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Utilization of tanniniferous feeds. 1. Chemical composition, tannin fractionation, amino acid and mineral profiles and *in vitro* digestibility of certain Indian agro-industrial byproducts

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ABSTRACT

Agro-industrial byproducts, viz. *Mangifera indica* (kernel), *Acacia nilotica* (pods), *Prosopis juliflora* (pods), *Shorea robusta* (cake), *Madhuca indica* (cake), *Camellia assamica* (waste), *Tamarindus indica* (seed meal), and *Acacia nilotica* (seed meal) were evaluated for compositional changes, fractionation of tannin and digestion kinetics. First 6 agro-industrial byproducts were also evaluated for compositional variation in amino acids profiles. *Acacia nilotica* seed meal, *Madhuca indica* and *Camellia assamica* contained highest level of crude protein followed by *Shorea robusta*, *Acacia nilotica* pods and *Prosopis juliflora*. Ether extract was highest in *Mangifera indica* and lowest in *Shorea robusta* while crude fibre content was highest in *Prosopis juliflora* and lowest in *Shorea robusta*. *Mangifera indica* and *Camelia assamica* contained high level of most essential amino acids. Lysine, cystein+methinoine, arginine and threonine content of *Mangifera indica* were 1.91, 7.65, 13.38 and 9.56g/100g protein respectively (on DM). Its threonine and cystein+methionine were even higher than whole egg protein. *Acacia nilotica* contained highest level of total phenol and total tannin followed by *Mangifera indica* and *Shorea robusta* while others contained within the variation of range. *In vitro DM* and OM digestibility were highest in *Mangifera indica* followed by *Camelia assamica*, *Acacia nilotica* seed meal, *Acacia nilotica* pods, *Prosopis juliflora* and other showed below 50% digestibility. *In vitro* CP digestibility was highest in *Acacia* seed meal and lowest in *Mangifera indica*. *In vitro* gas production was highest in *Camelia assamica* followed by *Acacia nilotica*, *Prosopis juliflora*, *Mangifera indica*, *Acacia* seed meal, *Shorea robusta*, *Madhuca indica*, *Tamarindus indica* during the first stage kinetic (0–24 h) while overall gas production in second stage kinetic (24–48 h) was highest in *Acacia nilotica* pods and lowest in *Madhuca indica*. However, overall gas production (0–48 h) was in the order of *Mangifera indica*>*Acacia nilotica* pods>*Camelia assamica*>*Prosopis juliflora*>*Shorea robusta*>*Acacia seed meal*>*Madhuca indica*>*Tamarindus indica*. The mineral content was higher in *Mangifera indica* and *Camellia assamica* in comparison to other 4 byproducts. It is inferred that *Mangifera indica* and *Camelia assamica* may be used as both energy and good source of essential amino acids while *Prosopis juliflora*, *Acacia nilotica* seed meal, *Shorea robusta*, *Madhuca indica* and *Tamarindus indica* as a source of protein, and *Acacia nilotica* pods both as a source of protein and energy.

Key words: Agro-industrial byproducts, Amino acids, Gas production, *In vitro* Digestibility, Tannins

In tropical region like India, the supply of quality feeds to support growth and productivity (milk) is not constantly available. An alternative approach to overcome the shortage of conventional feed ingredients such as grains and their milling byproducts and cake through use of new feed resources as animal feed (Rai and Barman 2004) has been build up steadily. Jain *et al.* (1996) estimated a deficit of about 18.9 MT digestible crude protein and 200 MT total digestible nutrients as animal feeds. Tanniniferous feeds are one of the unconventional feeds available annually in huge quantities in India (Table 1) but their use as an animal feed is limited due to the presence of 'tannins'-an anti-nutritional factor (Tripathy *et al.* 1984, Punj. 1988, Makkar *et al.* 1990, Makkar *et al.* 1991, Barman and Rai 2003). Therefore, there is an urgent need to investigate the presence of such anti-nutritional factors in various agroindustrial byproducts to predict their effect on the animal system. Tannins have a negative effect on digestibility of various nutrients (McLeod 1974, Kumar and Singh 1984, Barman and Rai 2000) and thus lower the productivity of the animals on one hand and causes toxicity to the vital organ on excessive and prolong feeding to the other (Gupta *et al.* 1977). However, tannins particularly condensed tannins reported to increase bypass

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Table 1. Availability, DCP and TDN value of various tanniniferous feeds

Feeds	Availability (10^5tonnes)	DCP (%)	TDN (%)	References
Agro-industrial byproducts				
mangoSeed Kernal (Mangifera indica)	10.0	6.1	70.0	Punj, 1988
Babul pods (Acacia nilotica)	na	10.5	73.5	Ranjhan, 1993
Babul seed (Acacia nilotica)	0.60	13.8	59.0	Punj, 1988
Vilayati babul pods (Prosopis juliflora)	10.0	6.1	70.0	Punj, 1988
Salseed meal (Shorea robusta)	0.70	0.1	57.8	Punj, 1988
Mohua Seed Cake (Madhuca indica)	3.0	8.0–9.3	49.8–60.0	Punj, 1988; Ranjhan, 1993
Tea waste (Camelia assamica)	0.05	7.5	58.0	Punj, 1988
Tamarind seed meal (Tamarindus indica)	0.87	5.3–12.6	60.1–63.9	Punj, 1988; Ranjhan, 1993

Na: not available.

