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Influence of *Moringa oleifera* foliage supplementation on feed intake, rumen fermentation and microbial profile of goats

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ABSTRACT

To study the effect of *Moringa oleifera* leaves (MOL) on nutrient intake, rumen fermentation and microbial profile of goats, three rumen cannulated adult male goats (*Capra hircus*) with average body weight of 19±1.0 kg were allotted to three treatments in 3×3 latin square design. The treatments were, control: fed on basal diet (wheat straw and concentrate mixture in 50:50 ratios), MOL10: basal diet supplemented with MOL @ 10% of dry matter intake (DMI) and MOL20: basal diet supplemented with MOL @ 20% of DMI. To make isonitrogenous diet, three concentrate mixtures of 17.0, 15.2 and 13.0% crude protein (CP) for three groups, respectively, were prepared. The dry matter intake, rumen pH, concentration of rumen metabolites like total VFA, molar proportion of VFAs, NH_3-N and microbial enzymes were unaffected due to supplementation of MOL. There was no change in the rumen microbial population (Log $_{10}$ number) of total bacteria, methanogens, *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavifaciens, Butyrovibrio fibrisolvens*, rumen fungi and protozoa. The results indicated that feeding of *Moringa oleifera* leaves did not affect rumen environment hence rumen enzyme and microbial population also remained unchanged.

Key words: Goats, Microbial profile, *Moringa oleifera*, Rumen fermentation

Moringa oleifera is Himalayan tree species; at present widely distributed in most parts of the world (Soliva *et al.* 2005). The moringa can be grown in different climatic conditions, with its ability to grow in all types of soils except waterlogged. India is the largest producer of *Moringa* and yield upto 650 metric tonnes of green leaves per hectare can be achieved using optimum conditions for the cultivation (Rajangam *et al.* 2001). The *Moringa* leaves (MOL) are rich in protein with amino acid make up appropriate for human and animal nutrition (Kumar *et al.* 2017). Also, MOL is high in readily fermentable carbohydrates and ether extract and rich in several bioactive compounds like vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, oxalates, phytates etc (Leone *et al.* 2015). With the above characteristics, MOL can be considered as a nutritious fodder for the ruminants specially fed on poor quality roughages. MOL can also replace costly protein supplements like soybean meal, groundnut cake etc to some extent in the diet of the ruminants. As a source of different phytochemicals, MOL can also be explored as a modulator for rumen fermentation. Hence, to ascertain its use as rumen modifier, the present experiment was conducted to study the changes in feed intake, rumen fermentation and rumen microbial profile in goats by feeding MOL.

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MATERIALS AND METHODS

Animals, feeding management and dietary treatments: Three fistulated adult male goats (*Capra hircus*) with average body weight of 19±1.0 kg were maintained at Animal Nutrition sheds of Indian Veterinary Research Institute, Izatnagar. The MOL were harvested and air-dried. The animals were fed on a diet containing concentrate mixture and wheat straw in 1:1 ratio to meet their maintenance requirement as per ICAR (2013). The goats were allotted to three treatments in 3×3 latin square design. The three treatments were, control: fed on basal diet, ML10: basal diet supplemented with MOL @ 10% of DMI and ML20: basal diet supplemented with MOL @ 20% of DMI. Three concentrate mixtures (Table 1) were prepared for the three treatments to make iso-nitrogenous diets. In each phase, 21 d feeding was carried out and body weights were recorded at start and end of the each phase. Daily dry matter intake from wheat straw, concentrate mixture and MOL was recorded.

Chemical composition of feed: Crude protein, ether extracts and ash content was determined as per the methods of AOAC (1995). Neutral detergent fibre (NDF) was determined without decalin and sodium sulphite while acid detergent fiber (ADF) was analyzed without decalin.

Table 1. Physical composition of concentrate mixtures used for feeding to goats

Ingredients	Control	ML10	ML20
Crushed Maize Grains	37	40	43
Soyabean meal	17	11	04
Wheat bran	43	46	50
Mineral mixture	2	2	2
Salt	1	1	1
Total	100	100	100

Hemicellulose was calculated as the difference between percent NDF and ADF on DM basis (Van Soest 1991). Calcium and phosphorus estimations were done as per Talapatra *et al*. (1940) and AOAC (2012). Copper and zinc were estimated by Atomic Absorption Spectrophotometer [Electronics Corporation of India Ltd. (ECIL), Hyderabad, India, Model No. 4141] with hydride generator. Phenolics and saponins were estimated in MOL as per method given by Makkar (2000) and AOAC (1990), respectively.

