Research Article

Effect of Tannin from Acacia Nilotica Pods on *in vitro* and *in sacco* Nutrient Degradability and Rumen Fermentation Pattern

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The study was carried out on* Acacia nilotica *extracted tannins on* in vitro, in sacco *nutrient degradability and rumen fermentation pattern.The results revealed that different extraction processes tried for tannin recovery was comparatively higher in methanol and cold water as compared to others. Two levels of tannins of pure tannic acid, extracted tannin through cold water process and babul pods were compared with different substrates such as GNC alone, concentrate mixture and TMR using 50:50 of roughage and concentrate. The in vitro nutrient digestibility (IVDMD and IVOMD) and rumen fermentation (NH3 and CH4) were reduced in tannin supplemented groups as compared to control (without tannin). However, total gas production was found higher in treated groups over control. In Sacco effective degradability of TMR was decreased in both pure tannic acid and extracted tannin powder where as babul pods did not alter the degradability.

***Keywords:* *Tannin,* Acacia nilotica, in vitro, in sacco**

INTRODUCTION

Successful evolution of plants from environment to land was achieved largely by massive formation of plant phenolic compounds accounting for about 40% of the medicinal plants. Of the secondary substances, tannins are the most abundant and widely distributed phenolic polymers found in higher plants and in many habitats including sewage, sludge, forest litter and the rumen (Muller-Harwey *et al.* 1988). Trees, shrub foliage and agro-industrial by-products are of importance in animal production because they do not compete with human food and can provide good protein supplements, especially in the dry season. The main constraint in using these feed resources is their anti nutritional factors, particularly tannins which are natural compounds widely distributed in the plant kingdom. These compounds are present in roots, leaves, fruits and seeds. Tannin being anti-nutritional, limits the utilization of various agro-industrial by products by inhibiting the nutrient utilization and so the animal productivity (Barman and Rai, 2006a). It is therefore, necessary to have a greater understanding of different aspects of tannin metabolism and its interactions with digestible nutrients in animal system. Beneficial as well as detrimental effects of tannins have been reported (Barman, 2004). Babul tree is used extensively as a traditional/ ayurvedic medicine. Its bark, gum, leaves and pods are used medicinally in India and West Africa. Acacia nilotica leaves contain relatively uncommon catechin gallate tannins while pods and bark contain (-) epigallocatechin gallates (Ayoub, 1985). Tanner *et al.*, (1990) and Barman (2004) also reported catechin gallates in A. nilotica pods. These compounds even being of condense tannin can be degraded in rumen to yield gallic acid, catechin and epicatechin. Keeping the above facts in mind, the present study was done to evaluate the effect of extracted tannins from Acacia nilotica pods on rumen fermentation patterns *in vitro*.

MATERIALS AND METHODS

Various tannin extraction process were used to prepare crude extract from Acacia nilotica pods using different solvents viz cold water, hot water, methanol and acetone. The sample was dried at 55 10 C and ground to pass through a sieve

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size of 1mm diameter. Tannins extraction was done using 400 mg ground sample in conical flask with 40 ml diethyl ether containing 1 per cent acetic acid (v/v) and mixed to remove the pigment material. Carefully discarded the supernatant after 5 minute and 20 ml of 70 per cent aqueous acetone was added and sealed the flask with cotton plug covering with aluminum foil and kept in electrical shaker for 2 h for extraction. Then it was filtered through What man filter paper No. 1 and sample was kept in refrigerator at 4 0C until analysis. Tannin was estimated in all type of extracted powder and Acacia nilotica pods. The tannin rich Acacia nilotica pods powder, pure tannic acid and extracted tannin were added to Groundnut cake, concentrate and TMR at the rate of tannin equivalent to 2 & 4% (w/w) to evaluate the in vitro and in sacco nutrient degradability. Tannin fraction from Acacia nilotica pods were extracted with different methods were quantified. The protein precipitation capacity of tannins from Acacia nilotica pods and extracted powder i.e., formation of soluble tannin-protein complexes were assayed using BSA. TMR (13 % CP, 62 % TDN) was prepared with Concentrate: Roughage in the ratio of 50:50. Three rumen fistulated male crossbred (Holstein Friesian x Sahiwal) cattle were selected as a donor of rumen liquor for in vitro study and same were also used for in sacco study. Animals were maintained under well ventilated house and fed on 2.0 kg concentrate and ad libitum maize green fodder to each animal. Chemical composition of the feed used for *in vitro* and *in sacco* studies is presented in Table 1.

