at four different concentrations (% w/w) [(i) Potassium permanganate (PP); (ii) Urea; (iii) Wood ash (WA); (iv) Ground nut cake (GNC);]. On the basis of lower tannin content and higher rate of detannification observed, ***in vitro*** degradability, DE and ME values of urea treated tremal hay were estimated. For further *in vivo* evaluation, 12 growing gaddi goats (18.82±1.08kg**)** were divided into three groups (Gr) of four animals each. The goats in Gr 1(control), 2, and 3 were offered *ad lib Grewia optiva* (biul), tremal and 0.5% urea treated CSD (UTCSD) tremal hay, respectively. Concentrate mixture was supplemented in all the groups throughout the feeding trial (90 days) for 40g average daily gain (ADG). Daily feed intake, weekly changes in body weight and blood biochemical changes (0 and 90 days) were recorded. A metabolism trial in each goat was conducted at 77 days of feeding trial.

All the physical treatments were effective (P<0.01) in reducing all the polyphenols in both the leaves. Maximum reduction (P<0.01) was due to CSDG followed by chopped and CSD in bun leaves. Whereas, in tremal leaves maximum reduction of TP, TT and HT was due to chopping, and reduction of NTP and CT was maximum due to CSDG. On physical along with chemical treatments, effect of CSDG and PP was maximum in reducing (P<0.01) most of the polyphenols of bun and tremal leaves. Maximum reduction of TP, NTP, TT, CT and HT of bun was in Groups CSDG+7.5% GNC, CSD+0.4% PP, CSDG+3.4% GNC, CSDG+0.4% PP and CSDG+3.4% GNC treatments; and of tremal was in CSDG+0.4% PP, CSDG+0.1% PP, CSD+0.5% Urea, CSDG+5-10% WA, and Fresh +10% WA, respectively. DOM (%), DE and ME (MJ or Mcal /kg) values were higher (P<0.01) in 0.5% UTCSD tremal hay, followed by untreated hay, 1.5 and 2.5% urea treated CSDTH. Body weight gain (kg) and ADG (g) of goats was higher (P < 0.01) in Gr 1 (3.20 & 43.48) followed by Gr 2 (2.69 & 32.99) and 3 (1.86 & 27.49); total gain, feed/DMI:gain, CPI:gain being comparable between Gr 1(biul hay) and 2 (tremal hay). DM, OM and NDF digestibilities (P<0.01), N (P< 0.01) and Ca (P< 0.05) balances (g/d) and DCP (P<0.05) was higher in Gr 1 followed by Gr 3 and 2. However, TDN was higher in Gr 3, followed by Gr 1 and 2, difference being nonsignificant between 1 and 3; P balance and blood biochemical parameters being comparable among the groups. Thus, it is concluded that all the treatments were effective in reducing all the polyphenols; however, TT was reduced to the maximum extent on CSDG+0.4% PP in bun and CSD+0.5% Urea in tremal hays; and CSD tremal hays could be used as an alternate basal diet in gaddi goats without any adverse effect on performance.

लघु सारांश

8 लघु सारांश

प्रस्तुत अध्ययन स्थानीय रूप से उपलब्ध 'ट्रैमल' तथा 'बन' के पत्तों को टैनिन रहित कर गद्दी बकरियों हेतु आहार विकसित करने के लिये चार भौतिक उपचार ताजा, कटा, काटकर सुखया (सीएसीडी) तथा पीसकर भौतिक उपचार के बाद चार रासायनिक उपचार पौटेशियम परमैग्नेट (पीपी) युरिया लकड़ी राख (डब्ल्यूए) मूंगफली खल (जीएनसी) कम ट्रैनिन की मात्रा तथा अंत्र पात्र टैनिन विरहण की उच्च दर से युरिया उपचारित ट्रैमिल शुष्कीय पाचक तथा उपापचयी उर्जा आंकलित की गयी। अंत जीवे मूल्यांकन हेतु 12 बढ़ती हुयी गद्दी बकरियों (18.82±0.8 किग्रा.) को तीन सम समूहों में विभाजित किया गया। समूह प्रथम (नियंत्रित), द्वितीय एवं तृतीय समूह क्रमशः भरपेट बियुल, ट्रेमल एवं 0.5 प्रतिशत युरिया उपचारित की एसडी ट्रेमल भूसा दिया गया। 40 ग्राम प्रतिदिन के दर से दाना सभी समूहों में 90 दिन तक दिया गया। दैनिक आहारण साप्ताहिक शरीरभार तथा जैवरासायनिक परिवर्तन 0 से 90 दिन तक दर्ज किया गया। पोषण के 77वें दिन पर चयापचय परीक्षण भी किया गया।

सभी भौतिक उपचार दोनों ही पत्तों में पोलिफिनोल्स की मात्रा कम करने से प्रभावी थे। अधिकतम कमी क्रमशः सीएसडीजी, कटा हुआ तथा काटकर सुखये बन पत्तों में पायी गयी जबकि ट्रेमल पत्तों के क्रमशः टीपी, टीटी तथा एएसटी में अधिकतम कमी काटने से तथा एनटीपी एवं सीटी में अधिकतम कमी सीएसडीजी से हुई। भौतिक एवं रासायनिक दोनो का ही प्रभाव सीएसडीजी एवं पीपी का अधिकतम पोलिफिनोल कम पाया गया। बन के पीपी, एनटीपपी, टीटी, सीटी एवं एचटी में अधिकतम कमी सीएसडीजी ±7.5% जीएनसी, सीएसडी ±0.4% पीपी, सीएसडीजी ±3.4% जीएनसी, सीएसडीजी ±01% पीपी एवं सीएसडीजी ±3.4% जीएनसी उपचार समूह जबकि ट्रेमल पत्तों में क्रमशः सीएसडीजी ±0. 4%, पीपी, सीएसडीजी ±0.1% पीपी सीएसडीजी ±0.5% युरिया सीएसडीजी ±5–10% उब्ल्यूए तथा ताजा ±10% डब्ल्यूए में पाया गया। डीओएम (%) डीई एवं एमई (एमजे (या) एमकैल/किग्रा) उपचार मूल्य 0.5% युटीसीएसडी ट्रेमल शुष्क, अनउपचारित शुष्क तथा 1. 5% एवं 2.5% युरिया उपचारित सीएसडीटीएच शरीर भार वृद्धि (किग्रा) तथा औसत दैनिक वृद्धि (ग्राम) समूह 1 (3.20 एवं 43.48) समूह 2 (2.69 एवं 32.99) एवं समूह 3 (1.86 एवं 27. 49) कुल वृद्धि आहार डीएमआईः वृद्धि सीपीआईः वृद्धि समूह 1 (बियुल शुष्क) तथा समूह 2 (ट्रैमल शुष्क) में तुलनात्मक पायी गयी। डीएम, ओएम एवं एनडीएफ पाचंकतायें एन, सीए संतुलन एवं डीसीपी उच्च समूह प्रथम, तृतीय एवं द्वितीय पायी गयी। जबकि डीडीएन समूह 3 में अधिक तत्पश्चात समूह 1 एवं 2 तथा 1 एवं 3 में असार्थक पी संतुलन एवं जैवरायायनिक प्राचल समूहों के बीच तुलनात्मक पाये गये। अंततः यह निष्कर्षित किया गया कि सभी उपचार पोलिफिनोल कम करने में असरदार पाये गये। जबकि टीटी में अधिकतम कमी दिन में सीएमडीजी 0.4ः पपीपी से तथा ट्रेमल शुष्क से सीएसडी 0.5ः युरिया एवं सीएसडी ट्रेमल शुष्क पाई गयी वैकल्पिक आधारित आहार गद्दी बकरियों के सामान्य है दिन की हेतु बिना किसी प्रतिकूल प्रभाव के उपयोग किया जा सकता है।

References

Bibliography

A.O.A.C. 1990. Official Methods of Analysis (15th. ed.), Association of Official Analytical Chemists, Arlington,Virginia, U.S.A.