Sampling of rumen liquor and contents: In each phase, 21 d feeding was carried out after which the rumen liquor/content was sampled on 2 consecutive days before feeding. Rumen contents collected from different locations of rumen were churned in the presence of CO_2 gas. After squeezing the churned contents, liquid portion was processed for enzyme estimation. Churned contents of every collection were pooled in equal proportion day wise and animal wise for microbial DNA extraction.

Rumen metabolites: The pH of the strained rumen liquor was recorded immediately after collection, with an electronic pH meter (Model: pH Spear, EC- PHWPSEN04; Eutech Instruments, Malaysia) calibrated against standard buffer solutions. Estimation of VFA was done using Nucon-5765 gas chromatograph (AIMIL, New Delhi, India) equipped with a double flame ionization detector and the glass column (4 ft length and 1/8 inch diameter) packed with chromosorb 101 as per method described by Agarwal *et al.* (2008). The gas flows for nitrogen, hydrogen and air were 30, 30 and 320 ml/min, respectively. Temperature of injector oven, column oven and detector were 270°C, 172°C and 270°C, respectively. Ammonia nitrogen was estimated by the method of Weatherburn (1967).

Extraction and estimation of enzymes: The enzymes from the rumen contents were extracted by using lysozyme and carbon tetrachloride followed by incubation at 39°C with continuous shaking for 3 h and lastly freezing to terminate the reaction. The activity of carboxymethyl cellulase (CMCase), avicelase, xylanase and amylase was estimated using carboxymethyl cellulose, avicel, xylan and starch as substrate, respectively (Agarwal *et al.* 2000). The activities were expressed as µmole reducing sugars produced $min^{-1}ml^{-1}$ of enzyme sample under assay conditions. Acetyl esterase activity was determined using p-nitrophenyl acetate as substrate activity was defined as µmole p-nitrophenol produced $min^{-1}ml^{-1}$ (Huggins and Lapides 1947). Protease activity was measured by using azocasein as the substrate (Iversen and Jorgensen 1995) and the activity was expressed as µg protein hydrolyzed $min^{-1}ml^{-1}$. Urease activity was determined by measuring amount of NH_3 released during incubation of sample with urea (Weatherburn 1967). The protein content of enzyme samples was estimated (Lowry *et al*. 1951) and specific activity of the enzymes was presented as units/mg protein.

Rumen microbial profile by real time PCR: The absolute quantification of different microbial groups (total bacteria, total fungi, total protozoa, methanogen, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Butyrovibrio fibrisolvens*) was done by real-time PCR (C×1000 Touch BIORAD). Total genomic DNA was extracted using Qiagen Stool kit and amplified using specific primers (Table 2). The purified PCR product was cloned in pGEMT easy vector (Promega) and transformed in *Escherichia coli*. The plasmid with insert was extracted and copy number was calculated. The plasmid was serially diluted to make standard curve and the copy number in the unknown sample was calculated (Ritalahti *et al.* 2006). The PCR reactions were performed in a total volume of 20 µl, containing 2 ng of template DNA, 10 µl of 2× kappa SYBR master mix, 0.6 µl of each primer (10 µM) and nuclease free water. The amplification reactions were performed in a total volume of 20 µl, containing 2 ng of template DNA, 10 µl of 2× kappa SYBR master mix, 0.6 µl of each primer (10 µM) and nuclease free water.

Statistical analysis: Data for effect on plane of nutrition and microbial profile were analyzed using one-way ANOVA through General Linear Model approach. When F-test was significant ($P<0.05$), Tukey's test was utilized to compare significant differences ($P<0.05$) among the groups. Data for fermentation parameters were analyzed using factorial univariate ANOVA with contrast analysis using the model intercept+treatment+period+treatment×period to test the effect of treatment, period and their interaction and means were compared using Tukey's test using SPSS computer package.

Table 3. Chemical composition of concentrate mixtures and fodders used for feeding to goats

Component	Control	ML10	ML20	MOL	Wheat straw
Crude protein	17.0	15.2	13.0	26.2	3.48
Ether extract	2.70	2.82	2.95	6.4	1.67
Neutral detergent fibre	36.5	37.7	39.5	18.4	82.2
Acid detergent fibre	9.03	9.04	9.11	14.5	58.0
Hemicellulose	27.5	28.7	30.4	3.90	24.2
Ash	5.81	5.61	5.40	12.7	7.31
Calcium	0.55	0.53	0.51	2.58	0.21
Phosphorus	0.89	0.88	0.88	0.43	0.14
Copper	0.08	0.08	0.07	0.07	0.04
Zinc	0.56	0.55	0.55	0.14	0.12

MOL, *M. oliefera* leaves; ML10, *M. oleifera* @ 10% of DM; ML20, *M. oleifera* @ 20% of DM.