RESULTS AND DISCUSSION

The in vitro fermentation data of groundnut cake with tannin supplementation has been presented in Table 2, 3 & 4. Acacia nilotica pods supplementation at the rate of 2 and 4% tannin equivalent significantly (P<0.01) reduced the IVTDMD (from 91.84 to 82.95 %), IVTOMD (from 92.41 to 84.14) and IVTCPD (from 95.08 to 91.66 %) in comparison to control. The incubation period ranged from 24-48 hrs. Total gas and methane production was not affected by treatment supplementation. However, ammonia production was significantly reduced (P<0.01) at higher tannin i.e., 4% level of pure tannic acid, extracted tannin powder and babul pods powder and ranged from 18.20 to 10.30 mg/dl. The in sacco digestion kinetics and effective degradability of dry matter, organic matter and crude protein (Table.5) were not affected by tannin supplementation either by pure tannic acid or Acacia pods or extracted

Cold water extraction	Hot water extraction	Methanol extraction	Acetone extraction
↓ 100gm sample Add1000ml cold water kept for overnight ↓ Filter it by double layer muslin cloth ↓ Kept in oven for drying at 60c in stainless bowel ↓ Remove dried sample Used for tannin estimation	↓ 100gm sample Add 1000ml water ↓ Boil it for 1hr at 60c ↓ Filter it by double layer muslin cloth ↓ Kept in oven for drying at 60c in stainless bowel ↓ Remove dried sample Dried sample for tannin estimation	↓ 100gm sample Add 700ml methanol ↓ 2 hr shaking ↓ Filter it by double layer muslin cloth ↓ Kept in oven for drying at 60c in stainless bowel ↓ Remove dried sample Dried sample for tannin estimation	↓ 100gm sample Add 700ml acetone ↓ 2 hr shaking ↓ Filter it by double layer muslin cloth ↓ Kept in oven for drying at 60c in stainless bowel ↓ Remove dried sample Dried sample for tannin estimation

Table 1 : Chemical composition (%DM) of substrates used in *in vitro* and *in sacco* studies

Parameter	GNC	Concentrate	Acacia nilotica pods	Berseem	Wheat straw
DM	95.59	95.67	92.59	83.73	91.27
OM	92.60	94.60	94.65	87.44	93.81
CP	38.26	22.70	14.05	17.22	2.20
EE	6.72	3.42	1.47	7.90	1.17
CF	8.44	8.43	14.90	22.17	42.91
NFE	33.39	60.23	62.38	39.15	46.84
Ash	7.40	8.26	5.35	12.56	6.19
NDF*	29.74	22.13	31.28	52.58	75.43
ADF*	12.98	8.33	19.07	38.11	54.74

Ingredient composition of concentrate mixture

Feed Ingredients	Percentage
Maize grain	33
Ground nut cake	21
Oiled mustard cake	12
Wheat bran	20
Rice bran	11
Mineral mixture	02
Common salt	01

Table 2 : Effect of treated Groundnut cake (GNC) with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH3 production (24 h incubation)

	GNC (C)	Pure tannic acid		Extracted acacia tannin powder		Acacia nilotica pods			
Parameter		2% tannin	4%tannin	2% tannin	4% tannin	2% tannin	4% tannin	SEm	Sig.
IVTDMD%	91.84^{c}±0.32	89.83^{bc}±0.35	89.42^{b}±0.13	90.09^{bc}±0.36	89.30^{b}±0.37	82.95^{a}±1.08	83.99^{a}±0.59	0.29**	0.01
IVTOMD%	92.41^{d}±0.30	91.01^{cd}±0.32	90.31^{c}±0.11	90.53^{cd}±0.35	90.02^{c}±0.35	84.14^{a}±1.01	86.50^{b}±0.50	0.26**	0.01
IVTCPD%	95.08^{c}±0.19	94.81^{bc}±1.01	94.02^{bc}±0.15	95.36^{c}±0.17	94.63^{bc}±0.19	93.05^{ab}±0.44	91.66^{a}±0.31	0.24**	0.01
Total gas(ml/g/h)	5.50±0.55	7.22±0.63	6.63±0.19	7.28±0.45	7.06±0.22	5.56±0.52	7.06±0.26	0.40NS	0.06
Methane (ml/500gm)	7.80±0.83	6.75±0.64	6.78±0.96	9.37±0.81	9.37±1.02	8.71±1.18	7.45±0.84	0.49NS	0.20
Ammonia (mg/dl)	15.74^{cd}±0.16	16.35^{d}±0.14	14.39^{bc}±0.11	18.20^{d}±0.39	13.94^{b}±0.61	16.13^{d}±0.14	10.30^{a}±0.62	0.23**	0.01

