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Cornell net carbohydrate and protein system for nutritional evaluation of tree leaves, shrubs and grasses

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ABSTRACT

Five species each of tree leaves, shrubs and grasses were nutritionally evaluated for their chemical constituents, carbohydrates, proteins and nitrogen fractions, following the Cornell net carbohydrate and protein system (CNCP). A summative approach was also used to derive the TDN (total digestible nutrient, (%DM) at 1X maintenance) values of these feeds so that more realistic values can be obtained to decide their appropriate level of incorporation in total mixed ration of the ruminants. The CP varied from 12.27 to 21.79, 11.27 to 17.49 and 3.49 to 4.27% in tree leaves, shrubs and grasses, respectively. Among the tree leaves, CP was highest in *Leucaena leucocephala* (21.79%) followed by *Albizia lebbek* (20.85%) and lowest in *Anogeissus pendula* (17.49%). In shrubs, the CP was maximum in *Dichrostachys cinerea*. Grasses were the richest in total carbohydrates compared to shrubs and tree leaves. Non-structural carbohydrate content varied greatly among the tree leaves, shrubs and grasses. Significantly lesser quantity of fraction C was present in *Grewia optiva*, *Securengia virosa* and *Heteropogan contortus* in tree leaves, shrubs and grasses respectively. The instantly degradable fraction A of nitrogen was higher in tree leaves followed by grasses and shrubs, whereas rapidly degradable fraction B1 was higher in grasses followed by shrubs and tree leaves. Among the tree leaves and shrubs, *Grewia optiva, Leucaena leucocephala, Securengia virosa* and *Zizyphus xylophyrus* can be used as good sources of quality protein whereas, among the grasses, *Heteropogan contortus* and *Dichanthium annulatum* are better energy sources to the ruminants. The TDN estimates (at 1*X level of maintenance) showed a significant ($P<0.01$) positive correlation with *in vitro* DM ($r^2=0.42$) and CP ($r^2=0.59$) digestibility, thus indicated the trustworthiness of the mathematical model used to derive these TDN estimates.

Key words: CNCP system, Carbohydrate, Digestibility, Grasses, Protein quality, Shrubs, TDN, Tree leaves

The methods of feed analysis (proximate and detergent systems) applied for determination of nutritive value supply the information only about the concentration or quantity of feed nutrients and give little indication about their availability in animal system. Also there is considerable variability in the utilization pattern of different fractions of particular nutrient. For example different fractions of carbohydrate (soluble sugar, starch, non-starch polysaccharides) and protein (non-protein nitrogen, soluble peptides and soluble tree protein) are utilized by different rumen microbes and animals differently (Nocek and Russel 1988, Sniffen *et al.* 1992), indicating their importance of estimation in feedstuffs. Accurate prediction of forage biological value and performance of animals fed forages has been proposed by Russel *et al.* (1992), using Cornell net carbohydrate and protein system (CNCP). The present study therefore was undertaken to provide information on the nutrient components especially in term of carbohydrate and protein fractions of some tree leaves, shrubs and grasses, available in semi-arid region of India using CNCP system. A summative approach was also used to derive the TDN (total digestible nutrient, (% DM at 1X maintenance) values of these feeds so that more realistic values can be obtained to decide their appropriate level of incorporation in total mixed ration of the ruminants.

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MATERIALS AND METHODS

Feeds and their processing

Samples (15), 5 of each of tree leaves (*Albizia lebbek, Leucaena leucocephala, Hardwickia binata, Grewia optiva* and *Anogeissus pendula*), shrubs (*Acacia catechu, Securengia virosa, Zizyphus xylophyrus, Dichrostachys cinerea* and *Helictris isora*) and grasses (*Heteropogan contortus, Sehima nervosum, Chrysopogon fulvus, Cenchrus ciliaris* and *Dichanthium annulatum*) were collected, when ambient temperature varied from 16.0° to 35 °C, from the Central Research Farm of Indian Grassland and Fodder Research Institute. Initially the samples were dried in shed and then in an oven at 60 °C till constant weight.