RESULTS AND DISCUSSION

Chemical composition of feed and plane of nutrition of goats: Concentrate mixtures differ in CP content (17.0% for control, 15.2% for ML10 and 13.0% for ML20) by reducing the soybean meal by 35.3 and 76.5% in the concentrate mixture of ML10 and ML20, respectively (Table 3). The level of other components was comparable for all the three concentrate mixtures. The CP content of MOL in the present study was similar to that reported by Dey *et al*. (2014) but was lower than that reported by Melesse *et al.* (2012). The MOL were high in calcium and low in phosphorus and zinc as compared to concentrate mixtures. Level of Cu was similar for MOL and concentrate mixtures. The tannin (2.23% DM) and saponin (7.8% DM) contents in MOL were lower and similar as reported by Pal *et al*. (2015) and Indriasari *et al*. (2016), respectively. Variability in level of secondary metabolites in the plants is a usual phenomenon and depends on many factors like season, maturity, storage of harvesting, vareities etc. Nouman *et al.* (2014) considered MOL as a very nutritious fodder because of its richness in nutrients with very low secondary metabolites contents. The intakes of DM and CP were similar among the groups (Table 4). Feeding of MOL to sheep @ 400g/d/h did not influence intake of DM and CP (Chaudhary *et al.* 2006). Jelali and Salem (2014) also reported similar feed intake in lambs receiving either soybean meal or MOL as protein supplement on oat hay based diet. But the DMI improved when, MOL was fed solely (Sultana *et al.* 2015). The use of concentrates as supplements to low-quality roughage is known to improve intake and digestibility of roughages (Nurfeta 2010). In the present study intake might not be affected as MOL were supplemented to basal diet including concentrate mixture.

Rumen fermentation and microbial enzyme profile: Rumen pH, fermentation metabolites like total VFA, molar proportion of VFAs and NH_3-N were unaltered (P>0.05) by MOL supplementation (Table 5). Jelali and Salem (2014) also reported similar rumen pH in lambs receiving either soybean meal or MOL as protein supplement on oat hay based diet. In contrast to our findings, addition of MOL to roughage based diet (roughage to concentrate ratio 70/30) increased VFA production without affecting NH_3-N (Sarkar 2016). However, in the present study, the high level of concentrate might be responsible for masking its effect on VFA production as concentrate portion contributes more to the VFA than roughage.

Diverse array of enzymes contributing to feed degradation are those that degrade plant cell wall polymers (e.g. avicelase, carboxymethylcellulse, xylanases, etc),

Table 2. Primers used for real time PCR

Primer name	Primer sequence	reference
Bacteria	F-5'CGGCAACGAGCGCAACCC-3' R-5'CCATTGTAGCACGTGTGTAGCC-3'	Denman *et al*. (2006)
Fungi	F-5'GAGGAAGTAAAAGTCGTAACAAGGTTTC-3' R-5'CAAATTCACAAAGGGTAGGATGATT-3'	
Methanogen	F-5'-TTCGGTGGATCDCARAGRGC-3'R R-5'-GBARGTCGWAWCCGTAGAATCC-3'	
Ruminococcus flavefaciens	F-5'CGAACGGAGATAATTTGAGTTTACTTAGG3' R-5'CGGTCTCTGTATGTTATGAGGTATTA-3'	
Fibrobacter succinogenes	F-5'GTTCGGAATTACTGGGCGTAAA-3' R-5'CGCCTGCCCCTGAACTATC-3'	
Ruminococcus albus	F-5'CCCTAAACAGTCTTAGTTCG-3' R-5'CCTCCTTGCGGTTAGAACA-3'	Koike and Kobayashi (2001)
Butyrovibrio fibrisolvens	F: TAACATGAGAGTTTGATCCTGGCTC R: CGTTACTCACCCGTCCGC	Denman and McSweeney
Protozoa	F-316f, 5'-GCTTTCGWTGGTAGTGTATT-3' R-539r, 5'-CTTGCCCTCYAATCGTWCT-3'	Sylvester *et al*. (2004)

Table 4. Effect of feeding *M. oleifera* leaves on the intake of nutrients in goats