A.O.A.C. 2000. Official Methods of Analysis,17th. ed., Association of Official Analytical Chemists, Washington D.C., U.S.A.

Aerts, R.J., McNabb, W.C., Molan, A., Brand, A., Barry, T.N. and Peters, J.S. 1999. Condensed tannins from *L. corniculatus* and *L. pedunculatus* exert different effects on the *in vitro* rumen degradation of Rubisco protein. *Journal of Science Food and Agriculture,* 79: 79-85.

Akter, Y., M. A. Akbar, M. Shahjalal and T.U. Ahmed. 2004. Effect of urea molasses multi-nutrient blocks supplementation of dairy cows fed rice straw and green grasses on milk yield, composition, live weight gain of cows and calves and feed intake. *Pakistan Journal of Biological Science,* 7(9):1523-1525.

Al-Dobaib.S.N. 2009.Effect of different levels of quebracho tannin on nitrogen utilization and growth performance of Najdi sheep fed alfalfa *(Medicago sativa)* hay as a sole diet.. *International Journal of Animal Science,* 80:532-541

Allain, C.A. 1974. *Clinical Chemistry,* 20: 470.

Alonso-Diaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A. and Hoste, H. 2010.Tannins in tropical tree fodders fed to small ruminants: A friendly foe? *Small Ruminant Research,* 89(2-3):164-173.

Amayta, S.M. 1990. Fodder Trees and their Lopping Cycle in Nepal. B.M. Amatya, Tahachal, Kathmandu, Nepal.

Anandan, S. and Dey, A. 2000. Nutritive value of oak (*Q. semecarpifola*) leaves for goats. *Indian Journal of Animal Nutrition,* 17: 84-86.

Animut, G, Goetsch, A,L., Puchala, R., Patra, A.K., Sahlu, T. and Varel, V.H. 2008. Methane emission by goats consuming different sources of condensed tannins. *Animal Feed Science and Technology*, 144:228-241.

Armstrong, W.D., Featherston, W.R., and Rogler, J.C. 1973. Influence of methionine and other dietary additions on the performance of chicks fed bird resistant, sorghum grain diets. *Poultry Science,* 52: 1592–1599.

Asquinth, T.N. and Butler, L.G. 1986. Interaction of condensed tannins with selacted proteins. *Phytochemistry*, 25: 1591-1593.

Athanasiadou, S., Kyriazakis, I., Jackson, F. and Coop, R.L. 2000. Effects of short term exposure to condensed tannins on adult *T. columbriformis.Veterinary Records,* 146: 728-732.

Aufrere, J., Dudilieu, M and Ponce, C. 2008. *In vivo* and *in situ* measurements of the digestive characteristics of sainfoin in comparison with lucerne fed to sheep as fresh forages at two growth stages and as hay. *Animal,* 2:1331 -1339.

Bagheripour, E., Rouzbehan, Y and Alipour, D. 2008. Effects of ensiling, air-drying and addition of polyethylene glycol on in vitro gas production of pistachio by-products. *Animal Feed Science and Technology,* 146:327–336.

Banerjee, G.C., 1988. Feeds and Principles of Animal Nutrition. Oxford and IBH Publishing Co Ltd., Calcutta.

Barahona, R., Lascano, C.E., Cochran, R., Morrill, J. and Totgemeyer, E.C. 1997. Intake, digestion, and nitrogen utilization by sheep fed tropical legumes with contrasting tannin concentration and astringency. *Journal of Animal Science,* 75: 1633-1640.

Barman, K. and Rai, S.N. 2000. Role of tannins in plant-animal relationship: a review. *Indian Journal of Dairy Sciemce,* 53: 390-410.

Barry, T.N and Manley,T.R.1984.The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. *British Journal of Nutrition,* 51:493-504.

Barry, T.N. 1985.The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. *British Journal of Nutrition,* 54: 211- 217.

Barry, T.N. and Manley, T.R. 1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus spp.* and their possible consequences in ruminant nutrition. *Journal of Science Food and Agriculture,* 37: 248-254.

Barry, T.N. and McNabb, W.C. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition,* 81:263-272.

Barry, T.N., McNeill, D.M. and McNabb, W.C.2001. Plant secondary compounds; their impact on forage nutritive value and upon animal production. *In: Gomide, J.A., Mattos, W.R.S., da Silva, S.C. (Eds.), Proceedings of the XIX International Grasslands Congress*, 2001/ 02, Sao Paulo, Brazil, pp. 445–452.

Beauchemin, K.A., McGinn, S.M., Martinez, T.F and McAllister, T.A. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *International Journal of Animal Science,* 85:1990-1996.

Begovic, S, E., Duzic, A., Sakirbegovic. and Tafro, A. 1978. Examination of tannase activity in ruminal contents and mucosa of goat fed oak leaves and during intraruminal application of 3 10% tannic acid. *Veterinaria*, 4: 445-457.

Belenguer, A., Hervás, G., Yáñez-Ruiz, D.R., Toral, P.G., Ezquerro, C and Frutos, P. 2010. Preliminary study of the changes in rumen bacterial populations from cattle intoxicated with young oak (*Quercus pyrenaica*) leaves. *Animal Production Science* 50, 228–234.

Belewu, M.A and Olajide , J.O 2010. Lesser known seed: A renewable pathway for sustainable animal production in Africa. Journal of Sustainable Development in Africa. 12 (3):179-188. *ISSN: 1520-5509; Clarion University of Pennsylvania, Clarion, Pennsylvania.*

Ben Salem, H and Smith, T. 2008. Feeding strategies to increase small ruminant production in dry environments *Small Ruminant Research.*, 77 (2-3), pp. 174-194.

Ben Salem, H ., Saghrouniab, L. and Nefzaouia, A. 2005. Attempts to deactivate tannins in fodder shrubs with physical and chemical treatments. *Animal Nutrition and Feed Technology,* 122 (1): 109-121.

Ben Salem, H., Nefzaouia, A., Ben Salem, L., and Tisserand, J.L.1999. Intake, digestibility, urinary excretion of purine derivatives and growth by sheep given fresh, air-dried or polyethylene glycol-treated foliage of *Acacia cyanophylla* Lindl. *Animal Nutrition and Feed Technology*, 78 (3): 297-311.

Benchaar, C and Chouinard, P.Y. 2009. Assessment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. *Journal of Dairy Science* 92:3392 -3396 .

Benchaar,C., McAllister, T.A and Chouinard, P.Y. 2008. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extracts. *Journal of Dairy Science,* 91:4765-4777.

Bhar, R. and Katiyar, R.C. 1996. Effect of feeding alkali treated mahua (Bassia latifolia) seed cake on important rumen parameters (in vivo) and nutrient utilization in growing crossbred bulls. *Indian Journal of Animal Sciences,* 66: 919-923.

Bhat, T.K., Singh, B. and Sharma, O.P. 1998. Microbial degradation of tannins - A current perspectives. *Biodegradation*, 9: 343-357.

Bhat, T.K., Singh, B. and Sharma, O.P. 2009. Plant secondary metabolites for animal production: Chemistry to function. *Proceedings of Animal Nutrition Association World Conference.* 14-17 Feb 2009, New Delhi, pp 178-180.

Bhatta, R., Krishnamurty, U and Mohammed, F. 2000. Effect of feeding tamarind *(Tamarindus indica)* seed husk as a source of tannin on dry matter intake, digestibility of nutrients and production performance of cross-bred dairy cows in mid lactation. *Animal Feed Science and Technology,* 83:67-74.