IVTDMD-*in vitro* true dry matter degradability; IVTOMD-*in vitro* true organic matter degradability; IVTCPD-in vitro crude protein degradability; IVGP-*in vitro* gas production; S.E.M.: standard error of the mean
Mean values with different superscripts a,b,c within a column differ significantly (NS-Non significant; **-P<0.01).

Table 3. Effect of treated concentrate with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH3 production (24 h incubation)

	Concentrate(C)	Pure tannic acid		Extracted acacia tannin powder		Acacia nilotica pods			
Parameter		2% tannin	4%tannin	2% tannin	4% tannin	2% tannin	4% tannin	SEm	Sig.
IVTDMD%	80.30^{bc}±1.64	75.30^{abc}±1.39	76.50^{abc}±0.67	81.80^{c}±0.85	77.07^{abc}±1.33	73.90^{ab}±0.49	70.90^{a}±3.08	0.84**	0.01
IVTOMD%	85.17^{c}±1.26	78.10^{ab}±1.22	77.73^{ab}±0.64	84.67^{c}±0.68	82.23^{bc}±1.02	74.00^{a}±0.49	71.83^{a}±2.99	0.76**	0.01
IVTCPD%	83.67^{b}±1.35	82.37^{b}±0.98	82.93^{b}±0.46	85.57^{b}±0.68	82.43^{b}±1.02	79.80^{b}±0.38	71.73^{a}±2.99	0.75**	0.01
Total gas (ml/g/h)	8.36^{c}±0.40	6.36^{ab}±0.30	5.64^{a}±0.23	8.52^{c}±0.13	8.03^{c}±0.34	7.32^{bc}±0.16	7.89^{c}±0.33	0.15**	0.01
Methane (ml/500mg)	19.87^{b}±1.45	13.50^{a}±0.17	10.83^{a}±0.26	19.73^{b}±1.07	20.07^{b}±1.13	20.87^{b}±2.28	18.60^{b}±0.59	0.64**	0.01
Ammonia (mg/dl)	22.67^{b}±0.27	15.87^{a}±0.93	13.07^{a}±0.93	15.49^{a}±0.81	14.00^{a}±0.00	15.87^{a}±0.93	14.00^{a}±0.00	0.37**	0.01

IVTDMD-in vitro true dry matter degradability; IVTOMD-in vitro true organic matter degradability; IVTCPD-in vitro crude protein degradability; IVGP-in vitro gas production; S.E.M.: standard error of the mean
Mean values with different superscripts a,b,c within a column differ significantly (NS-Non significant; **-P<0.01).

Table 4. Effect of treated total mixed ration (TMR) with different types and levels of tannin on *in vitro* true nutrient digestibility, gas, methane & NH3 production (48 h incubation)

	TMR (C)	Pure tannic acid		Extracted acacia tannin powder		Acacia nilotica pods			
Parameter		2% tannin	4%tannin	2% tannin	4% tannin	2% tannin	4% tannin	SEm	Sig.
IVTDMD%	85.05^{e} ± 0.42	76.97^{bc}±1.18	77.67^{bc}±0.28	76.57^{b}±0.31	73.81^{a}±0.76	81.54^{d}±0.38	79.40^{cd}±0.24	0.32**	0.01
IVTOMD%	77.73^{b}± 0.62	73.30^{a}±1.37	71.70^{a}±0.36	76.58^{b}±0.31	77.98^{b}±0.64	70.94^{a}±0.74	70.65^{a}±0.35	0.38**	0.01
IVTCPD%	93.85^{b} ± 0.17	94.13^{b}±0.31	94.04^{b}±0.37	90.16^{b}±0.14	88.20^{a}±0.36	91.72^{ab}±0.80	93.53^{b}±0.08	0.61**	0.01
Total gas (ml/g/h)	6.45b^{c} ± 0.23	4.33^{a}±0.11	4.04^{a}±0.16	5.14^{a}±0.37	6.68^{c}±0.26	5.05^{a}±0.33	5.33^{ab}±0.45	0.16**	0.01
Methane (ml/500gm)	18.77^{c} ± 0.36	10.20^{a}±3.19	13.05^{ab}±0.00	19.72^{c}±0.11	18.10^{bc}±1.15	12.96^{ab}±0.42	16.74^{bc}±0.02	0.69**	0.01
Ammonia (mg/dl)	34.53 ± 3.37	30.80±4.85	28.93±2.47	35.47±0.93	38.27±2.47	29.87±2.47	28.93±2.47	1.57NS	0.23

