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Utilization of tanniniferous feeds. 6. Chemical composition, tannin fractionation, *in vitro* digestibility and gas production of some top foliage

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ABSTRACT

Top foliages namely *Robinia pseudoacacia* (Robinia), *Fiscus religiosa* (Pipal) and *Quercus incana* (oak) were evaluated for chemical composition, tannin fractionation, *in vitro* digestibility of nutrients and gas production. Crude protein was higher in *Quercus incana* followed by *Robinia pseudoacacia* and *Fiscus religiosa*. Nitrogen free extract content was ranged from 36.2 to 59.4%. Total tannin (tannic acid equivalent) and condensed tannin (leucocyanidin equivalent) content was in the order of *Quercus incana*>*Robinia pseudoacacia*> *Fiscus religiosa*. *In vitro* dry matter, organic matter and gas production was higher in *Fiscus religiosa*, followed by *Robinia pseudoacacia* and *Quercus incana*. *In vitro* crude protein digestibility was in the order of *Fiscus religiosa* > *Quercus incana* >*Robinia pseudoacacia*. It is concluded that *Robinia pseudoacacia* and *Fiscus religiosa* may be used as a maintenance fodder for livestock while *Quercus incana* can be used as a lowgrade fodder in hilly areas where there is scarcity of fodders.

Key words: *In vitro* evaluation, Tannins, Top foliages

Top foliages may form a part of basal diet of ruminants in hilly areas (Kumar 1983, Makkar *et al.* 1986). It forms an integral part of sheep and goat feeding regime and nearly 60% of total feeds available to sheep and goat comes from top foliages (Barbind *et al.* 1994). However, their use as animal feed are limited even through there is shortage of grains and their by-products, because of the presence of 'tannins' – an anti-nutritional factor (Panda *et al.* 1983, Tripathy *et al.* 1984, Makkar *et al.* 1991). It is essential to estimate such anti-nutrient factor in top foliages as they have negative effect on nutrients digestibility (McLeod 1974 Kumar and Singh 1984, Barman and Rai 2000, 2003 a, c). In the present paper 3 species of top foliages have been evaluated for their nutritive value, tannin levels and the *in vitro* nutrient digestibility and gas production.

MATERIALS AND METHODS

The sample was dried at 55 ± 1°C to constant weight and ground to pass through a sieve of 1 mm diameter and stored in plastic containers with lid for further analysis. Proximate analysis (AOAC 1990) and fibre constituents were analyzed (Goering and Van Soest 1970).

For tannin extraction, 400mg 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.

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. Condensed tannin (leucocyanidin equivalent) was estimated by butanol- HCl method of Porter *et al.* (1986). Hydrolysable tannin was claculated from the difference between total tannins phenol and condensed tannin.

In vitro *nutrient digestibility and gas production*

Three rumen fistulated male crossbred (Holstein Friesian ×Sahiwal) cattle (body weight 238.0, 228.0 and 185.0 kg respectively) were selected and 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. Rumen liquor was collected by using a vacuum suction 3h post feeding and

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watering. The *in vitro* nutrient digestibility was estimated as per Tilley and Terry (1963).

Statistical analysis

The data were analyzed by one-way ANOVA as per Snedecor and Cochran (1989). Regression of effect of tannins (tannic acid equivalent) on *in vitro* dry matter (IVDMD), organic matter (IVOMD), crude protein digestibility (IVCPD) and gas production (IVGP) was estimated. Correlation coefficient was calculated to see the relationship between IVOMD and IVGP.

RESULTS AND DISCUSSIONS

Crude protein, crude fibre and fibre fractions in oak, ether extract in pipal and NFE in *Robinia* were higher (P<0.0.5) than in other leaves (Table 1). Higher crude protein (11.6%) content of Oak leaves was also reported by Lohan *et al.* (1983). Ranjhan (1993) also reported a similar CP content of *Fiscus religiosa* and *Quercus incana* leaves.

The total phenol and total tannin content of *Quercus incana* was higher (P<0.05) than that in *Robinia pseudoacacia* and *Fiscus religiosa* (Table 1). Hydrolysable tannins content was in the order (P<0.05) of *Robinia pseudoacacia<Fiscus religiosa <Quercus incana.* Condensed tannins content of *Robinia pseudoacacia* and *Quercus incana* leaves was higher (P<0.05) than *Fiscus religiosa*. Higher level of condensed tannins (2.50% DM) in *Quercus incana* leaves was also reported by Lohan *et al.* (1983).

