SUBSTITUTION OF TRADITIONAL CONCENTRATES WITH GREWIA OPPOSITIFOLIA LEAVES IN SHEEP

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SUBSTITUTION OF TRADITIONAL CONCENTRATES WITH *GREWIA OPPOSITIFOLIA* LEAVES IN SHEEP

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ABSTRACT
An experiment was conducted in a 4 x 4 Latin Square Design, involving four cross bred wethers (F1, Kaghani x Rembouilet) of 50 kg ± 2 kg body weight, four diets and four feeding periods. The diets were chopped sorghum hay as basal diet (diet A), basal diet with cottonseed cake (diet B), basal diet with maize oil cake (diet C) and basal diet with *Grewia oppositifolia* leaves (diet D). The wethers were kept in individual metabolic crates. The experiment was conducted at NWFP, Agricultural University Dairy Farm Malakandair and was lasted for sixty days consisting four feeding periods. Each period was consisted of ten days adaptation followed by five days data collection. The crude protein contents were highest (26.04%) in cottonseed cake followed by *G. oppositifolia* leaves (17.25%), maize oil cake (16.21%) and was lowest in the basal diet (4.77%). Supplements affect the intake of basal diet; the higher total dry matter intake (TDMI) of the basal diet was recorded with diets C and B followed by diet D. No difference (P>0.05) in the dry matter and organic matter digestibility were observed among the four diets. The nitrogen digestibility of the basal diet was significantly lower than that containing leaves (P<0.05). Based on the present findings, it has undoubtedly confirmed that *G. oppositifolia* leaves, the cheapest among the purchasable sources of N in the country, has improved the utilization of basal diet through increased total dry matter intake and nitrogen digestibility. Therefore, it is concluded that *G. oppositifolia* leaves can successfully replace the traditional protein supplements in sheep.

INTRODUCTION
Sheep and goats are truly multiple purposes small ruminant which plays an important role in the economy of Pakistan. Together they account for the production of meat, skins, wool and hair. The available feed resources in Pakistan are green forages, dry roughages and by-products of oil and cereal grains. Livestock in the country are heavily relying on crop residues and grazing area. Due to overgrazing and high carrying capacity of animals in the country, the grazing potentials of the ranges are progressively in declining. Furthermore, the nutrients supplied from the feed resources are not enough to meet the requirements of the existing livestock population in Pakistan and thus resulting low productive, under nourished and diseased livestock. To fulfill the existing shortage gap of nutrients demand for growing livestock population in the country, there is a need to explore alternate ways of feed resources. One of the alternative ways is the supplementation of feed, but conventional feed supplements such as cottonseed cake, maize oil cake, mustard seed cake and cereal brans are usually very expensive and most of our farmers cannot afford to feed these adequately to their livestock. Therefore, supplementation of feeds with suitable agro-industrial by-products, or with tree leaves, which are economical (Ondiek *et al*. (2000), easily available and rich in protein and energy, may overcome the said problem and meet the deficiencies demand of nutrients in the country. Ondiek *et al*. (2000) suggested that the replacement of conventional concentrate with tree leaves would be cheaper than the conventional concentrate. According to Baumer (1991) tree herbage is an integral part of the ruminant diets and constitutes significant source of protein, mineral and vitamins. In Pakistan large varieties of tree leaves in both plain and hilly regions are available. Some of these are extensively used for livestock feeding. Nutritive value of some of the local tree leaves is very high and comparable to concentrates for ruminant. Among tree leaves, *G. oppositifolia* leaves are the one growing widely in the province of N.W.F.P. The said tree leaves contain more than 12% crude protein and is higher (more 70%) in *in sacco* degradability (Shabana 1998). The concentrates were purchased from the local market and the leaves were collected from communal land of Gadoon Amazia area which cost only labors and transportation charges. The available information suggested that the nutritive value of *G. oppositifolia* leaves is close to conventional oil cakes. Therefore, the present study was planned to compare the substitution effect of conventional oil cake (cottonseed cake and maize oil cake) with *G. oppositifolia* leaves in ruminant ration. For this purpose a basal diet of sorghum hay was supplemented with cottonseed cake, maize oil cake and *G. oppositifolia* leaves to investigate the effect of the supplements on nutrient intake and *in vivo* digestibility in sheep, with the following objectives.

1. To compare the substitution effect of *Grewia oppositifolia* leaves, cottonseed cake and maize oil cake on *in vivo* digestibility fed to sheep.
2. To compare nutrient composition of *G. oppositifolia* leaves, cottonseed cake and maize oil cake.

