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EFFECT OF *GREWIA OPPOSITE OF OLIA* LEAVES AND CONVENTIONAL OIL CAKES AS FEED SUPPLEMENT ON CELL WALL INTAKE, DIGESTIBILITY AND NITROGEN RETENTION IN SHEEP

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ABSTRACT

An experiment was conducted in a 4 x 4 Latin Square Design, involving four cross bred Kaghani x Rembouilet wethers of 50 kg ± 2 kg body weight, four diets and four feeding periods. There were four diets such as basal diet (A)consisted chopped sorghum hay, diet B composed of basal diet with cottonseed cake (CSC), diet C composed of basal diet with maize oil cake (MOC)) and diet D consisted of basal diet with Grewia oppositifolia leaves. The wethers were kept in individual metabolic crates. There were four feeding periods and the experiment was lasted for sixty days. Each phase was consisted of ten days adaptation followed by five days data collection period. The basal diet and cottonseed cake were higher in NDF (64.94% and 62.38%, respectively) than maize oil cake and G. oppositifolia leaves (52.40% and 48.48%, respectively) and ADF (42.68%, 36.63% respectively) than maize oil cake and G. oppositifolia leaves (19.80% and 30.63% respectively). The NDF digestibility was the same in the basal diet, CSC and MOC, which were 12-18 (g/day) higher (P<0.05) than G. oppositifolia leaves. The sheep fed diets A, B, C and D, the percent NDF digestibility was the same for diets A, B and C (53.11, 47.87 and 50.37 respectively) and statistically different (P<0.05) for diet D (35.96). The ADF digestibility was different (P<0.05) among four diets, which can be ranked as A> C> D> B. An increase of 6.26-7.00 g/day in nitrogen intake in the basal diet was recorded when supplemented with G. oppositifolia leaves and other concentrate diets. The higher excretion of nitrogen was in the urine of the animals obtained from diet B followed by diet D and lower with diet A and C. The nitrogen excretion in feaces obtained of the animals from supplemented diets was higher than the basal diet. The higher nitrogen retention (55-60%) was recorded with supplemented diets than the basal diet when expressed in percentage. Based on the above findings, it is concluded that G. oppositifolia leaves can successfully replace the tested protein supplements by improved utilization of basal diet through higher nitrogen intake and retention.

Key Words: **Grewia *Oppositofolia* leaves, Sorghum, Digestibility, Chemical analysis, Sheep, NDF, ADF, Tree leaves, Nitrogen retention and intake**

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INTRODUCTION

Livestock is relying on crop residues and grazing area. The major feed resources in Pakistan are crop residues and grazing land that provide 90% of the livestock feed (57% and 33%, respectively), while cultivated fodders and concentrates contributes only 8% and 2%, respectively (Habib *et al*., 2003).

The nutrients supply from the above mentioned feed resources are not enough to meet the requirements of the existing livestock population in the country thus resulting in undernourished and diseased animals, that produce below their production potential. There is a need of 110.3 million tons of total digestible nutrient (TDN) (Economic Survey of Pakistan, 2006) to fulfill the demands of livestock by the efficient utilization of feed resources for increasing livestock production (Younas and Yaqoob, 2005). To fulfill the existing of nutrients for growing livestock population, there is a need to explore alternate feed resources which do not compete with human feed chain (Raghuvansi *et al*., 2007, Chema *et al*., 2011). One of the alternative ways is supplementation of conventional feed concentrates such as cottonseed cake, maize oil cake, mustard seed cakes and wheat bran are generally very expensive and present commodities prices put them beyond most of the farmers' affordability. Therefore, supplementation of feeds with other more economical, readily available and nutrients rich supplements may help overcome the fore mentioned problems and meet the demand of nutrients in the country. Therefore, there is a need to explore alternate feedstuffs which are easily accessible to farmers and compatible in values to traditional concentrates. Tree leaves is believed to be one of the alternate suitable choice, which are economical, easily available and adequate in nutrients (Reddy, 2006). Large varieties of tree leaves in both plain and hilly regions are available in the country some of which are extensively used as feedstuff. Among tree leaves, *G. oppositifolia* leaves are the one growing widely in the province of Khyber Pakhtunkhwa and contain more than 12% crude protein with 70%) *in- sacco* protein degradability (Shabana, 1998). The available information suggested that the nutritive value of *G. oppositifolia* leaves may be beneficial to replace conventional oil cakes. Therefore, the present study was designed to compare the substitution effect of conventional oil cakes (cottonseed cake and maize oil cake) with *G. oppositifolia* leaves in ruminant ration with the following objectives.

i. To compare the substitution effect of *Grewia oppositifolia* leaves on the *in vivo* digestibility of neutral detergent (NDF) and acid detergent fiber (ADF) with conventional oil cakes.

ii. To estimate nitrogen retention efficiency in sheep in response to feeding of cottonseed cake, maize oil cake and *G. oppositifolia* leaves.