IVTDMD-*in vitro* true dry matter degradability; IVTOMD-*in vitro* true organic matter degradability; IVTCPD-*in vitro* crude protein degradability; IVGP-*in vitro* gas production; S.E.M.: standard error of the mean
Mean values with different superscripts a,b,c within a column differ significantly (NS-Non significant; **-P<0.01)

Table 5. Effect of treated groundnut cake with different types and levels of tannin on *in sacco* degradability

Parameter	GNC(C)	Pure tannic acid		Extracted acacia tannin powder		Acacia nilotica pods		SEm	Sig.
		2% tannin	4%tannin	2% tannin	4% tannin	2% tannin	4% tannin		
Dry Matter (% of DM)									
a	17.01±1.61	13.24±4.73	15.58±1.95	10.94±1.83	11.72±1.18	14.39±3.26	16.79±2.26	1.42NS	0.58
b	67.23±5.37	63.03±0.49	57.03±1.94	68.94±0.46	67.56±1.63	70.26±5.90	66.43±0.40	1.70NS	0.14
c	0.07±0.02	0.06±0.02	0.06±0.03	0.08±0.03	0.07±0.04	0.06±0.01	0.09±0.02	0.01NS	0.95
EDMD	52.31±4.41	43.81±11.90	39.69±12.63	47.40±11.93	42.49±16.04	53.22±1.47	60.08±1.49	5.44NS	0.80
R^2	0.86	0.94	0.94	0.95		0.98	0.95	0.92	
Organic Matter (% of OM)									
a	12.48±1.61	6.92±4.66	11.12±1.84	8.25±1.58	9.20±1.04	11.29±3.39	12.68±2.16	1.39NS	0.65
b	71.52±4.67	70.24±0.09	62.22±2.01	71.10±0.42	69.40±1.66	71.17±6.36	69.60±0.82	1.69NS	0.44
c	0.07±0.02	0.06±0.02	0.06±0.03	0.08±0.03	0.07±0.04	0.07±0.01	0.11±0.02	0.01NS	0.78
EOMD	51.19±4.52	43.59±11.59	37.62±13.48	46.30±12.21	41.27±16.44	53.30±1.47	60.50±1.52	5.57NS	0.75
R^2	0.87	0.95	0.94	0.95	0.95	0.98	0.93		
Crude Protein (% of CP)									
a	15.12±1.25	11.83±4.18	8.49±1.48	8.37±0.96	13.12±0.92	5.70±2.98	5.01±2.05	1.21*	0.05
b	67.82±0.11	80.75±8.64	75.23±2.14	80.36±0.25	73.59±1.37	81.71±4.74	83.34±3.62	2.18NS	0.16
c	0.09±0.02	0.08±0.02	0.07±0.02	0.08±0.03	0.07±0.04	0.09±0.01	0.09±0.01	0.01NS	0.98
ECPD	57.95±3.91	57.74±5.22	48.80±8.68	54.54±10.16	48.16±14.96	57.16±1.23	57.20±1.74	4.28NS	0.94
R^2	0.89	0.96	0.97	0.97	0.99	0.97	0.94		

a-rapidly degradable, b-slowly degradable, c-rate of disappearance, R2- better adjustment to the non-linear model, S.E.M.: standard error of the mean, ED-Effective degradability, k=0.05, Mean values with different superscripts a,b,c,d within a column differ significantly (NS=Non significant; *=P<0.05).