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MATERIALS AND METHODS

A study was conducted to determine the nutrients digestibility and nitrogen retention in sheep given a basal diet of sorghum hay supplemented with *Grewia oppositifolia* leaves, cottonseed cake and maize oil cake. Research work with sheep was performed at experimental unit of Animal Nutrition Department at the University Dairy Farm Malakandahre. The experiment was conducted in a 4 x 4 Latin quare design involving four wethers, four diets and four feeding periods. The animals were F1 adult wethers (Kaghani x Rambouillet) of the same age and were kept in individual metabolic crates with separate feeding arrangement, watering, collection of feaces and urine. The four crates were kept in a well illuminated and ventilated room. The animals were treated for endo-parasites a week before staring the study. The diets used in the experiment were chopped sorghum hay as a basal diet (A), basal diet with cotton seed cake as diet B, basal diet with maize oil cake as diet C and basal diet with *G. oppositifolia* leaves as diet C. The *G. oppositifolia* leaves were collected from Gadoon Amazai area and were dried in shade. The oil cakes were purchased from the local market. About 40% of the total nitrogen requirement of the sheep was met from the supplements based on cottonseed cake, maize oil cake or dried *G. oppositifolia* leaves. The nitrogen requirement of the sheep was estimated using the standard recommendation of NRC (1985). For the said purpose cottonseed cake, maize oil cake and *G. oppositifolia* leaves were analyzed for dry matter (DM) and total nitrogen (N) before starting the experiment and the quantity for each diet was calculated to supply the same amount of N (40% of the requirement). There were four different feeding periods; each period was consisted of 10 days adaptation followed by 5 days data collection. The arrangement of the four diets A, B, C and D and wethers in four different periods were as under:

EXPERIMENTAL LAYOUT

Periods	Wethers			
	I	II	III	IV
1	A	D	C	B
2	B	C	A	D
3	D	A	B	C
4	C	B	D	A

The diets were offered once a day at 9.00 hrs. Sorghum hay was offered as 10% in excess of the pervious day consumption. The supplement was offered in small container and the feed refusal of the pervious day was recorded daily before offering the fresh feed. Clean drinking water was made available to individual animal at all time in the containers fixed in the crates.

Collection of Feaces and Urine

During the experimental period, data on daily feed intake and excretion of feaces by the animals were recorded on daily basis. Each day feaces were weighed, thoroughly mixed and a sub sample equal to 20% of the total weight, was collected in a labeled polythene bag and stored in a freezer until analyzed. Similarly, urine excreted during the last 24 hours was collected in bottles containing 100 ml of 5N sulfuric acid solution. Volume of the urine was measured, mixed and sub sample equivalent to 10% of the total volume was collected in a labeled bottle and freeze. Representative samples of feed ingredients were also collected daily and pooled for each experimental period.

On completion of each experimental period, the samples of feaces, urine and feed was thawed, and pooled for each animal and was mixed thoroughly.

About 50g of the pooled sample of feaces and feed in duplicate were taken for dry matter analysis and the remaining were dried at 60^{o}C for 72 hours. The air dried sample were ground in a laboratory mill to 1mm particles size and stored in labeled bottles for further chemical analysis. Urine samples after thawing and mixing were analyzed for total nitrogen content.

Chemical analysis

Ground samples of feed and feaces in duplicate were analyzed for dry matter (DM), and ash according to the standard procedures of AOAC (1990). Crude protein (N x 6.25) was determined by the Kjeldahl method (AOAC, 1990). In this method samples were digested with concentrated sulphuric acid followed by distillation and titration.

Feed Intake and digestibility

Intake was measured as the difference of feed offered and feed refused while digestibility of dry matter was calculated as the difference between the nutrients consumed and voided in feaces by the sheep during 24 hours.

Statistics

The data were analyzed with ANOVA according to the 4 x 4 Latin square designs (Steel and Torrie, 1980). Dietary means were compared with the LSD procedure. A statistical package SAS (1991) was used for analysis.