MATERIALS AND METHODS

Research work with sheep was performed at experimental unit of Animal Nutrition Department, Agricultural University, Peshawar, Pakistan. The animals F1 i.e. Kaghani x Rambouillet of the same age were kept in individual metabolic crates with separate arrangement for feeding, watering and collection of feaces and urine. The animals were treated against endo-parasites prior a week of starting study. The experimental animal were given chopped sorghum hay as a basal diet (A), basal diet with cotton seed cake (diet B), basal diet with maize oil cake (diet C) and basal diet with *G. oppositifolia* leaves (diet D). The nitrogen requirement of the sheep was estimated using the standard recommendation of NRC (2007). About 40% of the total nitrogen requirement of the sheep was met from feeding supplements. There were four feeding periods with 10 days adaptation followed by 5 days data collection periods. The diets were offered once a day at 9.00 hrs. Small container was used for supplements feeding.Feed refusal of the pervious day was recorded daily before offering the fresh feed. Clean drinking water was available *ad libitum*.

Collection of Feaces and Urine

During the experimental period, data on daily feed intake and excretion of feaces by all four animals were recorded on daily basis for a period of 80 days. A total of 80 feaces samples were collected, weighed, thoroughly mixed and transferred to polythene bags 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 on daily basis throughout the experimental periods. Volume of the urine was measured, mixed and sample was taken in bottle and freezed. Representative samples of feed ingredients were also collected daily and pooled for each experimental period.

Preparation of Samples and Chemical Analysis

On completion of experiment, the samples of feaces, urine and feed was taken out from freezer, thawed and pooled categorically for each animal and was mixed thoroughly. About 50g of the pooled sample of feaces and feed were taken in duplicate for dry matter analysis and the remaining were air dried at 60 ^{o}C for 72 hours and then grinded in a laboratory mill to 1-mm sieve particle size and transferred to labeled clean and dry bottles and stored for further chemical analysis. Urine samples after thawing and mixing were analyzed for total nitrogen content (AOAC, 2006). Grinded samples of feed and feaces in duplicate were analyzed for dry matter (DM) and nitrogen contents according to the standard procedures of AOAC (2006), neutral detergent fibre (NDF) and acid detergent fiber (ADF) according to the method of Van Soest and Wine (1967).

Nitrogen Retention

Nitrogen retention (g/day) was calculated by subtracting the total nitrogen excreted in feaces and urine (g/day) from the total dietary nitrogen (g/day) consumed by the animals (Iqbal *et al.* 2002).

Statistics

The ANOVA were calculated according to the 4 x 4 Latin square designs (Steel *et al*., 1997) by using four diets, four feeding periods and four animals in the experiment. Dietary means were compared with the LSD procedure. A statistical package SAS (1991) was used for analysis.

The ANNOVA model 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 AND DISCUSSIONS

Chemical Composition

The basal diet and cottonseed cake were higher in NDF and ADF contents than NDF and ADF contents in maize oil cake and *G. oppositifolia* leaves. The NDF contents in basal diet, cottonseed cake, maize oil cake and *G. oppositifolia* leaves were 64.94%, 62.38%, 52.40% and 48.48%, respectively while the ADF contents in the said diets were 42.68%, 36.63% 19.80% and 30.63%, respectively (Table I).

Table I *Dry matter, neutral detergent fibre and acid detergent fibre of sorghum hay as basal diet with supplements fed to sheep (In percent)*

Allotment	DM	NDF	ADF
Sorghum hay	95.57 ±1.251	64.94±0.351	42.68±0.152
Cottonseed cake	96.19 ±1.522	62.38±0.125	36.63±0.412
Maize oil cake	96.13 ±0.252	52.40±1.421	19.80±1.256
G. oppositifolia leaves	94.33 ±1.051	48.48±1.246	30.63±1.146

Cell Wall Intake and Digestibility

Results of daily intake and digestibilty of NDF and ADF are shown in Table II. The higher intake of neutral detergent fibre was with diets B and C and lower with diets A and D. The intake of acid detergent fibre content was much lower in diet B than the other supplemented diets (C & D) and of diet A. The NDF digestibility was the same with diets A, B and C and was lower with diet D (P<0.05) (Table II). The animals fed diets A, B, C and D, the mean percent NDF digestibility was 53.11, 47.87, 50.37 and 35.96 respectively. The ADF digestibility with all four diets were different (P<0.05). The animals fed diets A, B, C and D, the mean percent ADF digestibility was 48.02, 10.82, 38.00 and 25.53 respectively. The ADF digestibility can be ranked as A> C > D> B).

Table II *Intake and the digestibility of neutral detergent fiber and acid detergent fiber of sorghum hay as a basal diet with supplements fed to sheep*

Diets	Total DMI (g/d)	NDF intake (%)	ADF intake (g/d)	NDF digestibility (%)	ADF digestibility (%)
A	546.86^{b}	394.63±2.592	257.19±2.573	53.11^{a}	48.02^{a}
B	821.04^{a}	436.92±4.128	162.72±3.173	47.87^{a}	10.82^{d}
C	825.68^{a}	471.07±3.217	282.39±2.956	50.37^{a}	38.00^{b}
D	796.77^{a}	392.85±2.861	243.64±2.563	35.96^{b}	25.53^{c}