Attribute	Control	ML10	ML20	SEM	P value
Body weight (kg)	20.4	20.2	20.4	0.19	0.91
Metabolic body weight (kg $W^{0.75}$)	9.59	9.54	9.61	0.07	0.93
Dry matter intake (g)	473.1	481.5	481.1	16.4	0.97
Dry matter intake (g/kg $W^{0.75}$)	49.4	50.5	50.2	1.75	0.98
Dry matter intake (% of BW)	2.33	2.38	2.36	0.08	0.98
Crude protein intake (g/d)	47.9	52.6	57.0	2.24	0.28
Crude protein intake (g/kg $W^{0.75}$)	5.00	5.52	5.93	0.23	0.30

ML10, *M. oleifera* @10% of DM; ML20, *M. oleifera* @ 20% of DM.

Table 5. Effect of feeding *M. oleifera* leaves on rumen metabolites, and specific activity of microbial enzymes in goats

Attribute	Control	ML10	ML20	SEM	P- value		
					T	P	T*P
pH	6.6	6.7	6.7	0.08	0.85	0.42	0.95
Concentration of rumen metabolites							
TVFA (mmol/dl)	7.59	7.63	7.58	0.35	1.0	0.17	0.60
Acetate (%)	65.4	64.5	63.7	0.92	0.78	0.62	0.09
Propionate (%)	20.8	22.5	23.1	1.16	0.70	0.79	0.17
Butyrate (%)	12.8	12.3	12.4	0.30	0.78	<0.001	0.38
A:P ratio	3.2	3.2	2.8	0.17	0.60	0.94	0.10
Ammonia-N (mg/dl)	12.4	14.5	13.3	0.99	0.70	0.43	0.42
Activities of rumen enzymes (specific activity, units/mg protein)							
Carboxymethyl cellulase	0.64	0.59	0.54	0.10	0.92	0.06	0.89
Avicelase	0.46	0.57	0.41	0.04	0.27	0.44	0.06
Xylanase	1.37	1.96	1.06	0.15	0.76	0.10	0.95
Acetyl esterase	0.47	0.46	0.49	0.10	1.00	0.30	0.47
Urease	0.37	0.40	0.55	0.04	0.15	0.15	0.58
Protease	8.73	8.94	7.28	0.38	0.21	0.14	0.34

ML10, *M. oleifera* @10% of DM; ML20, *M. oleifera* @ 20% of DM.

Table 6. Effect of feeding *M. oleifera* leaves on ruminal microbes (Log $_{10}$ value) in goats

Rumen microbe	Control	ML10	ML20	SEM	P value
Total bacteria	10.96	10.99	10.99	0.12	0.82
Methanogens	6.38	6.66	6.58	0.15	0.79
Fibrobacter succinogenes	9.56	9.72	9.51	0.07	0.50
Ruminococcus albus	6.65	6.57	6.52	0.14	0.95
Ruminococcus flavifaciences	7.85	7.48	7.05	0.26	0.52
Butyrovibrio fibrisolvens	10.14	10.44	10.43	0.10	0.40
Fungi	6.58	7.38	6.89	0.26	0.51
Protozoa	8.10	8.89	9.06	0.32	0.48

ML10, *M. oleifera* @10% of DM; ML20, *M. oleifera* @ 20% of DM.

amylases, proteases are microbial origin present in the rumen. Efficient digestion of poor quality lignocellulosic feed depends on the activity of these enzymes. In the present study, feeding of MOL did not affect the activities of these enzymes indicating (Table 5) that the tannins and saponin contents present in MOL was not upto the level which can impose deleterious effect on these rumen enzymes.

Rumen microbial profile: The rumen microbial ecosystem contains diverse microbial groups responsible for feed digestion. In the rumen, *F. succinogenes*, *R. flavefaciens* and *R. albus* are considered as the primary fibrolytic bacteriag responsible for degradation of plant fibre. In the present study, the population density of these microbes along with total bacteria, fungi, protozoa and methanogens did not change by feeding MOL indicating that the rumen environment as well as microbial profile did not affect by feeding MOL supplemented diet (Table 6). Saponin is a well documented antiprotozoal agent and the effect is in dose dependent manner (Jayanegara *et al.* 2014). However, no effect on protozoa population in the present study might be due to low inclusion level of MOL so that the saponin level did not reach to the level which could influence protozoa population.

From the results of the present study, the inference was drawn that feeding of *Moringa olifera* leaves up to 20% of the dry matter intake to goats did not affect rumen environment, enzyme and microbial profiles and therefore can be used as a protein supplement in livestock.

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