Chemical analysis

The dried samples were ground to pass through 1 mm sieve size and analysed for total nitrogen (N), ether extract (EE) and ash (AOAC 1995), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (ADL), nitrogen that is insoluble in neutral detergent and acid detergent solutions (Van Soest *et al.* 1991), soluble protein (Krishnamoorthy *et al.* 1983), non protein nitrogen (NPN) (Licitra *et al.* 1996) and starch (AOAC 1995). Non-structural carbohydrate fraction was computed directly (Van Soest *et al.* 1991: 100@–(NDF-NDFP)+fat +ash).

In vitro *dry matter (DM) and crude protein (CP) digestibility*

The *in vitro* DM digestibility (IVDMD) was estimated as per Tilley and Terry (1963). The rumen liquor was obtained from fistulated Hariana bullock maintained on a wheat straw based diet (roughage to concentrate ratio 65:35). Water was offered twice daily. This schedule was followed for 21 days before collecting the rumen liquor. The rumen liquor was collected just after 4 h post feeding. The collected rumen liquor was immediately brought to laboratory and strained through four layers of cheesecloth. The strained rumen liquor (SRL) was flushed with CO_2 for 2 min. The 20 ml quantity of SRL (20 ml) was transferred in to each *in vitro* bottle containing 0.5 g substrate and 10 ml of McDougal's artificial saliva. The bottles were incubated for 48 h in a water-bath at 39.0±1.0 °C with automatic shaker. The *in vitro* crude protein digestibility (IVCPD) was estimated taking in to account the initial CP content of samples before incubation and the CP content after in vitro incubation. The data were analysed using SPSS 10.0 package.

Estimation of total digestible nutrients (TDN)

To derive the TDN values (at 1X level of maintenance), a summative approach was applied (NRC 2001) by estimating the concentration (% of DM) of truly digestible non-fibre carbohydrate (NFC), CP, EE and NDF for each feed (Weiss *et al.* 1992). Digestible NDF was obtained using a 48-h rumen *in vitro* assay.

RESULTS AND DISCUSSION

Chemical composition

The proximate and cell wall constituents of tree leaves, shrubs and grasses are presented in Table 1. The CP varied in tree leaves, shrubs and grasses. Among the tree leaves, CP content was highest in *Leucaena leucocephala* followed by *Albizia lebbek* and lowest in *Anogeissus pendula*. In shrubs, the CP content was maximum in *Dichrostachys cinerea*. In grasses no significant variation in CP content was observed. The fibre fractions (NDF, ADF and cellulose) were higher (P<0.01) in grasses compared to tree leaves and

Table 1. Chemical composition of tree leaves, shrubs and grasses (% of DM basis)

Name	CP	EE	NDF	ADF	Lignin	Ash	Cellulose	Hemi cellulose	TDN*
Tree leaves									
Albizia lebbek	20.85	4.74	47.75	29.12	11.01	9.51	17.97	18.63	54.85
Leucaena leucocephala	21.79	5.22	27.14	16.74	6.40	11.82	10.63	10.40	65.09
Hardwickia binata	15.86	2.53	35.88	26.87	8.49	8.82	18.22	9.01	60.37
Grewia optiva	16.70	4.20	34.37	21.02	3.93	13.47	17.06	13.35	63.81
Anogeissus pendula	12.27	4.11	37.58	22.22	6.96	9.02	15.48	15.36	62.62
Mean (Tree leaves)	*17.49*	*4.16*	*36.54*	*23.19*	*7.36*	*10.53*	*15.87*	*13.35*	*61.35*
Shrubs									
Acacia catechu	11.84	4.14	31.46	19.48	6.79	9.20	9.44	11.98	65.05
Securengia virosa	11.27	1.87	25.78	16.47	3.40	9.59	13.04	9.31	67.57
Zizyphus xylophyrus	11.83	2.37	26.32	12.98	4.99	8.11	8.57	13.34	67.18
Dichrostachys cinerea	17.18	1.55	40.34	28.87	13.39	11.64	13.01	11.47	47.85
Helictris isora	13.40	3.15	39.66	25.10	6.00	11.55	17.95	14.56	60.65
Mean (Shrubs)	13.10	2.63	32.71	20.58	6.91	10.02	12.40	12.13	61.66
Grasses									
Heteropogan contortus	3.69	1.05	48.91	43.97	4.65	7.45	38.29	4.94	60.43
Sehima nervosum	3.55	1.41	71.55	43.80	5.13	12.86	36.31	27.75	48.07
Chrysopogon fulvus	3.49	1.34	72.91	40.62	6.61	8.44	33.50	32.29	49.29
Cenchrus ciliaris	4.13	1.50	69.40	40.89	5.71	10.93	34.14	28.51	49.62
Dichianthium annulatum	4.27	1.46	71.07	42.42	4.93	10.64	34.78	28.65	51.01
Mean (Grasses)	3.83	1.35	66.77	42.34	5.41	10.07	35.40	24.43	51.69
P	**	**	**	**	**	**	**	**	**
SEM	0.27	0.08	0.54	0.21	0.25	0.25	0.20	0.59	0.60