Bhatta, R., Krishnamoorthy, U and Mohammed, F. 2001. Effect of tamarind *(Tamarindus indica)* seed husk tannins on *in vitro* rumen fermentation. *Animal Feed Science and Technology,* 90:143-152.

Bones, R.W. and Taussky, H.H. 1945. On the colorimetric determination of creatinine by Jaffe reaction. *Journal of Biological Chemistry*, 158: 581-591.

Brandis D. 1984. Indian trees. BSMPS, Dehra, India.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L and Ferret, A. 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science,* 90, 2580–2595.

Carulla, J.E, Kreuzer, M., Machmuller, A and Hess, H.D. 2005. Supplementation *of Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agriculture Research,* 56:961 -970 .

Chen, X.B. and M.J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details. An occasional publication of the Rowett Research Institute. Bucksburn, Aberdeen.

Cheng, K.J, McAllister, T.A., Popp, J.D., Hristov, A.N., Mir, Z and Shin, H.T. 1998. A review of bloat in feedlot cattle. *Journal of Animal Science,* 76:299-308.

Chiquette, J., Cheng, K.J., Rode, L.M and Milligan, L.P.1989. Effect of tannin content in two isosynthetic strains of birdsfoot trefoil *(Lotus corniculatus)* on feed digestibility and rumen fluid composition in sheep. *Canadian Journal of Animal Science* 69:1031 -1039

Coop, R.L. and Holmes, P.H. 1996. Nutrition and parasitic interaction. *International Journal of Parasitology*, 26: 951-962.

CSIR. 1969. The Wealth of India. A dictionary of Indian raw materials and industrial products. Raw materials, Vol. 8: Ph-Re, New Delhi, India pp 151-153.

Das, A.J., Battles, J.J., van Mantgem, P.J., Stephenson, N.L., 2008. Spatial elements of mortality risk in old-growth forests. *Ecology* 89, 1744–1756.

Deshpande, S.S., Cheryan, M. and Salunkhe, D.K. 1986. Tannin analysis of food products. *CRC Critical Review of Food Science and Nutrition.* 24: 401–449.

Devarajan, S. 1999. Effect of tannins on the ruminal degradation kinetics of locally available tree forages. M.V.Sc. Thesis. Deemed University, IVRI, Izatnagar, India. pp 59- 64.

Dhyani, D., Shalini, R.K and Maikhuri, K.G.2011. Energy budget of fodder harvesting pattern along the altitudinal gradient in Garhwal Himalaya, India. *Biomass & Bioenergy*, 35: 1823-1832.

Dollahite, J.W., Pigeon, R.F. and Camp, B.J.1962. The toxicity of gallic acid, pyrogallol, tannic acid, and Quercus hauardi in the rabbit. *American Journal of Veterinary Research*, 23:1264.

Douglas, G.B., Stienzen, M., Waghorn, G.C and Foote, A.G. 1999. Effect of condensed tannins in birdsfoot trefoil *(Lotus corniculatus)* and sulla *(Hedysarum coronarium)* on body weight, carcass foot depth, and wool growth of lambs in New Zealand. *New Zealand Journal of Agriculture Research,* 42:55 -64 .

Doumas, B.T. Watson, W.A. and Briggs, H.G. 1971. Albumin standards and the measurement of serum albumin with Bromocresol green. *Clinica Chimca Acta*, 31: 87-96.

Fadel Elseed, A.M.A. 2005. Effect of supplemental protein feeding frequency on ruminal chracteristics and microbial N production in sheep fed treated rice straw. *Small Ruminant Research,*57:11-17.

Fadel Elseed, A.M.A., Sekine, J., Hishinuma, M and Hamana, K. 2003. Effects of ammonia, urea plus calcium hydroxide and animal urine treatments on chemical composition and in sacco degradability of rice straw. *Asian-Ausralian Journal of Animal Science,* 16:368-373.

FAO, 2010. FAOSTAT. Food and Agriculture Organisation of the United Nations. Rome.http://faostat.fao.org/default.aspx

Field, J., Lettinga, G and Habets, L.H.A. 1990. Measurement of low molecular weight tannins: Indicators of methanogenic toxic tannins. *Journal of Fermentation and Bioengineering*, 69 (3), pp. 148-153.

Field, J.A and Lettinga, G. 1987. The methanogenic toxicity and anaerobic degradability of hydrolysable tannins. *Water Research,* 21:367-394.

Fillipich, L.J., Zhu, J., Oelrichs, P., Alsalami, M.T., Doig,A.J., Cao, G.R. and English, P.B. 1991. Hepatotoxic and nephrotoxic principles in *Terminalia oblongata. Research in Veterinary Science,* 50*:* 170-177.

Finlay, B.J., Esteban, G., Clarke, K.J., Williams, A.G., Embley, T.M and Hirt, R.R. 1994. Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letters,* 117:157-162 .

Fish, B.C. and Thompson, L.U.J. 1991. Lectin tannin interactions and their influence on pancreatic amylase activity and starch digestibility. *Journal of Agricultural and Food Chemistry*, 39: 727-731.

Foo, L.Y., Newman, R., Waghorn, G.C., McNabb, W.C and Ulyatt, M.J. 1996. Proanthocyanidins from *Lotus corniculatus. Phytochemistry* 41:617-624

Foo, L.Y., McNabb, W.C., Waghorn, G.C. and Ulyatt, M.J. 1997. Proathocyanidin from *Lotus pedunculatus*. *Phytochemistry*. 45: 1689-1696.

Fowler, M. E. and Richards, W. P. C. 1965. Acorn poisoning in a cow and a sheep. *Jounal of American Veterinary Medicine.*147:1215–1220.

Freeland, W.J., Calcott, P.H. and Anderson, L.R. 1985. Tannins and saponins: interaction in herbivore diets. *Biochemical Systematics and Ecology*, 13: 189-193.

Frutos, P., Hervas, G., Giraldez, F.J and Mantecon, A.R. 2004. Review. Tannins and ruminant nutrition. *Spanish Journal of Agriculture Research,* 2:191 -202 .

Gamble, G.R., Akin, D.E., Makkar, H.P.S. and Becker, K, 1996. Biological degradation of tannins in *Sericea lespedeza* by the white-rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus* analyzed by solid state 13C NMR spectroscopy. *Applied Enviromental. Microbiology.* 62: 3600–3604.

Garg, S,K., Makkar, H.P.S., Nagal, K.B., Sharma, S.K., Wadhwa, D.R. and Singh, B.1992. Toxicological investigations into oak *(Quercus incana)* leaf poisoning in cattle. *Veterinary and Human Toxicology,* 34*:* 161-164.

Getachew, G., Pittroff, W., Dutnam, D.H., Dandekar, A., Goyal, S and Depeters, E.J. 2008. The influence of addition of gallic acid, tannic acid, or quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. *Animal Feed Science and Technology* 140:444-461 .

Getachew, G., Makkar, H.P.S. and Becker, K. 2001. Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. *Animal Feed Science Technology*, 92: 51–57.

Goering, H.K. and Van Soest, P.J. 1970. Forage fibre analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379, ARS-USDA, Washington, DC.

Gomeri, G. 1942. Determination of inorganic phosphate in serum and plasma. *Journal of Laboratory and Clinical Medicine*, 29: 955 – 956.

Graham, H.N. 1992. Green tea composition, consumption and polyphenol chemistry. *Preventive Medicine,* 21: 334-350.

Grainger, C., Clarke, T., Auldist, M.J., Beauchemin, K.A., McGinn, S.M and Waghorn, G.C. 2009. Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* 89:241 -251.

Hart, K. J.,Yanez-Ruiz, D.R.,Duval, S.M., McEwan, N.R and Newbold, C.J. 2008 Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology,* 147:8-35.

Haslam E. 1966. *Chemistry of vegetable tannins.* Academic Press, London and New York.