protein value of feeds (Barry 1989, Waghorn *et al.* 1990, Rodriguez *et al.* 1999) by forming tannin-protein complex, which is less soluble and less accessible to proteolytic enzymes at the ruminal pH, thereby showing the rate of ruminal degradation (Broderic 1978), and low abomasal pH (<) detached the complex and protein become available for digestion and absorption. High rumen bypass protein leads to increase in weight gain, feed conversion efficiency (Dumanovski and Sotosek 1998, Queshik *et al.* 1998), wool growth (Min *et al.* 1999, Barry and McNabb 1999), reproductive performance and milk yield (Barry and McNabb 1999). Tannins also have anti-bloating (Barry and Manley 1986) and anti-nematodal (Barry and McNabb 1999) action. In addition to condensed tannins, hydrolysable tannins also found to be beneficial in high producing dairy cows (Errante *et al.* 1998). Studies on various tannin containing feeds are in progress worldwide to formulate economic rations for livestock and poultry. Tannins are broadly classified into 2 groups, namely, hydrolysable and condensed tannin (Gupta and Haslam 1980). Hydrolysable tannin contain carbohydrate moiety in which hydroxyl groups are esterified to gallic acid (gallotannin) or ellagic acid (ellagitannin). Hydrolysable tannins are readily hydrolysed by acid (Barman and Rai unpublished data), based or enzyme tannase. Condensed tannin contains phenol of flavon type and produced anthocyanidin on acid hydrolysis. Because of that reason, it is also known as proanthocyanidin. Here in the present communication an attempt has been made to evaluate the nutritive value, amino acid composition, tannin levels, mineral content and *in vitro* digestibility and gas production of few tanniniferous feeds.

MATERIALS AND METHODS

Chemical composition: Proximate analysis of feed was done as per AOAC (1990). Fibre constituents were analysed by the method of Georing and Van Soest (1970).

Sample preparation and tannin extraction: The sample was dried at 55±1° C to constant weight and ground to pass through a sieve of 1 mm diametre and stored in plastic containers with lid till analysis. For tannin extraction, 400 mg of ground sample in triplicate was taken in conical flask and 40 ml diethyl ether containing 1% acetic acid (v/v) was added and mixed to remove the pigment material. Carefully discarded the supernatant after 5 min and 20 ml 70% 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 Whatman filter paper No. 1 and sample was kept in refrigerator at 4°C until analysis.

Estimation of total phenols and tannins: Total phenol and tannins (tannic acid equivalent) were analyzed as per Makkar *et al.* (1993). Non-tannin phenol was estimated after precipitating the tannin with polyvenylpolypyrrolidone from the tannin extract. 200 mg of polyvenylpolypyrrolidone was taken in test-tube in triplicate and 2 ml distilled water with 2 ml tannin extract was added. The content was vortexed and kept under refrigeration at 4°C for 15 min. Content was again vortexed and filtered through Whatman filter paper No. 1. Supernatant was used for non-tannin phenol like total phenol estimation.

Preparation of standard curve: Tannic acid standard curve was prepared by dissolving 2.5 mg tannic acid in 5 ml distil water and from this 5μl, 10μl, 15μl, 20μl and 25μl was taken in test-tube and processed essentially as per total phenol estimation. Concentration of total phenol and non-tannin phenol was calculated from the standard curve. Total tannin phenol was calculated from the difference between total phenol and non-tannin phenol.

Estimation of condensed tannins

Condensed tannin (leucocyanidin equivalent) was estimated by butanol-HCl method of Porter *et al.* (1986). The concentration of condensed tannin was calculated (0% DM) from the formula A_{550nm}×78.26×dilution factor/% DM.

Estimation of hydrolysable tannins

Hydrolysable tannin was calculated from the difference between total tannin phenol and condensed tannin.

Minerals profile of feed

Minerals were estimated by using atomic absorption spectrophotometer except phosphorus, which was estimated through spectrophotometer.

Preparation of feed samples: Feed (1g) was digested in 100 ml Kjeldahl flask containing glass beads to avoid bumping at very low heat initially by adding adequate amount of tri acid mixture (HNO_3 : $HClO_4$: H_2SO_4 2.5 : 1 : 1 ratio) and then at higher temperature till the contents are clear and perchloric acid ($HClO_4$) fumes decreased to come out. The final volume was made to 25 ml and filtered to remove silica and other insoluble residues from digested samples.

Analytical procedure: Ca, Mg, Co, Cu, Mn and Zn was estimated with the help of atomic absorption spectrophotometer using acetylene as fuel and air as oxidant. Specific hollow cathode lamp was used for each mineral.

Estimation of phosphorous: Phosphorus in sample was estimated by phosphomolybdovandate complex formation method (AOAC 1990).

Amino acid composition of feed

Amino acid was analysed by hydrolysis and pre-column derivatization of sample followed by analysis in reverse phase HPLC. The individual amino acid is expressed as g/100g protein on DM.

Selection, housing, management and feeding of experimental animals: Three rumen fistulated male crossbred (Holstein Friesian ×Sahiwal) cattle (body weight 238.0, 228.0 and 185.0 kg respectively) were selected for experimental purpose and were housed in a well-ventilated byre. Animals were daily brushed and washed and were provided with fresh-water twice daily. Animals were maintained on a fixed diet comprising 1.5 kg concentrate mixture (wheat grain–49 part, wheat bran–30 part, groundnut-cake–18 part, mineral mixture–2 part and common salt–1 part salt) per animal; per day and green maize *ad lib.* as basal roughage.

Collection of rumen liquor: Rumen liquor was collected through vacuum suction, in the morning before feeding and watering. Rumen liquor was filtered through double layer of muslin cloth into a previously CO_2 flushed pre-warm thermos. After bringing to laboratory, rumen liquor was flushed with CO_2 gas and then fractionated for *in vitro* studies.

In-vitro *nutrient digestibility and measurement of gas production:* The *in-vitro* nutrient digestibility was estimated as per Tilley and Terry (1963). Gas production was measured through transducer fitted with 60 cc plastic syringe and 3 way valve at 6,12,18,24,30,36,42 and 48 h of incubation and expressed as ml/g substrate and ml/h/g substrate by taking an average of zero to 24 h gas production as first stage kinetic (fast degradation) and then 24 to 48 h as second stage kinetic (slow degradation). For estimation of *in vitro* CP digestibility, post incubated content from the vial was filtered through Grade 1 filter paper (SONAR) and after filtration residues in the filter paper was as such digested for protein estimation (AOAC 1990). From the difference in the protein content of the sample before and after incubation digestibility was calculated.