*Estimated total digestible nutrients (TDN,%DM) at 1*X maintenance level; P, statistical significance; SEM, standard error of means; **=P<0.01.

Table 2. Carbohydrate fractions in tree leaves, shrubs and grasses

Name	Carbohydrates (%)	Non-structural carbohydrates (% of CHO)	Carbohydrate fractions (% of carbohydrates)			
			A	B_1	B_2	C
Tree leaves						
Albizia lebbek	64.89	28.71	13.35	15.36	30.57	40.72
Leucaena leucocephala	61.16	57.77	36.65	21.12	17.11	25.12
Hardwickia binata	72.99	52.06	31.42	20.65	19.92	28.02
Grewia optiva	65.62	49.38	26.14	23.24	36.23	14.38
Anogeissus pendula	74.59	50.22	30.10	20.12	27.38	22.40
Mean (tree leaves)	67.85	47.63	27.53	20.10	26.24	26.13
Shrubs						
Acacia catechu	74.82	58.88	38.61	20.27	19.33	21.79
Securengia virosa	77.27	67.09	53.17	13.92	22.35	10.57
Zizyphus xylophyrus	77.68	67.09	47.61	19.48	17.48	15.43
Dichrostachys cinerea	69.44	43.44	28.57	14.87	10.38	46.18
Helictris isora	71.89	46.37	28.59	17.79	33.59	20.04
Mean (shrubs)	74.22	56.58	39.31	17.27	20.63	22.80
Grasses						
Heteropogan contortus	87.80	44.38	20.57	23.81	42.90	12.72
Sehima nervosum	82.18	13.01	1.85	11.15	72.01	14.98
Chrysopogon fulvus	86.74	16.03	3.47	12.56	65.67	18.30
Cenchrus ciliaris	83.43	16.92	4.10	12.82	66.65	16.43
Dichianthium annulatum	83.62	15.09	1.86	13.23	70.76	12.15
Mean (grasses)	84.754	21.09	6.37	14.71	63.60	15.32
P	**	**	**	**	**	**
SEM	1.12	0.97	1.18	0.47	0.78	0.79

P, Statistical significance; SEM, Standard error of means; **, $P<0.01$.

shrubs. Despite the higher cell wall fractions in grasses, the ADL content remained at par with the tree leaves and shrubs and plausible reason seems to be the stage of harvesting the grasses i.e., in between last week of July to first week of August, where the sprouting reaches to completion (Mishra 2004). Among the tree leaves the ADL was lowest in *Grewia optiva* and highest in *Albizia lebbek*. The higher lignin content has already been reported in *Albizia lebbek* in earlier study (Bhadauria *et al*. 2002). The readily utilizable hemicellulose was highest ($P<0.01$) in grasses than that in tree leaves and shrubs.

Estimates of total digestible nutrients

Estimates of total digestible nutrients (TDN) values at 1X level of maintenance are presented in Table 1. Tree leaves and shrubs provided higher ($P<0.01$) estimates of TDN compared to various grasses. The estimates of TDN seem to be comparable with the TDN values obtained in conventional digestibility trial conducted with various other tree leaves (Tewtia and Khirwar 2002, Rajendran and Kadirvel 2002, Jeyaprakash *et al*. 2003).