Haslam, E. 1989 *Plant Polyphenols.* Cambridge University Press, Cambridge.

Haslam, E. and Tanner, R.J.N. 1970. Spectrophotometric assay of tannase activity. *Phytochemisry.* 9: 2305-2309.

Haslam, E., Haworth, R.D., Jones, K. and Rogers. H.J. 1961. *Journal of Chemistry, pp.*1829-1859.

Herbert, K. 1984. Lipids, In clinical Chemistry: Theory, analysis and co-relation, Kaplan LA And Pesce, AJ Eds., CV Mosby, Toronto, 1182-1230.

Hervas, G., Perez, V, Giraldez, F.J, Mantecon, A.R, Almar, M.M and Frutos, P.2003. Intoxication of sheep with quebracho tannin extract. *Journal of Comparative Pathology* 129:44-54.

Hess, H.D., Kreuzer, M., Diaz, T.E., Lascano, C. E., Carulla, J.E and Soliva, C.R. 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated fluid. *Animal Feed Science and Technology* 109:79-94.

Holliman, A. 1985. Acorn poisoning in ruminants. *Veterinary Records,* 116546

Horigome, T., Ohkuma, T. and Muta, M. 1984. Effect of condensed tannins of false acacia leaves on protein digestibility as measured with rats. *Japanese Journal of Zoological Techniques and Science*, 55: 299–306.

Hoskin, S.O., Wilson, P.R., Barry, T.N., Charleston, W.A.G. and Waghorn, G.C. 2000. Effect of forage legume containing different condensed tannin content on internal parasites in young red deer (*C. elephus*). *Research in Veterinary Science,* 68: 223-230.

Jiban, M .2000.Genetic improvement of fodder trees. *The Indian Journal of Genetics and Plant Breeding,* 60: 4.

John, A and Lancashire, J.A.1981. Aspects of feeding and nutritive value of Lotus species. *Proceedings of New Zealand Grasslands Association,* 42:152.

Jones, G.A., McAllister, T.A., Muir, A.D. and Cheng, K.J. 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied Environmental Microbiology,* 60: 1374-1378.

Jones, W.T and Mangan, J.L. 1977. Complexes of the condensed tannins of sainfoin *(Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by polyethelene glycol and pH. *Journal of Science Food and Agriculture,* 28:126-136.

Jones, W.T., Anerson, L.B and Ross, M.D. 1973. Bloat in cattle, XXXIX. Detection of protein precipitants (flavolans) in legumes. *New Zealand Journal of Agriculture Research,* 16:441-446.

Jung, H.C and Vogel, K.P. 1986. Influence of lignin on digestibility of forage cell wall material. *Journal of Animal Science*, 62: 1703-1712.

Kamra, D.N., Patra, A.K., Chatterjee, P.N., Kumar, R., Agarwal, N and Chaudhary, L.C. 2008. Effect of plant extract on methanogenesis and microbial profile of the rumen of buffalo: a brief overview. *Australian Journal of Experimental Agriculture,* 48:175-178.

Kawas, J.R., Andrade-Montemayor, H and Lu, C.D. 2010. Strategic nutrient supplementation of free-ranging goats. *Small Ruminant Research*, 89 (2-3), pp. 234-243.

Khanal .R.C and Upreti C.R . 2008. Evaluation of Selected Species of Tree Fodders Cultivated for Feeding Ruminants in the Hills of Nepal. *Pakistan Journal of Nutrition,* 7 (2): 297-302.

Khanal, R.C and Subba, D.B. 2001. Nutrional evaluation of leaves from some major fodder trees cultivated in the hills of Nepal. *Animal Feed Science and Technology,* 92(12): 17-32

Kiangi, E.M.I and Kategile, J.A. 1981. Different sources of ammonia for improving the nutritive value of low quality roughages. *Animal Feed Science Technology,* 6: 377-386.

Kingsbury, M.J., 1964. Poisonous Plants of the United States and Canada. Prentice-Hall, Engelwood Cliffs NJ, USA.

Komolong, M.K, Barber, D.G and McNeill, D.M. 2001. Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucene hay *(Medicago sativa)* with increasing level of quebracho tannins. *Animal Feed Science and Technology* 92:59-72.

Krishnamoorthy,U., Soller, H., Steingass, H. and Menke, K.H. 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies *in vitro*. *Animal Feed Science and Technology*. 52: 177-188.

Kumar, A, 1992.Legume trees and other fodder trees as protein sources for livestock, *FAO Animal Production and Health Paper, 102*.

Kumar, R and Vaithiyanathan, S. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. *Animal Feed Science and Technology,* 30:21-38.

Kumar, R. and Horigome,T. 1986. Fractionation, characterization and protein precipitating capacity of the condensed tannins from *R. pseudoacacia* leaves. *Journal of Agriculture and Food Chemistry,* 34: 487-489.

Kyarisiima, C.C., Okot, M.W. and Svihus, B. 2004. Use of wood ash in the treatment of high tannin sorghum for poultry feeding. *South African Journal of Animal Science,* 34 (2):110-115.

Lewis, J.A and Starkey, R.L. 1969. Decomposition of plant tannins by some soil microorganisms. *Soil Science,* 107: 235-241.

Li, Y.G., Tanner, G. and Larkin, T. 1996. The DMCA-Hcl protocol and the threshold proanthocyanidin content of bloat safety in forage legumes. *Journal of Science Food and Agriculture,* 70: 89-101.

Lohan, O.P., I.all, D., Vaid, J. and Negi, S.S. 1983. Utilization of Oak tree (Quercus incana) fodder in cattle rations and fate of Oak leaf tannins in the ruminant system. *Indian Journal of Animal Sciences,* 53: 1057-1063.

Lowry, J.B., McSweeney, C.S. and Palmer, B. 1996. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied Environmental Microbiology,* 60: 1374-1378.

Maia, M.R., Chaudhary, L.C., Figueres, L and Wallace, R.J. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. *Anton van Leeuwen,* 91:303-314.

Majumdar, B.N and Momin, S.A. 1960. Studies on tree leaves as cattle fodder. Part V The Nutritive value of young Gular leaves *(Ficus glomerata).* Indian *Journal of Dairy Science*, 13: 16-19.with use of surfactants as solubilizing agents. *Clinical Chemistry*, 20, 447-453.

Makkar, H. P. S. and Singh, B.1992. Effect of wood ash on tannin content of oak (*Quercus incana*) leaves. *Bioresource Technology.*41: 85–86.

Makkar, H. P. S., Singh, B. and Kamra, D. N. 1994. Biodegradation of tannin in oak (*Quercus incana*) leaves by *Sporotricum pulverulentum*. *Letters of Applied Microbiology*, 18: 42–44.

Makkar, H.P. S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds: review. *Small Ruminant Research.*49:241–256.

Makkar, H.P.S and Singh, B. 1991. Effect of drying conditions on tannin, fibre and lignin levels in mature oak (*Quercus incana*) leaves. *Journal of Science Food and Agriculture,*, 54: 323–328.

Makkar, H.P.S and Singh, B. 1993. Effect of storage and urea addition on detannification and *in sacco* DM digestibility of mature oak (*Quercus incana*) leaves. *Animal Feed Science and Technology,* 41: 247-259.

Makkar, H.P.S. and Becker, K, 1996. Effect of pH, temperature, and time on inactivation of tannins and possible implications in detannification studies. *Journal of Agriculture and Food Chemistry,* 44: 1291–1295.

Makkar, H.P.S., Blummel, M and Becker, K. 1995. *In vitro* effects and interaction between tannins and saponins and fate of tannins in the rumen. *Journal of Science Food and Agriculture,* 69:481 -493.

Makkar, H.P.S., Blummel, M and Becker, K. 1995a. *In vitro* effects and interaction between tannins and saponins and fate of tannins in the rumen. *Journal of Science Food and Agriculture,* 69: 481.