Statistical analysis

The data were analysed by one-way ANOVA as per Snedecor and Cochran (1989). Regression analysis was done to find out the effect of tannins (tannic acid equivalent) with increasing levels in different feeds on *in vitro* dry matter (IVDMD), organic matter (IVOMD), crude protein digestibility (IVCPD) and gas production (IVGP). Correlation coefficient was calculated to see the relationship between IVOMD and IVGP (feeds were arranged in one side

Table 2. Chemical composition of some of the agro-industrial byproducts (% DM)

Sample	OM	CP	EE	CF	NFE	Ash	NDF	ADF	Cellulose	HC	Lignin
Agro-industrial byproducts											
Mango seed kernel	97.2^{f}	7.6^{a}	13.5^{h}	1.5^{h}	74.7^{g}	2.8^{b}	25.2^{a}	5.8^{a}	9.2^{b}	19.4^{f}	1.1^{h}
(*Mangifera indica*)	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±1.4	±0.6	±0.1	±2.0	±0.1
Babul pods	94.8^{c}	13.2^{c}	1.4^{b}	14.9^{f}	65.3^{d}	5.2^{e}	30.4^{b}	25.2^{b}	22.5^{c}	5.2^{b}	1.9^{d}
(*Acacia nilotica*)	±0.1	±0.2	±0.0	±0.0	±0.1	±0.1	±0.2	±0.4	±0.2	±0.6	±0.2
Vilayati babul pods	96.1^{e}	12.3^{b}	4.3^{d}	33.1^{h}	46.4^{b}	3.9^{c}	52.3^{g}	42.8^{g}	36.6^{e}	9.6^{c}	5.7^{c}
(*Prosopis juliflora*)	±0.4	±0.1	±0.0	±0.0	±0.4	±0.4	±0.3	±0.6	±0.5	±0.4	±1.1
Salseed meal	93.3^{a}	14.0^{e}	1.1^{a}	1.1^{a}	77.2^{h}	6.7^{g}	38.0 c	26.6^{c}	23.0 c	11.3^{d}	0.9^{h}
(*Shorea robusta*)	±0.0	±0.2	±0.0	±0.0	±0.2	±0.0	±0.0	±0.1	±0.6	±0.1	±0.0
Mohua Seed Cake	94.1^{b}	18.8^{g}	1.5^{c}	4.9^{d}	68.8^{f}	5.9^{f}	49.6^{e}	47.6^{h}	0.5^{a}	2.0^{a}	0.1^{a}
(*Madhuca indica*)	±0.5	±0.0	±0.0	±0.1	±0.6	±0.5	±0.3	±0.3	±0.0	±0.5	±0.0
Tea waste	95.6^{d}	16.2^{f}	7.4^{f}	3.8^{c}	68.2^{e}	4.5^{d}	49.8^{e}	37.2^{e}	39.9^{f}	12.6^{e}	1.8^{cd}
(*Camelia assamica*)	±0.1	±0.1	±0.0	±0.1	±0.1	±0.1	±0.1	±0.6	±2.6	±0.6	±0.1
Tamarind meal	97.5^{g}	13.5^{d}	8.4^{g}	12.2^{e}	63.4^{c}	2.5^{a}	51.0^{f}	37.9^{f}	40.3g	13.0^{e}	6.6^{f}
(*Tamarindus indica*)	±0.3	±0.1	±0.0	±0.0	±0.3	±0.3	±0.6	±0.4	±0.1	±0.3	±0.5
Babul seed meal	93.4^{a}	28.3^{h}	7.0^{e}	18.6^{h}	39.5^{a}	6.6^{g}	45.7^{d}	36.1^{d}	34.7^{d}	9.6^{c}	1.5^{c}
(*Acacia nilotica*)	±0.2	±0.0	±0.0	±0.0	±0.1	±0.2	±1.2	±0.4	±1.7	±0.8	±0.5
SEm	1.13	0.05	0.01	0.025	0.15	0.13	0.36	0.23	0.56	0.43	0.24

SEm-Standard error of mean; Different superscript ^{a,b,c,d,e,f,g,h} in a column differ significantly (P<0.05), NS: Nonsignificant.

with increasing tannin concentration). T-test (two samples with unequal variance) was done to compare between agro-industrial byproducts to find out whether or not, other factors namely CF, NDF, ADF and NFE involved in variation of IVDMD, IVOMD and IVGP.