The ANNOVA model was as under:

$Y_{ij\ (k)} = \mu + \alpha_i + \beta_j + t_{(k)} + e_{ij(k)}$

where

$Y_{ij\ (k)}$ = Combine effect of periods, animals and diets

μ = Average effect

α_i = Effect of periods

B_j = Effect of animals

$t_{(k)}$ = Effect of diets

$e_{ij(k)}$ = Random error

RESULTS

The present study was carried out in the department of Animal Nutrition in order to test the effect of four diets on four adult rams in terms of intake and digestibility. The four diets used in the experiment were sorghum hay as basal diet (A), sorghum hay with cottonseed cakes (B), sorghum hay with maize oil cakes (C) and sorghum hay with *G. opositifolia* leaves (D). The basal diet was offered as *ad libitum* in combination with other supplements. The experiment continued for 60 days (four feeding periods)**.** Each period was consisted of 10 days adaptation followed by 5 days data collection period. The chemical compositions i.e. dry matter (DM), ash, organic matter (OM) and crude protein (CP) of the diets are presented in Table I. The ash contents were higher in *G. opositifolia* and lower in maize oil cake while intermediate in cottonseed cake and sorghum hay. The crude protein content was highest (26.04%) in cottonseed cake followed by *G. oppositifolia* leaves (17.25%) and maize oil cake (16.21%) and the lowest in the basal diet (4.78.

Feed Intake and digestibility

Results of daily feed intake and dry matter digestibility of sorghum hay as basal diet and the affect of supplements on total dry matter intake (TDMI) by the animals are shown in Tables II and III. The highest dry matter intake (DMI) of the basal diet was recorded with cottonseed cake followed by maize oil cake and *G. oppositifolia* leaves while the lowest with the basal diet. Supplements affect (P<0.05) the intake of basal diet (sorghum hay). The higher total dry matter intake (TDMI) of the basal diet was recorded with diets C and B followed by diet D respectively, however, the difference (P>0.05) among the supplement was not statistically significant. The sheep fed diets A, B, C and D, the mean dry matter intake (g/day) was 546.86, 821.04, 825.68 and 796.77 respectively.

Diet composition affects the nitrogen digestibility in animal; an increase of 24.23-26.31 folds (g/day) in nitrogen digestibility was recorded when the basal diet was supplemented with all three supplements (Table III). However, no differences (P>0.05) in nitrogen digestibility among the three supplements were recorded. The sheep fed diets A, B, C and D, the mean percent nitrogen digestibility was 30.92, 56.43, 55.15 and 57.23 respectively**.**

DISCUSSION

Dry matter intake

Results of daily feed intake of sorghum hay as basal diet and the affect of supplements on total dry matter intake (TDMI) by the animals are shown in Table II. The highest dry matter intake (DMI) of the basal diet was recorded with cottonseed cake followed by maize oil cake and *G. oppositifolia* leaves while the lowest with the basal diet. Supplements affect (P<0.05) the intake of basal diet (sorghum hay). The higher total dry matter intake (TDMI) of the basal diet was recorded with diets C and B followed by diet D respectively, however, the difference (P>0.05) among the supplement was not statistically significant. One of the reasons for higher total dry matter intake of the basal diet with *G. oppositifolia* leaves (diet D) in the present study might be due to the higher palatability, as tree leaves are palatable (Norton & Waterfall 2000). The higher intake of the total dry matter of the basal diet supplemented with cottonseed cake may be due to its high protein content (Table I) and its bulky nature or may be a good appetizer. Patra *et al*. (2003), Ondiek *et al*. (2000) and Preston and Leng (1984) suggested that most of the straw based basal feed are usually low in nitrogen, digestible nutrients and minerals matter so an ideal supplements to such feed not only compensate for the nutrient deficiency but also boost up the intake of the basal diet of the animals as happened with the basal diet supplemented in the present experiment. Tree leaves is less lignified and contains more (10-29%) protein (Brenda *et al.* 1997). The *G. oppositifolia* leaves in the present study also qualify the above statement of the Brenda et al. (1997) and other workers in terms of protein (Table I); so the higher protein contents of *G. oppositifolia* leaves might be the other reasons for higher intake of basal diet. Swanson *et al.* (2000) credited the better supply of escaped protein in the diet toward higher feed intake. Improvement in intake of low quality forages may also be attributed to an increased rate of forage digestion and passage as a result of supplementation (Ellis 1978); that may happened with basal diet in the present study. Egan (1965) also reported that the intake of low quality forages might be enhanced by ruminal escape of supplemental protein, which also supports the findings of the present study. Alayon *et al.* (1998) stated that the supply of highly degradable nitrogen to the rumen increases microbial nitrogen (N) supply to the small intestine, which in turn increases the intake of diet. So the incorporation of the supplements, cottonseed cake, maize oil cake and *G. oppositifolia* leaves into basal diet appears to increase the microbial N supply to the small intestine of sheep and may have increased the intake of the basal diet.