tannin powder from Acacia pods or as such Acacia pods at the level of 2 and 4 % tannin level in groundnut cake and it may be due to methods of tannin extraction used. However, the values of effective degradability of DM (60.08 to 39.69 %), OM (60.50 to 37.62 %) and CP (57.95 to 48.80 %) were reduced in tannic acid and tannin extracted powder from Acacia pods. It was non significantly affected by treatments. Bhargava *et al.* (1973) also reported that tannic acid (HT) @ 6% (w/w) with GNC reduced protein solubility by 76%. Sengar and Mudgal, (1982) found that treatment of tannic acid (HT) @ 10% (w/w) with GNC reduced in vitro NH3 production by 91%. Dey *et al.* (2006) concluded that tropical tree leaves (Artocarpus heterophyllus, Ficus glomerata, Ficus bengalensis, and Ficus infectoria) supplementation (at 1-2% condensed tannins equivalent levels) significantly reduced the in vitro nitrogen degradability of groundnut cake. Dentinho *et al.* (2007) observed that although the phenolic crude extract from Cistus ladanifer L.(0, 12.5, 25, 50, 100 and 150 g/ kg levels) effectively reduced the in sacco soybean protein degradation in the rumen, and increased flux of feed protein to post rumen, it also had a negative effect on *in vitro* intestinal digestibility. The effective DM and CP degradability reduced from 69 to 52 and 69 to 40 %, respectively as total phenolic concentrations increased from 0 to 150g/kg DM of soybean meal. Martinez et al., (2005) reported that use of graded levels of tannic acid (0, 10, 25 and 50 g/kg DM) decreased the effective protein degradability of barley grain. The IVTDMD and IVTOMD of concentrate (Table. 5) was reduced (P<0.01) by supplementation of 2 and 4% tannin equivalent of babul pods, pure tannic acid and 4% extracted powder. However, IVTCPD was significantly (P<0.01) reduced from 83.67 to 71.73 % only at 4% tannin equivalent babul pod. Total and methane gas production was reduced (P<0.01) only at 2 and 4 % pure tannic acid supplementation, However, ammonia level reduced (P<0.01) in all type of treatment compared to control. In concentrate the in sacco effective degradability (Table 6) of dry matter and organic matter was not affected, but of crude protein was reduced from 57.89 to 45.85 % in 2 and 4 % pure tannic acid level. However, the extracted tannin powder from pods or Acacia pods as such supplementation at 2 and 4% tannin level did not influence in sacco degradability of concentrate. Supplementation of different form of tannin in total mixed ration (Table 7) reduced the IVTDMD, but IVTOMD was not influenced by extracted babul pod powder. However, IVTCPD was reduced only at 4 % tannin equivalent extracted powder. Total gas and methane production reduced significantly by supplementation of 2 and 4% tannin equivalent pure tannic acid and babul pods. However, ammonia level did not influence in TMR by tannin supplementation. In TMR (Table 7) the in sacco effective degradability of dry matter (35.29 to 28.45 %), organic matter (41.14 to 30.29 %) and crude protein (48.84 to 31.37 %) were reduced

Table 6. Effect of treated concentrate with different type and level of tannin on in sacco digestion kinetics and nutrient digestibility