Carbohydrate fractions

The values of structural, non-structural carbohydrates (NSC) and different fractions i.e. fraction A (fast degradable), B1 (intermediate degradable), B2 (slow degradable and C (unable cell wall) are presented in Table 2. Grasses were the richest in total carbohydrates compared ($P<0.01$) to shrubs and tree leaves. Non-structural carbohydrate contents varied greatly among the tree leaves, shrubs and grasses. Among the tree leaves, the *Leucaena leucocephala* leaves contained the highest NSC, whereas in shrubs and grasses the NSC was highest in *Securengia virosa* and *Heteropogan contortus*, respectively. The carbohydrate fraction A was considerably lower ($P<0.01$) in grasses than the tree leaves and shrubs. Fraction B1 (intermediately degradable in rumen and mostly considered as starch) also followed the similar trend. The contents of carbohydrate fraction B2, which is strongly degradable and mostly representing available cell wall, was higher ($P<0.01$) in grasses followed by tree leaves and shrubs.

Table 3. Protein fractions and *in vitro* crude protein digestibility (IVCPD) in tree leaves, shrubs and grasses

Name	*In vitro* CP digestibility	% of CP			
		Soluble protein	Non-protein nitrogen	Acid detergent insoluble protein	Neutral detergent insoluble protein
Tree leaves					
Albizia lebbek	53.80	35.08	58.81	18.27	34.22
Leucaena leucocephala	88.97	35.06	81.47	19.33	27.67
Hardwickia binata	54.43	50.39	76.32	18.66	39.14
Grewia optiva	66.53	39.73	66.82	18.51	41.54
Anogeissus pendula	78.37	18.84	59.49	20.95	30.50
Mean (Tree leaves)	68.42	35.82	65.38	19.15	34.62
Shrubs					
Acacia catechu	68.75	22.79	54.08	25.01	42.85
Securengia virosa	82.58	16.94	45.31	21.30	27.81
Zizyphus xylophyrus	69.29	18.17	36.12	35.42	54.01
Dichrostachys cinerea	52.50	35.05	16.46	31.10	33.20
Helictris isora	73.72	31.57	71.58	21.36	61.99
Mean (Shrubs)	69.36	24.91	44.71	26.84	43.97
Grasses					
Heteropogan contortus	60.97	31.31	78.75	38.21	54.98
Sehima nervosum	60.39	25.53	53.94	47.88	52.29
Chrysopogon fulvus	60.25	24.91	45.60	65.77	69.01
Cenchrus ciliaris	61.27	31.45	26.78	47.62	55.88
Dichianthium annulatum	73.47	38.75	17.97	30.64	40.77
Mean (grasses)	63.27	30.39	44.61	46.02	54.59
P	**	**	**	**	**
SEM	1.63	1.70	5.40	1.02	1.03

P, Statistical significance; SEM, Standard error of means; **, $P<0.01$.

Significantly ($P<0.01$) lesser quantity of fraction C was present in *Grewia optiva, Securengia virosa* and *H. contortus* in tree leaves, shrubs and grasses respectively, indicating the superiority of carbohydrate quality present in these feedstuffs. Bhadauria *et al.* (2002) also observed the lowest quantity of carbohydrate fraction C in *S. virosa* (13.46%) among the various shrubs evaluated.

Protein and nitrogen fractions

Buffer soluble protein fractions of different tree leaves, shrubs and grasses are depicted in Table 3. The buffer soluble protein fraction among these feed resources was highest in *Hardwickia binata* (50.39%) and lowest in *S. virosa*. The non-protein nitrogen (NPN) was higher ($P<0.01$) in *L. leucocephala* and minimum in *D. cinerea*. Singh *et al.* (2002) also reported higher NPN content in *L. leucocephala* in comparison to other tree leaves studied. The ADIP content was lesser ($P<0.01$) in *A. lebbek, S. virosa, D. annulatum*, whereas NDIP content was lesser ($P<0.01$) in *L. leucocephala, S. virosa* and *D. annulatum*.