Digestibilities

Dry matter and organic matter digestibility

Results of dry matter, organic matter and nitrogen digestibility of diets are shown in Table III. No differences were recorded in DM, OM digestibility among all diets fed to sheep. The same digestibility of the basal diet with the one supplemented may be due to certain factors inhibit the digestibility of diets B, C and D. Dixon and Stockdale (1999) stated that the substitution affects are often low when animals are consuming forage of low to medium digestibility and the digestibility of such forage is most likely determined by the rate of forage fiber digestion in the rumen. Reduced rate of fiber digestion in the rumen is often due to low rumen pH and/or an insufficiency of essential substrates for rumen microorganisms. With increasing maturity crude protein content and digestibility decreases with associated increases in the contents of cell wall constituents in forage. The digestive interactions of the feed mainly concerned with the crude fiber fraction i.e. cell wall content of the feeds and the accessibility of microorganisms to cell wall content. The inaccessibility of microorganisms to secondary walls is a major limitation to wall digestion in forages due to time constrains for microbes to access walls for digestion and thus much potentially digestible cell wall in grass feeds escapes in the feacal particles (Wilson & Mertens 1995; Wilson 1997; Moghaddam & Wilman 1998; Wilman & Ahmad 1999). The forage fiber content and ease of forage breakdown have also a major influence on voluntary feed intake and digestibility (Wilson *et al.*. 1989b; Wilson and Kennedy 1996); which may be affected by the activities of rumen microorganisms, by the handling and fate of plant particles in the rumen, by the load (physical bulk) of plant residues and the contraction of the organ responsible for digesta movement (Allen 1996). The basic requirement for the forage to pass from the rumen is the breakdown of feed from large particles to small particles (McLeod & Minson 1988; Wilson et al. 1989b). These authors suggested that the particles, which are held on a 1.2 mm screen during wet sieving, have a high resistance to passage from the rumen. Chewing during eating and during rumination is considered to be virtually the only factor responsible for reducing the size of particles in the rumen (Ulyatt et al. 1986; Wilson 1994); which in turn enhances the access of rumen microorganisms to the forage cell walls and cell content (Mtengeti et al. 1996; Wilman & Moghaddam 1998b; Hatfield et al. 1999) and hence high digestibility. Anatomical characteristics of leaves and stems of tropical and temperate grasses and legumes have influence on the ease and pattern of breakdown, and the characteristics of the resultant fiber particles. Epidermal and vascular structures, and microbial digestion, determine the initial breakdown of organs, which is fast in leaves of legumes and high quality temperate grasses, but slow in tropical grass leaves (Wilson and Kennedy 1996) further strengthen the hypothesis that inaccessibility of cell walls for microbial attack limits digestive weakening in both large and small particles. The basal diet of sorghum hay used in the present study seem enough mature and thus suggest greater cell wall contents and incomplete accessibility of microorganisms to the cell wall contents before the diet escapes from the rumen and hence low in *in vivo* digestibility. The diets containing cereals, interactions could be explained by drop in rumen pH and decrease in cellulolytic activity, due to which the digestibility of fibrous portion (NDF) decreases (Carro *et al*. 2000; Huhtanen and Jaakkola 1994; Berge *et al*. 1991 and Garces-Yepez 1997), as it might be the reason of low digestibility with the diet supplemented with MOC in the present experiment. The adverse effect of concentrate level on cell walls digestibility increases with maturity of the grasses and also higher cell walls contents of the grass (Huhtanen and Jaakkola 1994) as might be expected with the basal diet and cottonseed cake in the present study. With diet containing cottonseed cake (diet B) in the present experiment may be associated to its high hulls contents and the interaction can be explained above all by a large increase in *ad libitum* intake (Table III). In case a probable increase in digesta flow, and higher fibrous content (hull) in cottonseed cake contents may have prevented the access of microorganism from the cell wall contents before they escape in faecal particles (Wilson and Mertens 1995), can jointly explain an important decrease in diet B digestibility. The said suggestion can further be strengthen by that of Allen (1996), who reported that fibrous feed generally ferments and passes from the gastrointestinal tract more slowly than other dietary constituents, it has a greater filling effect over time than non-fibrous feed components and thus affect digestibility (Dixon and Stockdale 1999). Several other workers (Bitende and Ledin 1996; Woodward and Reed 1995) have demonstrated that tree leaves contain an anti nutritional factors particularly tannin that depressed the digestibility of the diet. Ayers *et al.* (1996) stated that the negative ADF and NDF digestibility is probably due to formation of CP-tannin complexes in the gut of the animal that might be the reason for low digestibility of the basal diet supplemented with *G. oppositifolia* leaves in the present study.