Makkar, H.P.S., Blummel, M. and Becker, K. 1995b. Formation of complexes between polyvinylpolypyrrolidone or polyethylene glycols and tannins and their implications in gas production and true digestibility in *in vitro* techniques. *British Journal of Nutrition,* 73:897.

Makkar, H.P.S., Singh, B and Dawra, R.K. 1987. Tannin-nutrient interactions- a review. *International Journal of Animal Science,* 2:127.

Makkar, H.P.S., Singh, B and Dawra, R.K. 1988. Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. *British Journal of Nutrition,* 60: 287-296.

Mandal, A. B., S. S. Paul, G. P. Mandal, A. Kannan and N. N. Pathak. 2005. Deriving nutrient requirements of growing Indian goats under tropical condition. *Small Ruminant Research,* 58:201-217.

Mandal, S.C., Mukherjee, P.K., Seha, K., Pal, M and Saha, B.P. 1997. Antidiarrhoeal evaluation of *Ficus racemosa* Linn. leaf extract. Natural Products Science. 3, 2,100-103.

Mangan, J.L. 1988. Nutritional effects of tannins in animal feeds. *Nutritional Research Reviews,* 1:209-231.

Marquardt, R.R., Ward, A.T., Campbell, L.D and Cansfield, P,E. 1977. Purification, identification and characterization of a growth inhibitor in faba beans (*Vicia faba* L. minor) and its depressive effect on the appetite and nutrient availability in the chick. *Journal of Nutrition*. 107: 1313–1324.

Mbatha, K.R., Downs, C.T and Nsahlai, I.V. 2002. The effects of graded levels of dietary tannin on the epithelial tissue of the gastro-intestinal tract and liver and kidney masses of Boer goats. *International Journal of Animal Science,* 74:579-586.

McAllister, T.A., Martinez, T., Bae, H.D., Muir, A.D., Yanke, L.J and Jones, G.A. 2005. Characterization of condensed tannins purified from legume forages: chromophore production, protein precipitation and inhibitory effects on cellulose digestion. *Journal of Chemical Ecology,* 31:2049- 2068.

McDonald, P., Edwards, R.A., Greenhagh, J.G.D. and Morgan, C.A. 1995. System for expressing the energy value of feeds. *Animal nutrition (5th edition),* Longman group Ltd, Singapore pp, 236.

McMahon, L.R., Majak,W., McAllister, T.A, Hall, J.W, Jones, G.A and Popp, J.D. 1999. Effect of sainfoin on *in vitro* digestion of fresh alfalfa and bloat in steers. *Canadian Journal of Animal Science,* 79:203-212.

McMahon, L.R., McAllister, T.A., Berg, B.P., Majak, W., Acharya, S.N and Popp, J.D. 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science,* 80:469-485.

McNabb, W.C., Peters, J.S., Foo, L.Y., Waghorn, G.C and Jackson, F.S. 1998. Effect of condensed tannin prepared from several forages on the *in vitro* precipitation of ribulose-1, 5-bisphosphate carboxylase (EC 4.1.1.39;Rubisco) protein and its digestion by trypsin (EC. 2.4.21.4) and chymotrypsin. *Journal of Science Food and Agriculture,* 77: 201-212.

McNabb, W.C., Waghorn G. C., Barry, T. N and Shelton, I. D. 1993. The effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. *British Journal of Nutrition*, 70:647.

McSweeney, C.S., Palmer, B., Bunch, R. and Krause, R. 1999. *In vitro* quality assessment of tannin containing tropical shrub legumes: protein and fibre digestion. *Animal Feed Science and Technology,* 82: 227-241.

McSweeney, C.S., Palmer, B., Mc Neill, D.M. and Krause, D.O. 2001. Microbial interactions with tannins : nutritional consequences for ruminants. *Animal Feed Science and Technology,* 91: 83-93.

Menke, K H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. 'Ihe estimation of the digestibility and metabolisable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agriculture and Food Science,* 93: 217-222.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained by chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Developments,* 28: 7.

Mia, W.H., Majumdar, B.N., Sahai, B and Kehar, N.D. 1960. Studies on tree leaves as cattle fodder: 4. The nutritive value of Pipal leaves (Ficus religiosa). *Indian Journal of Dairy Science*, 13:9-15.

Min, B.R. and Hart, S.P. 2003. Tannins for suppresion of internal parasites. *Journal of Animal Science,* 81: 102-109.

Min, B.R., Attwood, G.T, McNabb, W.C, Molan, A.L and Barry, T.N. 2005. The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Animal Feed Science and Technology* 121:45-58.

Min, B.R., Barry T.N., Attwood, G.T and Mcnabb, W.C. 2003. The effect of condensed tannins on the nutrition of ruminants fed fresh temperate forages: a review. *Animal Feed Science Technology*, 106: 3-19.

Min, B.R., McNabb, W.C., Barry, T.N., Kemp, P.D., Waghorn, G.C. and McDonald, M.S. 1999. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. *Journal of Agricultural Science,* 132: 323-334.

Min, B.R., Pinchak, W.E., Anderson, R.C, Fulford, J.D and Puchala, R. 2006. Effects of condensed tannins supplementation level on weight gain and *in vitro* and *in vivo* bloat precursors in steers grazing winter wheat. *Journal of Animal Science* 84:2546-2554.

Mohammadabadi, T M. Chaji, T.M and S. Tabatabaei. 2010 . The Effect of Tannic Acid on *in vitro* Gas Production and Rumen Fermentation of Sunflower Meal . *Journal of Animal and Veterinary Advances*, 9(2): 277-280 .

Molan, A.L., Duncan, A., Barry, T.N. and McNabb, W.C. 2000a. Effects of condensed tannins and sesquiterpene lactones extracted from chicory on the viability of the deer lungworm larvae. Proc. *New Zealand Society of Animal Production,* 60: 26-29.

Molan, A.L., Hoskin, S.O., Barry, T.N. and McNabb, W.C. 2000b. Effects of condensed tannins and sesquiterpene lactones extracted from four forages on the viability of the deer lungworm larvae and gastrointestinal nematodes. *Veterinary Records,*147:44-48.

Mueller-Harvey, I. 2006. Unravelling the conundrum of tannins in animalnutrition and health. *Journal of Food Science and Agriculture,* 86:2010-2037.

Muhammed, S., Stewart, C.S and Acamovic, T. 1995. Effects of tannic acid, ellagic acid, gallic acid and catechin on cellulose degradation by the rumen fungus *Neocallimastix frontalis* strain RE1. *International Journal of Animal Science,* 60:550.

Mukuru, S.Z. 1992. Traditional technologies in small grain processing. *In: Utilisation of sorghum and millets*. Eds. Gomez, M.I., House, L.R., Rooney, L.W. & Dendy, D.A.V., International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. pp. 47-56.

Mullen Jeffrey, A. and Koller, H. R .1988. Trends in carbohydrate depletion, respiratory carbon loss, and assimilate export from soybean leaves at night. *Plant physiology* 86(2):517-521.

NARC News letter , A quarterly news letter of Nepal Agricultural research council, Volume 15 (1).

Negi, S.S., Pal, R.N and Ehreich, C. 1979. Tree fodders in Himachal Pradesh. German Agency for echnical Co-operation, Eschbom, Germany, 41 pp.

Nesser, J.A., Coettzer, J.A.W., Boomker, J and Cable, H. 1982. Oak (*Quercus robur*) poisoning in cattle. *Journal of South African Veterinary Association,.*, 53:151-155.

Niezen, J.H., Robertson, H.A., Waghorn, G.C and Charleston,W.A.G. 1998. Production, faecal egg count and worm burdens of ewe lambs which grazed six contrasting forages. *Veterinary Parasitology,*. 80:15-27.