The instantly degradable fraction A of nitrogen (Table 4) was higher ($P<0.01$) in tree leaves followed by grasses and shrubs, whereas rapidly degradable fraction B1 was higher ($P<0.01$) in grasses followed by shrubs and tree leaves. Pichard (1977) also reported higher proportion of B1 fraction of N in forages and grasses. However, B3 fraction of N was similar in tree leaves and shrubs, whereas its content in grasses was significantly ($P<0.01$) lesser compared to former two. These results corroborate the findings of Krishnamoorthy *et al.* (1992), wherein grasses, forages and barley grains contained higher proportion of B3. Conversely to B3 fraction, the fraction C of N, which cannot be degraded by the ruminal bacteria and does not provide amino acid post ruminally (Krishnamoorthy *et al.* 1982) was substantially higher ($P<0.01$) in grasses than tree leaves and shrubs. Within the tree leaves the variation in fraction C was of lesser magnitude and ranged from 18.27 to 20.95%. In shrubs the fraction C was highest in *S. virosa* and *D. cinerea* and maximum in *Z. xylophyrus*. Earlier report also confirms the higher content of C fraction in *Z. xylophyrus* (Bhadauria *et al.* 2002). In

Table 4. Nitrogen fractions in tree leaves, shrubs and grasses

Name	Nitrogen fractions (%)				
	A	B1	B2	B3	C
Tree leaves					
Albizia lebbek	20.66	14.42	30.70	15.95	18.27
Leucaena leucocephala	28.55	6.51	37.27	8.34	19.33
Hardwickia binata	38.44	11.95	10.47	20.48	18.66
Grewia optiva	26.63	13.10	18.72	23.04	18.51
Anogeissus pendula	11.08	7.76	50.66	9.55	20.95
Mean (tree leaves)	25.07	10.75	29.57	15.47	19.15
Shrubs					
Acacia catechu	12.22	10.56	34.37	17.84	25.01
Securengia virosa	7.66	9.29	55.24	6.50	21.30
Zizyphus xylophyrus	6.66	11.52	27.82	18.58	35.42
Dichrostachys cinerea	5.77	29.29	31.74	2.10	31.10
Helictris isora	22.47	9.10	6.44	40.63	21.36
Mean (shrubs)	10.95	13.95	31.12	17.14	26.84
Grasses					
Heteropogon contortus	24.56	6.74	13.71	16.78	38.21
Sehima nervosum	14.04	11.50	22.17	4.41	47.88
Chrysopogon fulvus	11.11	13.80	6.09	3.23	65.77
Cenchrus ciliaris	8.26	23.19	12.67	8.26	47.62
Dichianthium annulatum	6.68	32.07	20.48	10.13	30.64
Mean (grasses)	12.93	17.46	15.02	8.57	46.02
P	**	**	**	**	**
SEM	1.50	2.05	1.64	1.95	1.02

P, Statistical significance; SEM, Standard error of means; **, $P<0.01$.

grasses, *C. fulvus* contained the maximum proportion of C fraction of N whereas in *D. annulatum*, it was the lowest.

Relationship among cell wall constituents and dry matter digestibility (in vitro)

The correlation coefficients among cell wall constituents and IVDMD revealed that in all the feed resources (tree leaves, shrubs and grasses) *in vitro* DM digestibility was highly ($P<0.01$) negatively correlated with NDF, ADF, cellulose and ADL content, whereas these cell wall constituents were positively correlated with each other (Table 5). However, the hemicellulose part of the cell wall constituents was positively correlated ($P<0.01$ and $r^2=0.58$) with *in vitro* DM digestibility. Thus it appears that the hemicellulose provides readily available nutrients to the rumen microbes for enhancing the degradation of organic matter in straws and fibrous feeds.

Table 5. Relationship of cell wall fractions with *in vitro* DM digestibility (IVDMD) of tree leaves, shrubs and grasses

Attributes	NDF	ADF	Cellulose	Hemi cellulose	ADL	IVDMD
NDF	–	0.92**	0.89**	0.87**	0.04	−0.33
ADF	0.92**	–	0.96**	0.61**	−0.01	−0.45
Cellulose	0.89**	0.96**	–	0.60**	−0.26	−0.32
Hemicellulose	0.87**	0.61**	0.60**	–	−0.08	0.48
ADL	−0.04	−0.01	−0.26	−0.07	–	−0.48
IVDMD	−0.33*	−0.45**	−0.32	0.48*	−0.48**	–

*and **, Correlation is significant at the $P<0.05$ and $P<0.01$ level, respectively.