Parameter	GNC(C)	Pure tannic acid 2% tannin	Pure tannic acid 4%tannin	Extracted acacia tannin powder 2% tannin	Extracted acacia tannin powder 4% tannin	Acacia nilotica pods 2% tannin	Acacia nilotica pods 4% tannin	SEm	Sig.
Dry Matter (% of DM)									
a	10.28±2.19	8.69±1.21	12.17±0.76	8.22±0.83	10.63±2.35	8.41±2.59	10.84±1.49	0.95NS	0.65
b	68.43±1.37	63.48±1.75	72.98±7.27	59.41±5.17	71.82±8.53	63.38±9.07	66.13±6.28	3.38 NS	0.73
c	0.06±0.02	0.07±0.01	0.04±0.01	0.05±0.01	0.05±0.02	0.07±0.01	0.06±0.01	0.01 NS	0.71
EDMD	45.38±4.23	44.75±1.54	43.56±1.40	37.89±1.04	44.08±1.62	44.97±0.19	46.23±3.09	1.20 NS	0.25
R^2	0.92	0.97	0.96	0.93	0.97	0.93	0.97		
Organic Matter (% of OM)									
a	8.95±2.25	5.52±0.55	9.15±2.23	7.24±2.70	9.54±1.43	7.40±1.23	10.94±0.77	0.94 NS	0.45
b	70.73±1.78	59.46±4.04	68.49±3.87	65.57±8.96	66.31±5.41	65.52±1.92	74.28±7.06	2.84 NS	0.61
c	0.06±0.02	0.07±0.01	0.06±0.02	0.07±0.01	0.07±0.01	0.06±0.01	0.04±0.01	0.00 NS	0.76
EOMD	45.13±4.23	38.47±1.12	44.34±1.62	44.86±0.16	46.44±3.07	44.20±1.54	43.12±1.41	1.20 NS	0.33
R^2	0.92	0.95	0.97	0.93	0.97	0.97	0.96		
Crude Protein (% of CP)									
a	12.37^{ab}±1.84	13.07^{ab}±1.82	17.01^{bc}±2.97	8.85^{a}±0.87	23.58^{c}±1.00	11.12^{ab}±1.16	12.73^{ab}±0.78	0.89**	0.00
b	70.85^{b}±2.54	47.35^{a}±2.04	63.46^{b}±5.96	67.82^{b}±5.10	65.69^{b}±2.88	71.70^{b}±1.96	69.59^{b}±1.25	1.87**	0.00
c	0.09±0.01	0.14±0.05	0.07±0.03	0.12±0.03	0.05±0.01	0.07±0.00	0.09±0.00	0.01NS	0.18
ECPD	57.89^{b}±3.02	45.85^{a}±1.26	49.98^{ab}±1.67	55.80^{b}±0.37	57.49^{b}±2.24	51.95^{ab}±1.32	57.66^{b}±0.95	0.93**	0.00
R^2	0.96		0.85	0.90	0.95	0.98	0.96	0.97	

a-rapidly degradable, b-slowly degradable, c-rate of disappearance, R2- better adjustment to the non-linear model, S.E.M.: standard error of the mean, ED- Effective degradability, k=0.05, Mean values with different superscripts a,b,c,d within a column differ significantly (NS=Non significant; **= P<0.01).

Table. 7. Effect of treated total mixed ration with different type and level of tannin on in sacco digestion kinetics and nutrient digestibility

Parameter	TMR (C)	Pure tannic acid 2% tannin	Pure tannic acid 4%tannin	Extracted acacia tannin powder 2% tannin	Extracted acacia tannin powder 4% tannin	Acacia nilotica pods 2% tannin	Acacia nilotica pods 4% tannin	SEm	Sig.
Dry Matter (% of DM)									
a	9.75^{ab}±1.62	8.50^{ab}±0.68	13.26^{b}±1.27	9.71^{ab}±1.93	6.63^{a}±1.18	10.71^{ab}±1.26	10.61^{ab}±1.01	0.71NS	0.09
b	51.27^{a}±2.95	59.84^{a}±4.36	65.16^{ab}±5.66	82.45^{b}±8.06	59.33^{a}±1.48	71.25^{ab}±2.59	71.27^{ab}±1.23	2.35**	0.01
C	0.05^{b}±0.01	0.03^{ab}±0.00	0.02^{a}±0.01	0.02^{a}±0.01	0.02^{a}±0.01	0.03^{ab}±0.01	0.02^{a}±0.01	0.01**	0.01
EDMD	34.59^{b}±0.90	28.45^{a}±1.77	33.37^{b}±0.83	27.74^{a}±0.97	25.91^{a}±0.44	35.29^{b}±0.63	33.29^{b}±1.17	0.55**	0.01
R^2	0.94	0.91	0.95	0.97	0.96	0.96	0.95		
Organic Matter (% of OM)									
a	22.19^{c}±2.49	16.75^{abc}±0.66	22.85^{c}±0.92	11.78^{a}±1.83	19.0^{bc}±1.13	12.19^{a}±1.12	13.80^{ab}±0.74	0.75**	0.01
b	65.49±21.61	58.76±5.40	68.37±9.87	85.66±7.36	91.2±24.29	65.08±0.68	64.25±0.91	7.11NS	0.56
c	0.04±0.02	0.02±0.01	0.02±0.01	0.01±0.01	0.0±0.00	0.04±0.01	0.03±0.00	0.01	0.07
EOMD	41.14^{c}±1.06	34.88^{b}±1.60	39.47^{c}±0.70	30.29^{a}±0.94	32.8^{ab}±0.42	40.04^{ab}±0.55	40.02^{b}±1.11	0.53**	0.01
R^2	0.94	0.91	0.95	0.98	0.97	0.95	0.95		
Crude Protein (% of CP)									
a	12.29^{c}±1.66	8.24^{abc}±0.50	4.63^{a}±1.22	5.68^{ab}±0.07	6.17^{ab}±2.55	9.32^{abc}±0.67	11.47^{bc}±0.73	0.70**	0.01
b	67.20±1.51	72.40±2.36	76.07±6.63	85.97±4.99	77.20±6.37	78.51±0.68	73.27±0.70	2.20NS	0.13
c	0.04^{ab}±0.01	0.03^{ab}±0.00	0.03^{ab}±0.00	0.03^{a}±0.00	0.04^{ab}±0.01	0.04^{ab}±0.00	0.05^{b}±0.00	0.00*	0.05
ECPD	43.42^{b}±0.79	36.59^{a}±1.55	31.37^{a}±0.45	33.91^{a}±1.26	36.00^{a}±2.53	43.60^{b}±0.34	48.84^{b}±0.92	0.71**	0.01
R^2	0.96	0.97	0.95	0.99	0.97	0.98	0.97		