RESULTS AND DISCUSSIONS

Chemical composition

Acacia nilotica seed-meal, *Madhuca indica* and *Camelia assamica* contained highest ($P<0.05$) protein content followed by *Shorea robusta, Tamarindus indica, Acacia nilotica*, Acacia *nilotica pods, Prosopis juliflora* while *Mangifera indica* contained the lowest level of protein (Table 2). Similar level of protein was also reported by Punj (1988). High protein content of *Madhuca indica* cake was also reported by Tiwari *et al.* (1996). Ether extract was highest in *Mangifera indica* followed by *Tamarindus indica, Camelia assamica* and *Acacia nilotica seed meal* and others contained in the range of 1.44 to 4.32% DM. It is clear from the chemical composition that *Camelia assamica* as well as *Madhuca indica* can be used as protein supplement in the concentrate mixture of livestock ration. Other ingredients though contain relatively low protein, can be used for maintenance purposes. *Prosopis juliflora* contained the highest level ($P<0.05$) of crude fibre followed by *Acacia nilotica* seed meal, *Acacia nilotica* pods and *Tamarindus indica while* other contained in the range of 1.1 in *Shorea robusta* to 4.95% in *Madhuca indica*. Neutral detergent fibre content was highest in *Prosopis juliflora* followed by *Tamarindus indica, Camelia assamica* and *Madhuca indica* while other in the range of 25.2 in *Mangifera indica* to 38.0%, DM in *Shorea robusta*. Nitrogen free extract ranges from 39.5 to 77.2% in the order ($P<0.05$) of *Acacia nilotica* seed meal < *Prosopis juliflora* < *Tamarindus indica* < *Acacia nilotica* pods < *Camelia assamica* < *Madhuca indica* < *Mangifera indica* < *Shorea robusta*. Among these, *Shorea robusta, Mangifera indica, Madhuca indica, Camelia assamica, Acacia nilotica* pods and *Tamarindus indica can* be used as an energy feed in making concentrate mixture. Ranjhan (1993) reported a similar (CP (14.04%) content of *Acacia nilotica* pods. Similarly, Ngwa *et al.* (2001) found similar level of CP but low NDF and ADF. Low level of CF and high NFE content of *Shorea robusta* was also reported by Ranjhan (1993). He also reported a similar CP (15.4%) content of *Tamarindus indica*. Sidhuraju *et al.* (1996) also reported similar CP (23.4%) and EE (6.7%) of *Acacia nilotica* seed. High level of crude protein and nitrogen free extract of tea waste was also reported by Baruah (1997). He also found no negative effects on feed intake, milk yield and milk composition by replacing 15% wheat bran in concentrate mixture with tea waste of crossbred cows (Jersey×Assam local).

Tannin partitioning

Among the agro industrial byproducts *Acacia nilotica* pods, *Mangifera indica* and *Shorea robusta* contained highest level ($P<0.05$) of total tannin and total phenol (Table 3) while *Tamarindus indica, Madhuca indica* and *Camelia assamica* contained level for ruminant followed by *Prosopis juliflora* and *Acacia nilotica* seed meal containing the least ($P<0.05$). Condensed tannins content were highest ($P<0.05$) in *Acacia*

Table 3. Tannin content of some of agro-industrial byproducts (% DM)

Sample	Total	Non tannin	Total tannin[a]	Hydrolysable tannin	Condensed tannin[b]
Agro-industrial byproducts					
Mango seed kernel (*Mangifera indica*)	$9.59^{e}\pm0.28$	$1.54^{f}\pm0.05$	$8.39^{g}\pm0.59$	$8.27^{g}\pm0.58$	$0.12^{b}\pm0.01$
Babul pods (*Acacia nilotica*)	$21.67^{h}\pm0.63$	$2.96^{g}\pm0.06$	$18.71^{h}\pm0.68$	$17.31^{h}\pm0.68$	$1.40^{g}\pm0.02$
Vilayati babul pods (*Prosopis juliflora*)	$1.85^{b}\pm0.04$	$1.10^{d}\pm0.01$	$0.76^{b}\pm0.03$	$0.57^{b}\pm0.03$	$0.19^{cd}\pm0.01$
Salseed meal (*Shorea robusta*)	$9.08^{d}\pm0.43$	$1.24^{e}\pm0.05$	$7.98^{f}\pm0.45$	$7.92^{f}\pm0.44$	$0.06^{a}\pm0.01$
Mohua seed cake (*Madhuca indica*)	3.80 c$\pm$0.41	$0.61^{c}\pm0.04$	$3.19^{d}\pm0.42$	$1.98^{d}\pm0.33$	$1.21^{e}\pm0.16$
Tea waste (*Camelia assamica*)	$3.96^{c}\pm0.08$	$0.33^{b}\pm0.01$	$3.63^{e}\pm0.07$	$3.17^{e}\pm0.07$	$0.46^{e}\pm0.01$
Tamarind meal (*Tamarindus indica*)	$9.91^{f}\pm0.31$	$6.95^{h}\pm0.32$	$2.96^{c}\pm0.31$	$1.59^{c}\pm0.42$	$1.37^{f}\pm0.12$
Babul seed meal (*Acacia nilotica*)	$0.48^{a}\pm0.04$	$0.21^{a}\pm0.04$	$0.27^{a}\pm0.05$	$0.11^{a}\pm0.06$	$0.16^{bc}\pm0.00$
SEm	0.16	0.06	0.18	0.18	0.04

SEm-Standard error of mean, superscripts a,b,c,d,e,f in a column differ significantly ($P<0.05$), [a]Tannic acid equvalent, [b]Leucocyanidin equivalent.

Table 4. Minerals content (on DM) of different agro-industrial byproducts

Minerals	Acacia nilotica	Mangifera indica	Shorea robusta	Madhuca indica	Camellia assamica	Tamarindus indica
Calcium,%	0.34	0.10	0.18	0.16	0.48	0.20
Phosphorus,%	0.07	0.08	0.12	0.09	0.10	0.08
Magnesium,%	0.28	0.43	0.35	0.35	0.23	0.35
Zinc, ppm	17.00	26.75	29.00	26.25	29.75	18.50
Copper, ppm	12.50	12.50	17.50	10.00	10.00	15.00
Cobalt, ppm	2.50	3.75	3.75	6.25	5.00	3.75
Manganese, ppm	22.50	10.25	69.50	19.00	216.75	36.00

ppm=parts per million.

nilotica pods followed by *Tamarindus indica, Madhuca indica* whereas other contained traces. Hydrolysable tannins content was in the order ($P<0.05$) of *Acacia nilotica* pods > *Mangifera indica* > *Shorea robusta* > *Camelia assamica* > *Madhuca indica* >*Tamarindus indica* > *Prosopis juliflora* > *Acacia nilotica* seed meal.