Table 6. Relationship of carbohydrate fractions with *in vitro* DM digestibility (IVDMD) of tree leaves, shrubs and grasses

Attributes	Fractions of carbohydrate				*In vitro* DM digestibility (%)
	A	B1	B2	C	
Fraction-A	–	0.499**	–0.869**	0.005	0.443
Fraction B1	0.499	–	–0.562	–0.035	0.014
Fraction B2	–0.869**	–0.562**	–	–0.471**	0.089
Fraction C	0.005	–0.035	–0.471**	–	–0.364*
In vitro DM digestibility (%)	0.443	0.014	0.089	–0.364*	-

* and **, Correlation is significant at the P<0.05 and P<0.01 level, respectively.

Relationship among cell wall fractions and dry matter digestibility (in vitro)

Correlation coefficients workout for the different fraction of carbohydrates of tree leaves, shrubs and grasses with *in vitro* DM digestibility (Table 6) indicated that fraction C was negatively correlated with the IVDMD, whereas the fraction A was positively correlated to the IVDMD. However, B1 and B2 had a nonsignificant correlation with IVDMD.

Relationship of total digestible nutrients estimates (TDN) with dry matter and protein digestibility

The TDN estimates (at 1*X level of maintenance) showed a significant (P<0.01) positive correlation with *in vitro* DM (r^2=0.42) and CP (r^2=0.0.59) digestibility (Table 8). Further, the higher TDN estimates were found associated with the feeds containing comparatively lesser quantity of nutritionally less significant or poorly utilizable fractions/

Table 7. Relationship of protein and nitrogen fractions with *in vitro* CP digestibility (IVCPD) of tree leaves, shrubs and grasses

Attributes		Protein fractions			Nitrogen fractions				
	In vitro CP digestibility (IVCPD)	Non protein nitrogen (NPN)	Acid detergent insoluble protein (ADIP)	Neutral detergent insoluble protein (NDIP)	A	B1	B2	B3	C
ADIP	–0.345*	–0.353*	–	0.730**	–0.417*	0.184	–0.447**	–0.463**	–
NDIP	0.324*	–0.042	0.730**	–	–0.090	–0.029	–0.780**	0.269	0.730**
N-A0	0.063	0.844**	–0.417**	–0.090	–	–0.482**	–0.326*	0.471**	–0.417**
N-B1	0.327*	–0.833**	0.184	–.029	–0.482**	–	–0.206	–0.297*	0.184
N-B2	0.502**	–0.068	–0.447**	–0.780**	–0.326**	–0.206	–	–0.381**	–0.447**
N-B3	0.065	0.443**	–0.463**	0.269	0.471**	–0.297*	–0.381**	–	–0.463**
N-C	–0.345**	–0.353**	–	0.730**	-0.417**	0.184	–0.447**	–0.463**	–
IVCPD	–	0.188	–0.345**	0.324**	0.063	0.327*	0.502**	0.065	–0.345**

*and **, Correlation is significant at the P<0.05 and P<0.01 level, respectively.

Relationship of protein and nitrogen fractions with protein digestibility (in vitro)

Acid detergent insoluble protein (ADIP) fraction of tree leaves, shrubs and grasses was found to be negatively correlated with *in vitro* CP digestibility (Table 7), whereas in case of neutral detergent insoluble protein (NDIP) the relationship was inverted and a significant (P<0.01) positive correlation was observed. Among the nitrogen fractions, B1 and B2 were positively correlated with CP digestibility. The fraction C of N had the significant (P<0.01) negative correlation with CP digestibility substantiating the fact that the ruminal microbes poorly utilize it.

Table 8. Relationship of total digestible nutrients (TDNX) with *in vitro* DM (IVDMD) and CP digestibility (IVCPD) of tree leaves, shrubs and grasses

Attributes	IVDMD	IVCPD	TDNIX
IVDMD	-	.699**	.415**
IVCPD	.699**	-	.592**
TDNX	.415**	.592**	–

**Correlation is significant at the P<0.01 level.

components i.e. ADL, C fraction of carbohydrates and nitrogen, ADIP etc. In present investigation, the *S. virosa* and *Z. xylophyrus* were having the lower content of these nutritionally less significant or poorly utilizable fractions/ components and thus provided the higher (P<0.01) estimates of TDN compared to other tree leaves, shrubs and grasses.

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