The IVDMD and IVOMD of *Fiscus religiosa* leaves were higher (P<0.05) followed by *Robinia pseudoacacia* and *Quercus incana* (Table 2). With increasing level (P<0.05) of

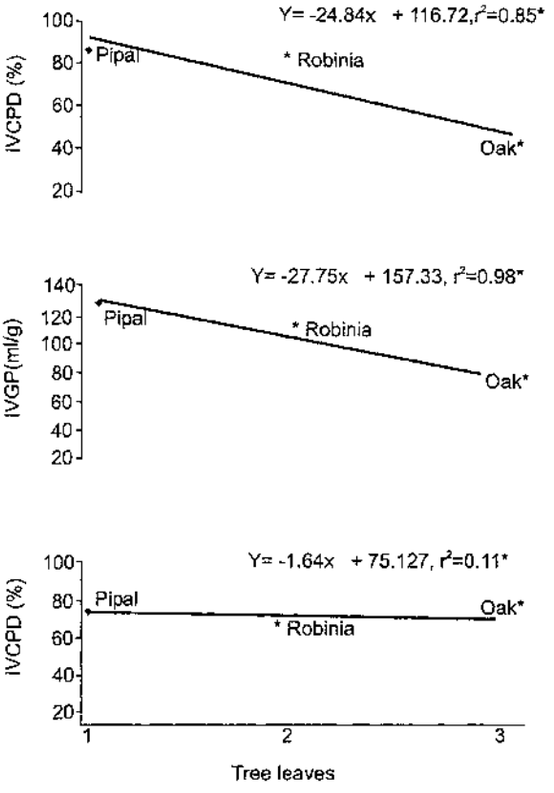

Fig. 1. Regression of IVOMD (%), IVGP (ml/g) and IVCPD (%) in different tree leaves (*Pipal, Ficus religiosa; Robinia, Robinia pseudoacacia*; oak, *Quercus incana*) with increasing tannin concentration.

Table 1. Chemical composition and tannin content of top foliages (%DM)

Parameters	Robinia (*Robinia pseudoacacia*)	Pipal (*Fiscus religiosa*)	Oak (*Quercus incana*)	SEm
OM	92.1±0.4	91.7±0.5	97.2±0.5	0.24^{NS}
CP	8.9^{b}±0.2	7.9^{a}±0.1	9.6^{c}±0.1	0.01
EE	3.2^{a}±0.0	10.1^{c}±0.0	4.1^{b}±0.0	0.01
CF	20.5^{b}±0.0	26.4^{a}±0.0	47.4^{c}0.0	0.01
NFE	59.4^{c}±0.2	47.3^{b}±0.5	36.2^{a}±0.3	0.18
Ash	7.9^{b}±0.4	8.3^{c}±0.6	2.8^{a}±0.5	0.24
NDF	54.5^{b}0.9	50.7^{a}±0.1	63.2^{c}±1.0	0.39
ADF	52.9^{b}±0.2	48.6^{a}±0.1	60.6^{c}±0.5	0.15
Cellulose	50.0^{b}±1.8	43.7^{a}±0.9	54.5^{c}±0.2	0.59
HC	1.6^{a}±0.1	1.9^{a}±0.3	2.6^{b}±0.5	0.16
Lignin	2.6^{a}±0.2	4.1^{b}±0.1	5.5^{c}0.3	0.11
Total phenol[1]	3.83^{b}±0.07	1.99^{a}±0.10	9.39^{c}±0.25	0.13
Non tannin phenol	1.0^{b}±0.12	0.47^{a}0.02	1.23^{c}±0.000	0.44
Total tannin[1]	2.83^{b}±0.08	1.52^{a}±0.08	8.16^{c}0.24	0.13
Hydrolysable tannin	0.15^{a}±0.06	1.41^{b}±0.06	5.33^{c}±0.12	0.07
Condensed tannin[2]	2.68^{b}±0.10	0.11^{a}±0.02	2.83^{b}±0.14	0.44

SEm, Standard error of mean; different superscript ^{a,b,c} in a row differ significantly (P<0.05), NS, nonsignificant; [1]tannic acid equivalent, [2]leucocyanidin equivalent.