Beneficial level of condensed tannins in ruminant was reported to be 2–4% in diet of ruminants (Barry 1989, Waghorn *et al.* 1990). Low condensed tannins content increases the rumen bypass protein (Barry 1989, Waghorn *et al.* 1990). A minimum level (27 g/kg DM) of condensed tannins in forage legume is required to yield 56% rumen bypass protein with a minimum degradation rate of 0.048/h (Broderic and Albrecht 1994). Duodenal non-ammonia nitrogen flow increased linearly with increased condensed tannins content up to 4%. Low condensed tannins content of *Robinia pseudoacacia* and *Quercus incana* (2.68 and 2.83% DM respectively) with moderate level of crude protein (8.0 and 9.6% DM respectively) may be favourable for increasing bypass protein value of leaves. Low condensed tannin content of forage like *Lotus corniculatus* increased milk yield (Woodward *et al.* 1999) and milk protein content (Bhatta *et al.* 2000). Tannins also reduce bloat and parasitic load in animal. The suitable concentration of condensed tannin in *Lotus* species for prevention of bloat and protection of protein should be given @ 30 to 40 g per kg dry matter (Barry and Manley 1986). However, McNeill *et al.* (2001) accentuated that condensed tannins at low concentration through *Leucaena* leaf meal did not improve protein utilization and N balances in sheep. Even they did not find improvement in N-balance by supplementing *Quebracho* condensed tannins in small quantity (2–60g/day) to basal diet containing lucerne hay fed to sheep. Condensed tannins vary in their ability to release protein in the acidic medium of abomasum (McNeill *et al.* 1998). The contradictory report regarding low condensed tannins and by-pass protein depended upon other

Table 5. Amino Acids Profiles of Some of the Tanniniferous feeds (g/100g protein on DM)

Amino Acids	*Acacia nilotica* pods	*Mangifera indica*	*Prosopis juliflora*	*Shorea robusta*	*Madhuca indica*	*Camelia assamica*	[1]Whole egg
Essentials							
Arginine	3.10	13.38	3.33	0.87	–	–	6.10
Valin	1.55	5.74	5.00	0.87	1.29	5.08	6.80
Histidine	6.21	–	–	0.72	1.18	5.08	2.40
Isoleucine	2.59	3.82	2.78	0.43	0.75	5.08	6.20
Leucine	–	1.91	0.56	0.14	0.11	–	8.80
Lysine	1.03	1.91	1.11	0.29	0.54	1.69	6.90
Cystein+Methionine	4.66	7.65	3.89	0.57	1.29	6.77	5.70
Phenylalanine	2.59	1.91	1.11	0.29	0.32	1.69	5.70
Threonine	–	9.56	4.44	–	1.18	10.16	–
Tyrosine	2.07	5.74	0.56	–	–	10.16	4.10
Non Essential							
Proline	13.45	19.12	3.33	2.02	1.18	49.11	–
Alanine	0.52	1.91	4.44	0.14	0.86	1.69	5.90
Serine	0.52	1.91	0.56	0.29	0.32	–	7.60
Glycine	0.52	3.82	2.22	0.43	0.75	3.39	3.30
Glutamic acids	–	5.74	–	0.29	0.32	1.69	12.30
Aspertic acids	8.28	1.91	1.67	0.43	0.32	1.69	9.60

Represent untraceable' [1]FAO, 1970.

factors like CT configuration, its molecular weight, size, conformation and charge of the protein molecule (Hagerman and Butler 1981). For formation of tannins-protein complex a minimum of about 350 molecular weight of tannins is necessary (Van Baren and Robinson 1969). When the molecular weight is higher than 5 000 condensed tannins lose their degree of protein precipitation because of decrease in solubility in physiological solutions. Maximum binding occurs when the ratio of tannins to protein is 1 : 1 to 1 : 3 (Van Baren and Robinson 1969). All proteins with the exception of proline rich protein which have molecular weight < 20 000 have low affinities for tannins (Haegerman and Butler 1981). Tannins-protein complexes are more hydrophobic than dietary proteins. High temperature enhances the hydrophobic interaction between tannin and protein (Hagerman and Butler 1978). In addition to condensed tannins, hydrolysable tannin was also beneficial in some cases (Errante *et al.* 1998). In addition to condensed tannins, hydrolysable tannins also form tannin-protein complex (TPC) through H-bond between the phenolic subunits of the polymer and the carbonyl groups of peptides of the proteins. However, it is reported that anaerobic bacteria failed to dissociate TPC associated with condensed tannins but can dissociate those complex with hydrolysable tannins (Osawa 1990, Osawa and Sly 1992, Osawa *et al.* 1993, Nemoto *et al.* 1995, Mcsweeney *et al.* 1999).

Mineral content of different agro-industrial byproducts

Minerals were estimated in *Acacia nilotica, Mangifera indica, Shorea robusta, Madhuca indica, Camellia assamica* and *Tamarindus indica* (Table 4). Most of the minerals were higher in *Mangifera indica* and *Camellia assamica* compared to other byproducts. Ngwa *et at.* (2001) reported that *Acacia nilotica* pods contained 0.64% Ca, 0.19% P, 0.16% Mg, 29.9 ppm Zn, 10.7 ppm Cu and 17.1 ppm Mn that were higher with the exception of Mg and Mn compared to the present studies. This might be due to geographical variations as the mineral contents of soil varies from one region to another.

Amino acid composition

Most essential amino acids were present in good amount in *Mangifera indica, Acacia nilotica, Prosopis juliflora, Shorea robusta, Madhuca indica* and *Camelia assamica* (Table 5). Among these *Mangifera indica* (kernel) contained highest amount of arginine, lysine, cystein+methionine and threonine. *Mangifera indica* and *Camelia assamica* contained higher levels of all the essential amino acids except histidine in *Mangifera indica* and arginine and leucine in *Camelia assamica* which were untraceable compared to other ingredients. *Mangifera indica* contained even higher level (g/100g protein on DM) of arginine, cystein+methionine, threonine and tyrosine than whole egg protein (FAO 1970). All the essential amino acids of *Shorea robusta* were lower amongst the above feed ingredients. *Acacia nilotica* contained higher level of histidine (6.21g/100g protein on DM) compared to other ingredients and its value is even higher than that of whole egg protein (2.07 g/100g protein DM, FAO, 1970). It becomes clear from the amino acid profiles of the above tanniniferous feed that *Mangifera indica* and *Camelia assamica* can be used as a source of amino acid. *Camelia assamica* can be used as energy, protein and essential amino acids supplement. A similar level of amino acids content of *Acacia nilotica* pods was also reported by Ngwa *et al.* (2001).