a-rapidly degradable, b-slowly degradable, c-rate of disappearance, R2- better adjustment to the non-linear model, S.E.M.: standard error of the mean, ED-Effective degradability, k=0.05, Mean values with different superscripts a,b,c,d within a column differ significantly (NS=Non significant; *=P<0.05 and **= P<0.01).

(P<0.01) by supplementation of pure tannic acid and extracted tannin powder at the level of 2 and 4 % tannin level (% DM). The Acacia pods as such supplementation at 2 and 4% tannin level did not influence in sacco degradability of total mixed ration. The effective crude protein degradability of TMR was reduced from 43.42 to 31.37 % in treatment group. Barman and Rai, (2006a) found that increasing levels of babul pods from 0 to 65 per cent in total mixed ration (TMR) significantly reduced IVDMD, IVOMD and IVCPD from 66.64 to 52.75; 67.19 to 54.71 and from 77.15 to 46.65 per cent, respectively. The in vitro gas production was also significantly reduced from 174.07 to 148.0 ml/ g substrate in TMRs ranging from 0 to 12 per cent tannin. Tannin concentration was negatively correlated with IVDMD, IVOMD, IVCPD and IVGP. Dubey (2007) replaced maize grain up to 66 percent with babul pods of 4 per cent tannin level in TMR along with Calcium hydroxide and PEG-4000 treatment and did not find reduction for *in vitro* digestibility of TMRs.

This shows that maize grain can be replaced with babul pods up to 66 per cent, without affecting digestibility if, tannin is chemically treated. Tandon (2009) found *in vitro* dry matter, organic matter and crude protein digestibility equal to control at Acacia pods tannin concentration of 3.1 to 3.5 per cent in TMR. The gas production was statistically non significant and was in accordance to IVOMD. Rioux *et al.*, (1995) reported that higher tannin (3.8%) cultivar of birdsfoot trefoil silage (Lotus corniculatus L.) did not influenced the effective degradability of DM (82%) and CP (94%) at k=0.5%/h. The efficiency of absorption from small intestine decreased from 58 to 50% when PEG was added to the diet, suggesting that tannins in some way enhanced absorption and utilization of nitrogen in small intestine. Herves *et al.* (1999) reported that quabracho tannins at a rate of 15g/100g of Soy bean meal (SBM) can be used as chemical additive to decrease rumen degradation. This enables proteins to by-pass degradation in the rumen and undergoes enzymatic hydrolysis in the abomasums and absorbed from small intestine (Min *et al.* 2001, 2002, 2003). Furthermore tannins form complexes with surfaces of bacterial cell wall and with bacterial enzymes which alter the bacterial growth and reduce proteolytic enzymes activities (Jones *et al.* 1994).

REFERENCES

Ayoub, S.M.H. 1985. Flavanol molluscicides from the sudan Acacias. *Int. J. Crude Drug Res.* **23**:87-90.