Norton, B. W. 2000. The significance of tannins in tropical animal production. In Proceedings of the International workshop on tannins in livestock and human nutrition (Ed. L. P. Brooker). ACIAR, Adelaide. pp. 14-21.

Norton, B.W and Ahn, J.H. 1997. A comparison of pressed and dried *Calliandra calothrysus* supplements for sheep given a basal diet of barley straw. *Journal of Agriculture and Science Cambridge,*. 129: 485-494.

Osti, N.P., C.R. Upreti, N.P. Shrestha and S. B. Pandey 2006. Nutrients contents in fodder trees leaves, grasses and legumes from buffalo growing areas of Nepal. Proceedings of 5th Asian Buffalo Congress held from April 18-22, 2006, Naning China. pp 366-371.

Osti, R., Tanaka, S and Tokioka, T. 2008. The importance of mangrove forest in tsunami disaster mitigation. *Disasters,* 33, 203–213.

Pal, R.N., Dogra, K.K., Singh, L.N and Negi, S.S. 1979. Chemical composition of some fodder trees in. Iimachal Pradesh. *Forage Research,* 5:109-115

Palmer, B. and McSweeney, C. S. 2000. Tannins in Calliandracalothyrsus: effect of polyethylene glycol (PEG) and an evaluation of 19 accessions. In: Brooker, J.D. (Ed.), Tannins in Livestock and Human Nutrition. *Proceedings of the International Workshop*, Adelaide, Australia, May 31–June 2, 1999. ACIAR Proceedings No. 92, pp. 36–39.

Parmar, C and Kaushal, M. K. 1982, Wild Fruits. Kalyani Publishers, New Delhi, India.

Patel, A. V., Rojas-Vera, J and Dacke, C. G. 2004.Therapeutic constituents and actions of *Rubus* species. *Current Medical Chemistry,* 11: 1501-1512.

Patra, A. K and Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of Science Food and Agriculture,* 91: 24–37. doi: 10.1002/jsfa.4152

Patra, A.K and Saxena, J. 2009. Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Anton van Leeuwen* 96:363-375.

Patra, A.K. 2010. Aspects of nitrogen utilization in sheep fed mixed diets containing foliages from trees and browses. *British Journal of Nutrition,* 103:1319-1330.

Patra, A.K., Kamra, D.N., Agarwal, N and Chatterjee, P.N. 2008. Effect of *Terminalia chebula* and *Allium sativum* on rumen fermentation, enzyme activities and microbial counts in buffalo. *Indian Journal of Animal Nutrition,* 24:251-255.

Patra, A.K., Kamra, D.N and Agarwal, N. 2006. Effect of plants containing secondary metabolites on *in vitro* methanogenesis, enzyme profile and fermentation of feed with rumen liquor of buffalo. *Animal Nutrition and Feed Technology,* 6:203 - 213.

Paul, S.S., Kamra, D.N., Sastry, V.R.B and Sahu, N.P. 2006. Effect of adding an anaerobic fungal culture isolated from a wild blue bull *(Boselophus tragocamelus)* to rumen fluid from buffaloes on *in vitro* fibrolytic enzyme activity, fermentation and degradation of tannins and tannin-containing kachnar tree *(Bauhinia variegata)* leaves and wheat straw. *Journal of Food Science and Agriculture,* 86:258-270.

Pearlman, F.C and Lee, R.T.Y. 1974. Detection and measurement of total bilirubin in serum,

Plumee, K. H., Jhonson, B and Galey, F. D. 1998. Disease in cattle dosed orally with oak or tannic acid. In toxic plants and other natural toxicants, CAB International, Wallinford, UK, pp. 549-553.

Prakash S., Bhat, S.R., Quiros, C.F., Kirti, P.B. and Chopra, V.L. 2009. Brassica and its close allies: cytogenetics and evolution. *Plant Breeding Reviews,* 31, 21–187.

Prasad, R. D. D., Reddy, M. R. and Reddy, G. V. N. 1998. Effect of feeding baled and stacked urea treated rice straw on the performance of crossbred cows. *Animal Feed Science and Technology,*73:347-352.

Price M.L., Buttler L. G., Rogler J.C and Featherston W.R. 1979. Overcoming the nutritionally harmful effects of tannin in sorghum grain by treatment with inexpensive chemicals. *Journal of Agriculture and Food Chemistry,*27:441-445.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L and Pennisi, P. 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: effects on lamb growth, performance and meat quality. *Journal of Animal Science* 78:810-816.

Pritchard, D. A., Stocks, D.C., O'Sullivan, B. M., Martin, P. R., Hurwood, I. S. and O'Rourke, P.K. 1988. The effect of polyethylene glycol (PEG) on wool growth and live weight of sheep consuming Mulga (*Acacia aneura*) diet. *Proceeding of Australian Society of Animal Production*, 17: 290–293.

Puchala, R, Min, B.R, Goetsch, A.L and Sahlu, T. 2005.The effect of a condensed tannin-containing forage on methane emission by goats. *International Journal of Animal Science,* 83:182-186.

Ram, C and Ray, S.C. 1943. Tree leaves as cattle fodder. 11.Digestibility coefficient and nutritive value of some tree leaves. *Indian Journal of Veterinary Science and Animal Husbandry,* 13:191-195.

Ramirez-Restrepo, C. A., Barry, T.N, Pomroy, W.E., Lopez-Villalobos, N., McNabb, W.C and Kemp, P.D. 2005a. Use of *Lotus corniculatus* containing condensed tannins to increase summer lamb growth under commercial dryland farming conditions with minimal anthelmintic drench input. *Anim Feed Science and Technology,* 122:197-217.

Ramirez-Restrepo, C.A., Barry, T.N., Lopez-Villalobos, N., Kemp, P.D and Harvey, T.G. 2005b. Use of *Lotuscorniculatus* containing condensed tannins to increase reproductive efficiency in ewes under commercial dryland farming conditions. *Animal Feed Science and Technology* 121:23-43.

Rawel, H., Rohn, S and Kroll, J. 2000. Reactions of selected secondary plant metabolites (glucosinolates and phenols) with food proteins and enzymes: Influence on physico-chemical protein properties, enzyme activity and proteolytic degradation. In "Recent Re-search Developments in Phytochemistry," ed. by S.G. Pandalai. Re-search Signpost, Trivandrum, India, 4, pp. 115–142.

Rayudu, G. V. N., Kadirvel, R., Vohra, P and Kratzer, F, H. 1970. Effect of various agents in alleviating the toxicity of tannic acid for chickens. *Poultry Science*, 49: 1323–1326.

Reed, J.D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science,* 73: 1516-1528.

Reitman, S. and Frankel, S. 1957. Determination of serum glutamic oxaloacetic transaminase and pyruvate transaminase by colorimetric method. *American Journal of Clinical Pathology,* 28: 56.

Robertson, J.B. and Vansoest, P.J. 1989. The detergent system of analysis and its application to human foods. Cornell Uni., Ithaca, New York.

Russell, R.W and Lolley, J, R.1989.Deactivation of tannin in high tannin milo by treatment with urea. *Journal of Dairy Science,* 72(9): 2427-30

Sahnoune, S., Besle, J.M., Chenost, M., Jouany, J.P., and Combes, D., 1991.Treatment of straw with urea. 1. Ureolysis in a low water medium. *Animal Feed Science and Technology,*, 34: 75-93.

Sahoo, A., Singh, B and Bhat, T.K. 2010. Effect of tannins on *in vitro* ruminal protein degradability of various tree forages. *Livestock Research for Rural Development, 22:119.*

Salem, H.B., S. Abidi, H.P.S. Makkar and A. Nefzaou, 2005. Wood ash treatment a cost-effective way to deactivate tannins in *Acacia cyanophylla* Lindl. foliage and to improve digestion by Barbarine sheep. *Animal Feed Science and Technology*, 122: 93-108.