In-vitro *digestibility of different nutrients*

The IVDMD and IVOMD values were highest (P<0.05) in *Mangifera indica* among the agro-industrial byproducts (Table 6) followed by *Camelia assamica* and *Acacia nilotica* seed meal. *Acacia nilotica* pods although showed high IVOMD than *Camelia assamica* and *Acacia nilotica* seed meal but did not differ significantly, however, differed (P<0.05) from *Mangifera indica*. *Prosopis juliflora* showed similar IVDMD with *Acacia nilotica* pods but IVOMD differ significantly (P<0.05) from that of *Acacia nilotica* pods. *Tamarindus indica, Shorea robusta* and *Madhuca indica* showed below 50% of IVDMD and IVOMD (Table 5) in the order of *Madhuca indica* < *Tamarindus indica* < *Shorea robusta*. *Shorea robusta* both *showed higher (P<0.05)* IVDMD and IVOMD compared to *Madhuca indica* and *Tamarindus indica*. Although, IVDMD was similar but IVOMD lower (P<0.05) in *Madhuca indica* than *Tamarindus indica*. Lower IVDMD and IVOMD of *Madhuca indica* might be due to the presence of mowrin an additional antinutrient factor along with tannins. *Madhuca indica*

Table 6. *In vitro* digestibility of different nutrients of certain agro-industrial byproducts

Feed stuffs	IVDMD (%)	IVOMD (%)	IVCPD (%)
Agro-industrial byproducts			
Mango Seed Kernel (*Mangifera indica*)	$83.33^{e}\pm0.50$	$85.55^{f}\pm0.40$	$59.74^{a}\pm5.80$
Babul pods (*Acacia nilotica*)	$63.12^{c}\pm3.82$	$75.91^{e}\pm7.45$	$64.14^{b}\pm1.66$
Vilayati babul pods (*Prosopis juliflora*)	$61.89^{c}\pm1.68$	$62.81^{d}\pm1.55$	$65.80^{b}\pm0.50$
Salseed meal (*Shorea robusta*)	$44.15^{b}\pm2.57$	$47.01^{c}\pm2.54$	$79.00^{d}\pm0.50$
Mohua Seed Cake (*Madhuca indica*)	$33.37^{a}\pm0.53$	$34.68^{a}\pm0.62$	$75.83^{c}\pm0.01$
Tea waste (*Camelia assamica*)	$73.04^{d}\pm2.58$	$72.54^{e}\pm2.91$	$77.54^{cd}\pm0.30$
Tamarind meal (*Tamarindus indica*)	$34.91^{a}\pm1.56$	$37.72^{b}\pm1.78$	$75.82^{c}\pm0.88$
Babul seed meal (*Acacia nilotica*)	$72.40^{d}\pm8.17$	$73.63^{e}\pm7.85$	$80.33^{e}\pm4.59$
SEm	1.77	2.07	1.10

SEm-Standard error of mean, superscripts ^{a,b,c,d,e,f} in a column differ significantly (P<0.05).

contained 26.04% DM mowrin (sapoglucoside) as reported by Tiwari and Patle (1985). Among the agro-industrial byproducts IVCPD value was highest ($P<0.05$) in *Acacia nilotica* seed meal (80.33±4.59%) and lowest ($P<0.05$) in *Mangifera indica* (59.74±5.85%). Others showed IVCPD in the order of *Acacia nilotica* < *Prosopis juliflora* < *Tamarindus indica* < *Madhuca indica* < *Camelia assamica* < *Shorea robusta. However*, IVCPD were similar in *Acacia* pods and *Prosopis*. Similarly, it was similar in *Madhuca indica, Tamarindus indica* and *Comelia assamica. Shorea robusta* although showed higher ($P<0.05$) IVCPD but it was similar to *Camelia assamica.* Lower *in vitro* digestibility of CP of *Mangifera indica* and *Acacia nilotica* pods was due to their higher tannins (tannic acid equivalent) content (Y=–1.576x79.367, r^2=0.24, $P<0.05$). Though *Shorea robusta* contained high-level tannins, it hydrolysed rapidly compared to *Acacia nilotica* and *Mangifera indica,* which hydrolysed slowly (Barman and Rai, unpublished data), which might lead to higher IVCPD. Protein precipitable capacity of total phenols of *Acacia nilotica* pods was highest followed by *Mangifera indica* and *Madhuca indica* (Makkar *et al.* 1990). IVCPD of agro-industrial byproducts was negatively correlated (0.47, n=8, $P<0.005$) with total phenol content. Higher IVCPD of *Camelia assamica* and *Madhuca indica* in spite of their higher condensed tannins content compared to *Mangifera indica* might be due to low content of free condensed tannins as soluble condensed tannin reduces crude protein digestibility (Hagerman *et al.* 1992, Rintter and Reed 1992) and their low content of total phenols as protein precipitation capacity is governed by total phenols not by condensed tannins (Makkar *et al.* 1990). However, significant reduction in crude protein digestibility was observed by Tiwari *et al.* (1996) by incorporation of 22% *Madhuca indica* cake in complete feed mixture of crossbred calves compared to control, which is contradictory to the high IVCPD value of *Madhuca indica* in the present study. Inclusion of different levels (0 to 12% on DM) of tannic acid equivalent) of *Acacia nilotica* pods in total mixed ration reduced the IVDMD, IVOMD, IVCPD and IVGP ($P<0.05$) (Barman and Rai unpublished data).