Barman, K. 2004. Biodegradation of tanniniferous feeds and their influence on nutrient utilization and productivity of the dairy animals. Ph.D thesis. Submitted to NDRI, Karnal.

Barman, K. and Rai, S.N. 2006a. Utilization of tanniniferous feed 2. Effect of tanniniferous feed on nutrient digestibility, gas production and tannin degradation products in cattle *in vitro. Indian J. Anim. Sci.*, **76**(10): 829-837.

Bhargava, B., Ranjhan, S.K. and Singh, U.B. 1973. Nutritional value of protected GNC for animal production solubility of groundnut cake from various sources and protection by tannic acid treatment. *Indian J. Anim. Sci.*, **43**:495-502.

Dentinho, M.T.P., Moreira, O.C., Pereira, M.S. and Bessa, R.J.B. 2007. The use of a tannin crude extract from Cistus ladanifer L. to protect soya-bean protein from degradation in the rumen. *Animal*, **1**:645-650.

Dey, A., Dutta, N., Sharma, K., and Pattanaik, A.K. 2006. Evaluation of condensed tannins from tropical tree leaves and its impact on in vitro nitrogen degradability of groundnut cake. *Anim. Nutr. Feed Technol.*, **6**: 215-222.

Dubey, D.K. 2007. Studies on degradation of tannins from Acacia nilotica pods and their influence on nutrient utilization, milk production and reproduction in dairy animals Ph.D. thesis submitted to NDRI, karnal, India.

Herves, H, Frutos, P., Serano, E., Clifton, G., Giraldez, F.J. and Mantecon, A.R. 1999. Effect of treatment with condensed tannins on ruminal degradation and intestinal digestibility of soybean oil meal. ITEA, Production Animal, 20: 499-501.

Jones, G.A., McAllister, T.A., Cheng, K.J. and Muir, A.D. 1994. Effect of sainfoin (Onobrychris viciifolia scop) condensed tannin on growth and proteolysis by 4 strains of rumen bacteria. *Appl. Environ. Microbiol.* **60**: 1374-1378.

Martinz, T. F.; Moyano, F.J. Diaz, M.; Barroso, F.G. and Alarcon, F.J. 2005. Use of tannic acid to protect barley meal against ruminal degradation. *J. Sci. Food Agric.* **85**:1371-1378.

Min, B. R., Barry, T. N., Attwood, G.T. and McNabb, W. C. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology*, **106**:(1-4)3-19

Min, B.R., Fernandez, J.M., Barry, T.N., McNabb, W.C. and Kemp, P.D. 2001. The effect of condensed tannins in Lotus corniculatus upon reproductive efficiency and wool production in ewes during autumn. *Animal Feed Science and Technology.* **92**:185-202.

Min, B.R., Mc Nabb, W.C., Peters, J.S, Barry, T.N. 2000. Solublization and degradation of proteins from white clover (Triflium ripens) and Lotus carniculatus by rumen microorganisms and the effect of condensed tannins on these processes. *J. Agric. Sci. Cam,* **134**: 324-334.

Mueller-Hervey, I. and McAllan, A. B. 1988. Tannins their biochemistry and nutritional properties. *Adv. Plant cell Biochem. Biotechnol.* **1**:151-217.

Rioux, R., Santos, G.T.D., Petit, H.V. and Proulx G. J. 1995. Effect of cultivars on in vitro nitrogen fraction and ruminal degradation in birdsfoot trefoil silage. *J Dairy Sci* **78**:1766-1773.

Sengar, S.S. and Mudgal, V.D. 1982. Effect of feeding treated and untreated proteins on growth and milk production in goats: effect on feed utilization and quality and quantity of milk produced. *Ind. J. Anim. Sci.*, **52**:1047-1051.

Tandon, M. 2009. Studies on Acacia nilotica pods replacing energy sources in ration of dairy cows on production performance and neutraceutical value of milk and milk products. Ph.D Thesis. Submitted to NDRI, (Deemed University) Karnal, Haryana.

Tanner, J.C., Reed, J.D. and Owen, E. 1990. The nutritive value of fruits (pods and seeds) from four Acacia sp. compared with extracted noug (Guizotia abyssinica) meal as supplements to maize stover for Ethiopian highland sheep. *Anim. Production,* **51**:127-133.