Salem, H.B, Nefzaoui, A, Salem, L.B, Tisserand, J.L, Ben-Salem, H and Ben-Salem, L. 1997. Effect of *Acacia cyanophylla* Lindl. foliage supply on intake and digestion by sheep fed lucern hay-based diets. *Animal Feed Science and Technology,* 68:101 -113.

SAS Institute. 2003. SAS User's Guide. Version 9.1. SAS Inst. Inc.,Cary, NC.

Sastry, V.R.., Kamra, D.N and Pathak, N.N. 1999. Laboratory Mannual of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, India.

Sen, K.C. and Ray, S.N. 1971. Nutritive *Value* of Indian Cattle feeds and Feeding of Animals. Mise Bull. 25, ICAR, New Delhi.

Sen, K.C., Ray, S.N and Ranjhan, S.K. 1978. Nutritive value of Indian Cattle Feeds and the Feeding of Farm Animals. ICAR. New Delhi.

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A.M. and Saura-Calixto, F. 2009. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food. Res.*, 53 (2): S310-329.

Sharma, D. D., Chawla, M. S and Negi, S. S. 1968. Chemical composition and nutritive value of Bamboo (*Bambusa arundinacea*) and Kachnar (*Bauhinia variegata*) tree leaves. *Agra University Journal of Research,* 5: 253-258

Sharma, R.K. 2002. Degradation kinetics and nutrient utilization of tannin rich top feeds. Ph. D Thesis, Indian Veterinary Research Institute, Izatnagar, India.

Sharma, R.K., Singh, B and Sahoo, A. 2008. Exploring feeding value of oak (*Quercus incana*) leaves: Nutrient intake and utilization in calves. *Livestock science*, 118 (1): 157-165.

Sharma, R.K., Singh, B. and Bhat, T. 2000. Nitrogen solubility, protein fractions, tannins and *in sacco* DM digestibility of tree fodders of Shivalik range. *Indian Journal of Animal Nutrition,* 17:1-7.

Shi, Z. C. 1988. Identification of the phenolic substances in bovine urine associated with oak leaf poisoning. *Research in Veterinary Science,*, 45:152.

Shrestha, R.K. and Tiwari, B.N. 1991. Nutritive value and tannin content of some common tree fodder species of the Western Hills of Nepal. LAC Seminar Paper 31/91. Lumle Agricultural Centre, Kaski, Nepal.

Silanikove, N., Gilboa, N. and Nitsan, Z. 1997. Interactions among tannins, supplementation, and polyethylene glycol in goats fed oak leaves. *Animal Science,* 64: 479–483.

Silanikove, N., Gilboa, N., Nir, I., Perevolotsky, A. and Nitsan, Z. 1996. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Quercus calliprinos*, *Pistacia lentiscus* and *Ceratonia siliqua*) by goats. *Journal of Agriculture and Food Chemistry,*. 44: 199–205.

Silanikove, N., Nitsan, Z. and Perevolotsky, A. 1994. Effect of daily supplementation of polyethylene glycol on intake and digestion of tannin containing feeds (Ceratonia siliqua) by sheep. *Journal of Agriculture and Food Chemistry,*. 42: 2844-2847.

Silanikove, N., Prevolotsky, A. and Provenza, F.D. 2001. Use of tannin binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Animal Feed Science and Technology,* 91: 69-81.

Singh P., Verma A.K., Dass R.S., Mehra U.R. 1999. Performance of pashmina kid goats fed oak (*Quercus semecarpifolia*) leaves supplemented with a urea molasses mineral block .*Small Ruminant Research*, 31 (3), pp. 239-244.

Singh, B and Makkar, H.P.S. 2000. The potential of mulberry tree foliage as an animal feed supplement in India. FAO Electronic Conference on Mulberry for Animal Production, April 2000, Rome, Italy.

Singh, B. and Bhat, T.K. 2001. Tannins revisited- changing perceptions of their effects on animal system. *Animal Nutrition and Feed Technology,*1: 3-18.

Singh, B., Sahoo, A,, Sharma, R and Bhat, T. K.2005 Effect of polyethylene glycol on gas production parameters and nitrogen disappearance of some tree forages. Animal Feed Science and Technology 123-124:351-364.

Singh, K.K., Samanta, A.K. and Srinivas, B. 1992. Nutritional evaluation of tree leaves and range legumes. *Indian Journal of Animal Nutrition,* 12: 234-236.

Singh, P., Verma, A.K., Dass, R.S and Mehra, U.R. 1999. Performance of pashmina kid goats fed oak (Quercus semecarpifolia) leaves supplemented with a urea molasses mineral block. *Small Ruminant Research,* 31 (3): 239-244.

Singh, R and Misri, B. 2004. Traditional animal rearing practices in mountains of Himachal Pradesh, *ENVIS Bulletin* Vol 12(1) : Himalayan Ecology.

Singh, R.V. 1982. Fodder trees of India. Oxford & IBH Co. New Delhi, India.

Singh, S., Paliwal, V.K and Gupta, P.C. 1995. Nitrogen solubility, degradability and dry matter disappearance of malt sprout, groundnut cake based combinations. *Indian Veterinary Journal,* 72: 1158.

Sinha, R.K., Das, A.B., Amiyangshu, C and Chatterjee, A. 1989. Evaluation of nutritional value in three legume trees as a source of fodder. *Indian Journal of Forestry,* 12: 285-288.

Sivakumaran, S., Molan, A.L., Meagher, L.P., Kolb, B., Foo, L.Y and Lane, G.A. 2004. Variation in antimicrobial action of proanthocyanidins from *Dorycnium rectum* against rumen bacteria. *Phytochemistry,* 65:2485-2497.

SkeneIK and Brooker, J.D. 1995. Characterization of tannin acylhydrolase activity in the ruminal bacterium *Selenomonas ruminantium. Anaerobes,*1:321 -327.

Sliwinski, B.J., Kreuzer, M., Wettstein, H.R and Machmuller, A. 2002a. Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins and associated emissions of nitrogen and methane. *Archives of Animal Nutrition,* 56:379-392

Sliwinski, B.J., Soliva, C.R., Machmuller, A and Kreuzer, M. 2002b. Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. *Animal Feed Science and Technology,* 101:101 -114.

Smith, A.H., Zoetendal, E.G and Mackie, R.I. 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microbial Ecology,* 50:197-205.

Spencer, C.M., Martin, R., Gaffney, S.H., Goulding, P.N., Magnolato, D and Lilley, T.H. and Haslam, E. 1988. Polyphenol complexation-some thought and observation. *Phytochemistry,* 27: 2397-2409.

Spier, S.J., Smith, B.P., Seawright, A.A., Norman, B.B., Ostrowski, S.R and Oliver, M.N. 1987. Oak toxicosis in cattle in Northern California: clinical and pathological findings. *Journal of the American Veterinary Medical Association*, 191(8):958-964.

Steel, R.G.D and Torrie, J.H. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc. NY. Pg. 173-176.

Steingass, H. 1983. Bestimmung des energetischen Futterwertes von Wirtschaftseigenen Futtermitteln aus der Gasbildung bei der Pansenfermentation *in vitro*. Dissertation, Universitat Hohenheim, Stuttgart.

Steingass, H. and Menke, K.H. 1986. Schatzung des energetischen Futterwertes aus der *in vitro* mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. *Tierenahrung*, 14:251.

Stienezen, M ., Waghorn, G. C and Doughlas, G,B. 1996. Digestibility and effects of condensed tannins on digestion of sulla (*Hedysarum coronarium*) when fed to sheep. *New Zealand Journal of Agriculture Research,* 39: 215–221.