In vitro *gas production (IVGP)*

IVGP in terms of ml/g and ml/h/g were significantly ($P<0.05$) higher in *Camelia assamica* followed by *Acacia nilotica* pods and *Prosopis juliflora* compared to other agro-industrial byproducts. However, IVGP in *Acacia* pods and *Prosopis juliflora* were insignificant. Others showed IVGP in the order of *Mangifera indica* > *Acacia* seed meal > *Shorea robusta* > *Maduca indica* > *Tamarindus indica* ($P<0.05$, Table 7). However, during the second stage kinetic IVGP (Table 8) as ml/g and ml/h/g were significantly higher ($P<0.5$) in *Acacia nilotica* pods and *Mangifera indica* than other agro-industrial byproducts. IVGP in others was in the order ($P<0.05$) of *Tamarindus indica* > *Shorea robusta* > *Acacia nilotica* seed meal > *Camelia assamica* > *Prosopis juliflora* > *Madhuca indica*. However, overall gas production (0–48 h) was highest ($P<0.05$) in *Mangifera indica* and *Acacia nilotica* pods followed by *Camelia assamica, Prosopis juliflora, Shorea robusta, Acacia nilotica* seed meal, *Madhuca indica* and *Tamarindus indica.* It was observed that with increasing level of tannins (tannic acid equivalent) in different agro-industrial byproducts, IVGP (ml/g) increased ($P<0.05$) in

Table 7. *In vitro* gas production (First stage kinetic) of certain agro-industrial byproducts

Feed stuffs	Incubation period				IVGP (ml)	IVGP (ml/g)	IVGP (ml/h/g)
	6h	12h	18 h	24h			
Agro-industrial byproducts							
Mango Seed Kernel (*Mangifera indica*)	$2.50^{a}\pm0.50$	$9.83^{b}\pm0.17$	$27.33^{f}\pm0.33$	$19.67^{h}\pm0.33$	$59.33^{d}\pm0.67$	$118.67^{d}\pm1.33$	$4.94^{d}\pm0.06$
Babul pods (*Acacia nilotica*)	$12.33^{e}\pm0.88$	$18.67^{e}\pm1.33$	$16.67^{de}\pm0.33$	$17.67^{de}\pm0.33$	$65.33^{e}\pm1.20$	$130.67^{e}\pm2.41$	$5.44^{e}\pm0.10$
Vilayati babul pods (*Prosopis juliflora*)	$14.67^{f}\pm2.61$	$18.33^{e}\pm1.67$	$16.67^{de}\pm1.20$	$14.00^{e}\pm0.58$	$63.67^{de}\pm4.71$	$127.33^{e}\pm9.42$	$5.31^{e}\pm0.39$
Salseed meal (*Shorea robusta*)	$8.33^{d}\pm0.88$	$13.33^{c}\pm3.39$	$11.33^{c}\pm0.88$	$15.33^{f}\pm0.33$	$48.33^{c}\pm2.91$	$96.67^{c}\pm5.82$	$4.03^{c}\pm0.24$
Mohua Seed Cake (*Madhuca indica*)	$9.33^{d}\pm0.33$	$10.67^{b}\pm0.88$	$10.33^{b}\pm1.20$	$9.83^{b}\pm0.60$	$40.17^{b}\pm1.59$	$80.33^{b}\pm3.18$	$3.35^{b}\pm0.13$
Tea waste (*Camelia assamica*)	$18.67^{g}\pm1.67$	$20.33^{f}\pm0.88$	$16.83^{e}\pm0.44$	$12.67^{d}\pm0.33$	$68.50^{f}\pm3.02$	$137.00^{f}\pm6.03$	$5.71^{f}\pm0.25$
Tamarind meal (*Tamarindus indica*)	$4.50^{b}\pm0.50$	$4.50^{a}\pm0.50$	$4.00^{a}\pm0.00$	$9.00^{a}\pm1.00$	$22.00^{a}\pm2.01$	$44.00^{a}\pm4.01$	$1.83^{a}\pm0.17$
Babul seed meal (*Acacia nilotica*)	7.00 c±1.73	$14.67^{d}\pm0.67$	$16.17^{d}\pm0.17$	12.00 c±0.00	$49.83^{c}\pm2.32$	$99.67^{c}\pm4.64$	$4.15^{c}\pm0.19$
SEm	0.67	0.75	0.36	0.21	1.26	2.52	0.10

IVGP: *in vitro* gas production; SEm-Standard error of mean, superscripts ^{a,b,c,d,e,f} in a column differ significantly ($P<0.01$).

Table 8. *In vitro* gas production (Second stage kinetic) of certain agro industrial byproducts