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

Nitrogen Intake and Retention

The nitrogen intake of the basal diet was increased (P<0.05) by 6.26-7.00 (g/day), when supplemented with cottonseed cake, maize oil cake and *G. oppositifolia* leaves (Table III). The higher feacal excretion of nitrogen was recorded in the feaces of animals obtained from supplemented diets (P<0.05). The higher (P<0.05) excretion of nitrogen was recorded in the urine of animals obtained from diet B and diet D and lower with diet A and diet C. The nitrogen excreted both in the feaces and urine obtained from animals fed all diets can be ranked as D> B> D> A. The lower (P<0.05) nitrogen retention (0.99 g/day) was in animals fed with basal diet (diet A) and the higher (P>0.05) nitrogen retention was in animals fed with supplemented diets. The total nitrogen retention when expressed as a percent revealed that all the three supplemented diets supported 55-60% higher nitrogen retention than basal diet (diet A). (The values are statistically described, then there is no reason to describe it in text form).

The NDF digestibility was the same with diets A, B and C and was lower with diet D (P<0.05) (Table II). The ADF digestibility with all four diets were different (P<0.05) and can be ranked as A> C > D> B). With increasing maturity crude protein content and digestibility decreases with associated increases in the contents of cell wall constituents in forage (Sultan *et al*., 2008)**.** 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 (Wilson and Mertens, 1995). The inaccessibility of microorganisms to secondary walls is a major limitation in forages due to time constrains for microbes to access cell walls for digestion and thus much potentially digestible cell walls in grasses escapes as feacal particles (Wilson and Mertens, 1995; Wilman and Ahmad 1999; Wilman and Moghaddam 1998b) that may be the case with *G. oppositifolia* leaves in the present study. Anatomical characteristics of leaves and stems of 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 further support the low digestibility of *G. oppositifolia* leaves. The *G. oppositifolia* leaves has lower NDF and ADF contents than sorghum hay and cottonseed cake (Table I), a much lower NDF and ADF digestibility were recorded when *G. oppositifo*lia leaves were supplemented with basal diet (Table II) again support the findings of Wilson and Mertens (1995) to cell wall contents before the diet escape from the rumen of the animals. Several other workers (Dey *et al*., 2006, 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**)** and Mlambo and Makkar (2005)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 other reason for the lowest digestibility with the diet supplemented with *G. oppositifolia* leaves in the present study.

Table III ***Nitrogen intake and retention of sorghum hay as a basal diet with supplements fed to sheep***

Diets	Total nitrogen intake (g/day)	Feacal nitrogen excreted (g/day)	Urinary nitrogen excreted (g/day)	Nitrogen retained (g/day)	Nitrogen retained (%)
A	4.78^{b}	3.26^{a}	0.53 ^{b}	0.99^{b}	20.15^{b}
B	11.78^{a}	5.11 ^{a}	1.27 ^{b}	5.40^{a}	45.94^{a}
C	11.01^{a}	4.83^{a}	0.66 ^{b}	5.52^{a}	49.16^{a}
D	11.41^{a}	4.83^{a}	1.41^{a}	5.17^{a}	44.63^{a}

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

The diet containing cottonseed cake (diet B) in the present experiment, cell wall digestibility may be associated to its high hulls contents as evidence from the higher NDF (62.38%) and ADF (36.63%) 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 NDF 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). Differences in NDF, ADF could also be attributed to species genotypic differences in control of fibre accumulation in the plant (Reddy and Elanchezhian, 2008).

Nitrogen Intake and Retention

Supplements increased the intake of basal diet by 6.26-7.00 (g/day (Table III). The excretion of nitrogen recorded in bulk (both faeces and urine) was higher in the feaces of animals obtained from supplemented diets. As for as the excretion of N in the feaces of animals is concerned, it remained the sam , however, the higher excretion of N ($P<0.05$) was recorded in the urine of animals fed *G. oppositifolia* leaves. The total nitrogen retention when expressed as a percent revealed that all the three supplemented diets supported 55-60% higher nitrogen retention than basal diet (diet A) which is in line with that of Ravi and Prasad (2008) and Habib *et al*. (2008). The lower retention of nitrogen in 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 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). Satter and Slyter (1974) noted that a minimum level of 50mg/litre of rumen ammonia N are required for optimal microbial growth in the rumen. 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). Bhatta *et al*. (2005) suggested that supplementation of different tree leaves improved digestibility and rumen fermentation and improved rumen ammonia and N retention support the findings of the present in sheep of supplemented groups. Woodward and Reed (1997) also reported higher N retention for diets including *S. sesban* leaves are also in line with the findings of the present study. Based on the above discussions and the findings of present study it is recommend that *G. oppositifolia* leaves can be served as a best substitute for the tested protein supplement in terms of nitrogen intake and nitrogen retention when supplemented to a low quality basal diet, however, further studies such as weight gain in small ruminants, milk yield in lactating animals and the architecture of *G. oppositifolia* leaves also needs to be explored.

CONCLUSION AND RECOMMENDATIONS

G. oppositifolia leaves have improved the utilization of basal diet through increased nitrogen intake and retention and was compatible with conventional concentrates under study therefore can successfully replace the tested protein supplements.

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