Nitrogen digestibility

Results of nitrogen digestibility of all diets are shown in Table III. Diet composition affects the nitrogen digestibility ($P<0.05$) in animals; an increase of 24.23-26.31 (g/day) in nitrogen digestibility was recorded when the basal diet was supplemented with all three supplements. The diets with three supplements were similar ($P>0.05$) in nitrogen digestibility. The lower nitrogen digestibility of the basal diet (diet A) may be attributed to its lower nitrogen content and intake (4.78 g/day) and thus its lower ammonia-N concentration in the rumen as many workers i.e. Bryant and Robinson (1962); Woodward and Reed (1997) suggested that microbial-N synthesis in the rumen depend on the concentration of ammonia-N. Optimizing rumen fermentation digestion of forages requires adequate ammonia N in the rumen to supply the N required for microbial growth (Leng 1990). Hence, the ammonia N in the current study would be considered adequate in the diets supplemented with basal diet. The lower intake of sorghum hay, the increase intake of the diets supplemented confirms the importance of availability of a source of N for effective utilization of the basal diet. This effect might be associated with increased rate of fermentation in the rumen (Mehrez and Orskov, 1978) or improved N status of the animal (Egan, 1965) or both. The CP content of the supplemented diets was generally high and can be considered adequate for supplying nitrogen to the rumen microbes, assuming that the CP is adequately degraded in the rumen (Wekesa *et al.* 2006).

Supplementation of the basal diet with all three supplements in the present study tended to increase significantly ($P<0.05$) the total DMI (Table II) and nitrogen digestibility (Table III) suggesting that *G. oppositifolia* leaves can be successfully substituted with the more expensive cottonseed cake or maize oil cake, when fed to sheep.

Table I ***Chemical composition of sorghum hay as basal diet with supplements fed to sheep (In percent)***

Diets	Dry matter	Ash	Organic matter	Crude Protein
Sorghum hay	95.57 ±1.251	7.89 ±0.222	92.11 ±0.241	4.77 ±0.345
Cottonseed cake	96.19 ±1.522	5.30 ±1.420	94.70 ±1.522	26.04 ±1.242
Maize oil cake	96.13 ±0.252	1.48 ±0.543	98.52 ±1.254	16.21 ±1.234
G. oppositifolia leaves	94.33 ±1.051	12.50 ±1.251	87.50 ±1.311	17.25 ±1.541

Table II ***Daily dry matter intake of sorghum hay as basal diet with supplements fed to sheep***

Diets	Total DMI (g/d)	Sorghum hay DMI (g/d)
Sorghum hay (A)	546.86^{b}	546.86^{b}
Sorghum hay + cottonseed cake (B)	821.04^{a}	674.58^{a}
Sorghum hay + maize oil cake (C)	825.68^{a}	596.10^{a}
Sorghum hay + *G. oppositifolia* leaves (D)	796.77^{a}	570.85^{b}
Cv (%)	7.43	9.39

Means in same column with different superscript are statistically significant at 0.05 α level

Source of variance

Intake

Diet:	0.0007
Period:	0.2792
Animals:	0.0006

Table III ***Organic matter intake and the digestibility of organic matter and dry matter of sorghum hay as a basal diet with supplements fed to sheep (In percent)***

Diets	OM intake	DM digestibility	OM digestibility	Nitrogen digestibility
Sorghum hay (A)	551.97[b]	53.13	55.84	30.92[b]
Sorghum hay + Cottonseed cake (B)	733.17[a]	53.74	59.66	56.43[a]
Sorghum hay + maize oil cake (C)	732.43[a]	59.37	56.65	55.15[a]
Sorghum hay + *G. oppositifolia* leaves (D)	762.74[a]	52.77	57.39	57.23[a]
Cv (%)	12.27	11.23 NS	9.65 NS	15.37

Means in same column with different superscript are statically significant at 0.05 α level.

Source of variance

	Dig.	Nitrogen dig.
Diet:	0.0554	0.6255
Period:	0.4542	0.0122
Animals:	0.6215	0.2812

CONCLUSIONS AND RECOMMENDATION

The present study has undoubtedly confirmed that *G. oppositifolia* leaves has improved the utilization of basal diet through increased total dry matter intake and nitrogen digestibility. Therefore, the incorporation of *G. oppositifolia* leaves as protein supplements to any low quality basal diet is highly recommended. To get proper benefits from *G. oppositifolia* leaves further study such as weight gain in small ruminants and milk yield in lactating animal are also recommended to be explored.

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