Feed stuffs	Incubation period					IVGP (ml)	IVGP (ml/g)	IVGP (ml/h/g)
	24 h	30 h	36 h	42 h	48 h			
Agro-industrial byproducts								
Mango Seed Kernel (*Mangifera indica*)	$19.67^{h}\pm0.33$	$17.83^{f}\pm0.44$	$12.33^{d}\pm0.17$	$13.50^{f}\pm0.29$	$7.00^{d}\pm1.53$	$70.83^{d}\pm0.17$	$141.67^{e}\pm0.33$	$5.90^{e}\pm0.01$
Babul pods (*Acacia nilotica*)	$17.67^{g}\pm0.33$	$16.33^{e}\pm0.33$	$14.00^{e}\pm1.20$	$12.00^{e}\pm1.53$	$10.50^{e}\pm1.53$	$72.50^{e}\pm4.71$	$145.00^{d}\pm9.42$	$6.04^{d}\pm0.39$
Vilayati babul pods (*Prosopis juliflora*)	$14.00^{e}\pm0.58$	$8.33^{b}\pm0.44$	$6.50^{a}\pm0.50$	$5.00^{a}\pm0.00$	$3.50^{abc}\pm0.00$	$37.33^{b}\pm1.09$	$74.67^{b}\pm2.19$	$3.11^{b}\pm0.09$
Salseed meal (*Shorea robusta*)	$15.33^{f}\pm0.33$	$12.17^{c}\pm0.73$	$8.83^{d}\pm0.17$	$7.33^{d}\pm0.33$	$5.00^{d}\pm0.00$	$48.67^{d}\pm1.43$	$97.33^{d}\pm2.85$	$4.06^{d}\pm0.12$
Mohua Seed Cake (*Madhuca indica*)	$9.83^{b}\pm0.60$	$6.67^{a}\pm0.33$	$6.17^{a}\pm1.17$	$5.83^{a}\pm0.73$	$3.83^{bc}\pm0.44$	$32.33^{a}\pm1.37$	$64.67^{a}\pm2.73$	$2.69^{a}\pm0.11$
Tea waste (*Camelia assamica*)	$12.67^{d}\pm0.33$	$9.00^{d}\pm0.00$	$6.83^{a}\pm0.17$	$7.33^{d}\pm0.33$	$3.00^{d}\pm0.00$	$38.83^{d}\pm0.60$	$77.67^{b}\pm1.20$	$3.24^{b}\pm0.05$
Tamarind meal (*Tamarindus indica*)	$9.00^{a}\pm1.00$	$10.75^{d}\pm2.26$	$10.75^{e}\pm2.26$	$9.00^{e}\pm2.01$	$10.00^{e}\pm0.50$	$49.50^{d}\pm8.02$	$99.00^{d}\pm16.05$	$4.13^{d}\pm0.67$
Babul seed meal (*Acacia nilotica*)	$12.00^{d}\pm0.00$	$8.33^{b}\pm0.33$	$7.00^{b}\pm0.00$	$7.83^{d}\pm0.17$	$4.00^{d}\pm0.00$	$39.17^{b}\pm0.44$	$78.33^{b}\pm0.88$	$3.26^{b}\pm0.04$
SEm	0.21	0.30	0.39	0.38	0.29	1.24	2.48	0.10

IVGP: *In vitro* gas production; SEm-Standard error of mean, superscripts ^{a,b,c,d,e,f,g,h} in a column differ significantly (P<0.01).

both first stage kinetic and second stage kinetics as well as over all gas production. On the other hand, IVOMD increased with the exception in *Tamarindus indica* and *Madhuca indica* with increased tannins (tannic acid equivalent) content of ingredients and it was positively correlated (r=0.85, n =8; p<0.05) with IVGP. Thus it can be interpreted that tannins (tannic acid equivalent) of these agro-industrial byproducts even at higher concentration did not affect IVOMD and IVGP. In *Tamarindus indica* and *Madhuca indica* others like mowrin in *Madhuca indica* and some unknown factors in *Tamarindus indica* along with high (P<0.05) NDF and condensed tannins in both the ingredients and high (P<0.05) lignin content in the *Tamarindus indica* might influenced IVOMD as well as IVGP. Condensed tannins reduced the fibre digestion (Barry *et al.* 1986 Waghorn *et al.* 1987, Palmer and McSweeney 2000) by complexing with lignocellulose and preventing microbial digestion or directly inhibiting cellulolytic micro-organisms (McSweeney *et al.* 1998) or both. All ingredients, those contained > 8% tannins-tannic acid equivalent (*Mangifera indica* and *Acacia nilotica* pods) produced higher (P<0.05) IVGP during the second stage kinetic (24–48 h, Table 7) which indicates that high tannin lowers the gas production by slowing the release of nutrients into the incubation medium on prolonged incubation time. Unlike other low tannins containing agro-industrial byproducts, *Tamarindus indica* produced low (P<0.05) IVGP in first stage kinetics but more (P<0.05) in second stage kinetics like that of *Mangifera indica* and *Acacia nilotica* pods, which contained more >8% tannins (tannic acid equivalent). Reasons for this, might be the involvement of other factors namely high (P<0.05) non-tannin phenol (6.95%) and high condensed tannins (1.37%) of *Tamarindus indica*, which lead to slow release of nutrient by microbial action during first 24 h incubation which reduced the IVGP, and thereafter, in the next 24 h because of the adaptation of microbes to the tannins leads to high IVGP.

However, IVCPD in agro-industrial byproducts decreased (r^2=0.24, P<0.05) with increasing tannins (tannic acid equivalent). The low ratio of soluble to insoluble tannins in feed affect the digestibility of protein less than crude fibre and that of high ratio affect the digestibility of same in reverse manner (Hagerman *et al.* 1992 Rintter and Reed 1992, Smith and Brown 2001). In our case, soluble to insoluble tannins content might be high in agro-industrial byproducts which leads to lower (P<0.05) IVCPD without affecting IVDMD, IVOMD and IVGP.

The present study indicates that various agro-industrial byproducts contain good sources of energy, protein and amino acids. *Mangifera indica* and *Camelia assamica* may be used as a good source of energy as well variety of essential amino acids; *Prosopis juliflora*, *Acacia nilotica* seed meal, *Shorea robusta*, *Madhuca indica* and *Tamarindus indica* as a source of protein and *Acacia nilotica* pods both as a source of protein and energy. Tannins (tannic acid equivalent) content is in the order of *Acacia nilotica* seed meal < *Prosopis juliflora* < *Tamarindus indica* < *Madhuca indica* < *Camelia assamica* < *Shorea robusta* <*Mangifera indica* < *Acacia nilotica* pods. Tannins (tannic acid equivalent) did not influence IVDMD, IVOMD and IVGP in agro-industrial byproducts but decreased IVCPD (r^2=0.24, P<0.05). All those agro-industrial byproducts containing > 8% tannin resulted in lower (P<0.05) IVGP in first stage while higher (P<0.05) in second stage kinetic.

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