Table 2. *In vitro* digestibility (% DM) and gas production of top foliages

Parameters	Robinia (*Robinia pseudoacacia*)	Pipal (*Fiscus religiosa*)	Oak (*Quercus incana*)	SEm
IVDMD (%)	71.45^{b}±3.50	75.35^{c}±1.24	36.7^{a}±0.99	1.81
IVOMC (%)	78.84^{b}±3.38	85.95^{c}±5.42	36.27^{a}±0.44	3.01
IVCPD (%)	66.38^{a}±3.29	76.22^{b}2.19	72.94^{b}±1.91	2.06
Incubation period (h)				
1st stage kinetics				
6h	15.67^{r}±0.33	13.33^{q}±0.33	1.00^{p}±0.58	0.35
12h	9.33q±0.33	15.00^{r}±0.00	5.00^{p}±0.58	0.31
18h	7.00^{q}±0.00	10.67^{r}±0.33	6.50^{p}0.29	0.21
24h	8.00^{q}±0.00	10.00^{r}0.58	5.50^{p}±0.29	0.30
Total (ml)	40.00^{q}±0.00	49.00^{r}0.58	18.00^{p}±0.58	0.39
(ml/h/g)	80.00^{q}±0.00	98.00^{r}±1.16	36.00^{p}±1.16	0.77
Rate of production	3.33^{q}±0.00	4.08^{r}±0.05	1.50^{p}0.05	0.03
2nd stage kinetics				
24h	8.00^{q}±0.00	10.00^{r}±0.58	5.50^{p}0.29	0.30
30h	1.17^{p}±0.17	1.67^{q}0.33	4.00^{r}±0.00	0.18
36h	3.00^{p}±0.00	3.33^{q}±0.33	3.50^{q}±0.00	0.16
42h	2.67^{p}±0.33	2.67^{p}0.00	5.67^{q}±0.00	0.27
48h	2.33^{q}±0.33	2.00^{p}±0.00	2.00^{p}±0.00	0.16
Total (ml)	17.17^{p}±0.44	19.67^{q}±0.67	20.67^{r}0.60	0.47
Rate of production	1.43^{p}±0.04	1.64^{q}±0.06	1.72^{r}±0.05	0.04

SEm, Standard error of mean; IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; IVCPD, *in vitro* crude protein digestibility; superscripts ^{a,b,c} and ^{p,q,r} in a row differ significantly (P<0.05; P<0.01).

tannins (tannic acid equivalent) in the leaves IVDMD and IVOMD decreased significantly (P<0.01, Y= -19.325x+99.817, r^2=0.82; Y =-24.84x+116.72, r^2 0.85, Fig 1). However, IVCPD of *Robinia pseudoacacia* was significantly (P<0.05) lower compared to *Quercus incana* and *Fiscus religiosa*. Although IVCPD value of *Fiscus religiosa* was higher than *Quercus incana* but did not differ significantly (Y= -1.64x + 75.127, r^2=0.11, Fig. 1). Similar result was observed in protein digestibility, which was not affected by inclusion of *Quercus incana* leaves in the diet of cattle (Lohan *et al.* 1984).

The total IVGP (ml) and the rate of production (ml/h/g) was lower (P < 0.05) in *Quercus incana* leaves during first stage kinetics (Table 2). However, during the second stage kinetics, IVGP was in order (P < 0.05) of *Quercus incana* >*Fiscus religiosa* > *Robinia pesudoacacia* in terms of both ml/g and ml/h/g and ml/h/g. However, overall IVGP (ml/g) was in the rank of *Ficus religiosa* > *Robinia pseudoacacia* > *Quercus incana* (P<0.05, Y = -27.75 x +157.33, r^2 = 0.98, Fig. 1). Similar to agro-industrial byproducts (Barman and Rai 2006a, 2006b) *Quercus incana* containing >8% tannins, produced high (P<0.05). IVGP in second stage kinetics compared to first stage kinetics.

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). This may be the reason of low (P<0.05) IVDMD, IVOMD and IVGP without any affect on IVCPD in tree leaves.

It is concluded that *Robinia pseudoacacia* and *Fiscus religiosa* leaves may be used as a maintenance fodder for livestock while *Quercus incana* leaves may be used as a low-grade fodder in hilly areas where there is scarcity of fodders.

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