Subba Rao, A., Amrith Kumar, M.N. and Sampath, S.R. 1971. Studies on mulberry (*Morus alba*) leaf stalk palatability, chemical composition and nutritive value. *Indian Journal of Veterinary Science,* 48: 853-857.

Synge, R. L. M. 1975. Interactions of polyphenols with proteins in plants and plant products. *Plant food for human nutrition* (formerly Qualitus plantarum) 24(3-4):337-350

Talapatra, S.K., Ray, S.C and Sen, K.C. 1940. The analysis of mineral constituents in biological material. Part I. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in feedstuffs. *Indian Journal of Veterinary Science and Animal Husbandry,* 10: 243-258.

Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T and Sivakumaran, S. 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa,* and effects of extractable condensed tannin fractions on methanogenesis.*Animal Feed Science and Technology* 123 - 124:403 -419.

Terrill, T.H, Douglas, G.B, Foote, A.G, Purchas, R.W, Wilson, G.F and Barry, T.N. 1992. Effect of condensed tannins upon body growth and rumen metabolism in sheep grazing sulla *(Hedysarum coronarium)* and perennial pasture. *Journal of Agriculture Science, Cambridge,* 119:265-273.

Thorne, P., Subba, D.B., Walker, D.H., Thapa, B., Wood, C.D and Sinclair, F.L. 1999. The basis of indigenous knowledge of tree fodder quality and its implications for improving the use of tree fodder in developing countries. *Animal Feed Science and Technology,*, 81: 119-131.

Tiemann, T.T., Lascano, C.E., Wettstein, H.R., Mayer, A.C., Kreuzer, M and Hess, H.. 2008. Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. *Animal Feed Science and Technology,* 2:790-799.

Tietz, N.W., Rinker, A.D and Shaw, L.M. 1983. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. *Journal of Clinical Chemistry and Clinical Biochemistry*, 21: 731-748.

Trinder, P. 1960. *Analyst*, 85, 889

Upreti, C. R and Shrestha, B. K. 2006. Nutrient contents of feeds and fodder in Nepal, Animal Nutrition Division, NARC, Khatmandu, Nepal

Van Leeuwen, P., Jansman, A.J.M., Wiebenga, J., Koninkx, J.F.J.G and Mouwen, J.M.V.M. 1995. Dietary effects of fabbean *(Vicia faba* L.) tannins on the morphology and function of the small-intestinal mucosa of weaned pigs. *British Journal of Nutrition,* 73:31 -39.

Vashist, G. D., Mehta, P., Kumar, A., Sharma, S.K and Katoch, D.C. 2000. A study on socio-economic aspects of forage and fodder crops. *A case study of availability and requirement in Himachal Pradesh.* Department of Agricultural Economics, HPKV, Palampur (H.P). pp. 121

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G and Lanza, M. 2009. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *Journal of Animal Science,* 87:2674-2684.

Vitti, D.M.S.S., Nozella, E.F., Abdalla, A.L., Buenoa, I.C.S., Silva Filho, J.C., Costa, C., Buenod, M.S., Longo, C., Vieira , M.E.Q., Cabral Filho, S.L.S., Godoy , P.B and Mueller-Harvey, I. 2005. The effect of drying and urea treatment on nutritional and anti-nutritional components of browses collected during wet and dry seasons. *Animal Feed Science and Technology* 122:123-133.

Vu, D. D., Cuong, L. X., Dung, C. A and Hai, P. H. 1999. Use of urea-molasses-multinutrient block and urea-treated rice straw for improving dairy cattle productivity in Vietnam. *Preventive Veterinary Medicine,* 38:187-193.

Waghorn, G.C ., Ulyatt, M. J., John, A. and Fisher, M. T. 1987. The effect of condensed tannins on the site of digestion of amino.acids and other nutrients in sheep fed *Lotus corniculatus. British Journal of Nutrition* 57:115-126.

Waghorn, G.C. 1990. Effects of condensed tannin on protein digestion and nutritive value of fresh herbage. *Proceedings of the Australian Society of Animal Production,* 18:412-415.

Waghorn, G.C. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production - progress and challenges. *Animal Feed Science and Technology,* 147:116-139.

Waghorn, G.C., Shelton, I.D and McNabb, W.C. 1994. The effect of condensed tannin in *Lotus pedunculatus* on nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agriculture Science, Cambridge,*123:109-119.

Waghorn, G.C., Tavendale, M.H and Woodfield, D.R. 2002. Methanogenesis in forages fed to sheep. *Proceedings of New Zealand Grasslands Association,*64:167-171.

Wallace, R.J. 2004. Antimicrobial properties of plant secondary metabolites.*Proceedings of the Nutritional Society,* 63:621 -629.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B and Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. *Asian-Australian Journal of Animal Science,* 15:1458–1468.

Wang, Y., Alexander, T.W and McAllister, T.A. 2009. *In vitro* effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on rumen bacterial populations and fermentation. *Journal of Food Science and Agriculture,* 89:2252-2260.

Wang, Y., Douglas, G, B., Waghorn, G, C ., Barry, T, N and Foote, A, G. 1996a. Effect of condensed tannins in Lotus corniculatus upon lactation performance in ewes. *Journal of Agricultural Science*, *Cambridge* 126:87-98.

Wang, Y., Douglas, G. B., Waghorn, G. C., Barry, T. N., Foote, A. G and Purchas, R, W. 1996b. Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne. *Journal of Agricultural Science*, *Cambridge*, 126: 87-98.

Wang, Y., Waghorn, G, C., Barry, T, N and Shelton, I. D.1994. The effect of condensed tannins in *Lotus corniculatus* on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. *British Journal of Nutrition*, 72: 923–935.

West, J.W, Hill, G.M and Utley, P.R. 1993. Peanut skins as a feed ingredient for lactating dairy cows. *Journal of Dairy Science,* 76:590-599.

Woodfolk, J.A., Hayden, M.L., Miller. J.D., Rose.G., Chapman. M.D and Platts-Mills, T.A.1994. Chemical treatment of carpets to reduce allergen: a detailed study of the effects of tannic acid on indoor allergens. *Journal* of *Allergy and Clinical Immunology*, 94(1):19-26.

Woodward, S.L., Waghorn, G.C., Lassey, K.R and Laboyrie, P.G. 2002. Does feeding sulla *(Hedysarum coronarium)* reduce methane emission from dairy cows? *Proceedings of the New Zealand Society of Animal Production,* 62:227-230.

Woodward, S.L., Auldist, M.J., Laboyrie, P.J and Jansen, E.B.L. 1999. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production,* 59:152-155.

Wybenga, D.R., Giorgio, D and Pillegi J.V. 1971. Urea estimation by the diacetyl monoxime method. *Clinical Chemistry,* 19:891-895.

Yeruham, I., Avidar, Y., Perl, S., Yakobson, B., Shlosberg, A., Hanji, V and Bogin, E., 1998.Probable toxicosis in cattle in Israel caused by the oak *Quercus calliprinos*. *Veterinary Human Toxicology*. 40: 336-340.

Yu, F., Moughan, P. J and Barry, T. N.1996b. The effect of condensed tannins from heated and unheated cottonseed on the ileal digestibility of amino acids for the growing rat and pig. *British Journal of Nutrition*, 76: 359–371.

Yu, F., Moughan, P. J and Barry, T.N. 1996a. The effect of cottonseed condensed tannins on the ileal digestibility of amino acids in casein and cottonseed kernel. *British Journal of Nutrition*, 75: 683– 698.

Zhu, J., Filippich, L.J and Alsalami, M.T. 1992. Tannic acid intoxication in sheep and mice. *Research in Veterinary Science,* 53: 280-292.

Zucker, W. V.1983. Tannins: does structure determine function? An ecological perspective. *The American Naturalist.*, 121: 335-65.

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