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Effect of feeding *Prosopis cineraria* leaves on methanogens, rumen fermentation, growth performance, blood biochemical constitute and nutrient utilization, in rumen content of Lambs and Kids

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Abstract

This study was designed to investigate the effect of feeding *Prosopis cineraria* leaves with lamb ration on methanogens, growth performance, nutrient utilization, rumen fermentation and microbial hydrolytic enzymes status during pre weaning phase of Lambs and kids and they allowed suckling twice daily until 90 days of age, concentrate and forage were provided ad libitum. Lambs and kids were weighted at weekly intervals and metabolism study was conducted on eight representative lambs and kids of uniform body weight, at 180 days of age. Lambs and kids consumed 614.4 and 531.7 g feed (DM) daily, which accounted 2.64 and 2.82 % of body weight, and 57.9 and 58.7 g/kg$W^{0.75}$ (metabolic body weight) respectively. Animals were in positional nitrogen balance % intake and inter species variations did not observe. Serum biochemical and mineral content were also not different between, except total cholesterol which was (114.4mg/dl) higher in kids. Extra-cellular activity of α-amylase and carboxy-methyl-cellulase (CMCase) was higher in lambs, while cellular activity of α-amylase was higher in kids.. Therefore, it is concluded that under high plane of nutrition inter species variations in digestible capabilities and performance attributes did not exhibited in lambs and kids. Amplified PCR sequences of methanogen ribosomal RNAs were analyzed and submitted to Gene Bank. These sequences were BLAST aligned in NCBI, showing 99% similarity with other sequence of sheep, cattle, and buffalo with exception of one sequence (Accession no-KP752400), may be unique to this geographical location.

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Introduction

Animal husbandry has made sizable contribution to human being in the past century. Animal products provide one sixth of human food energy and more than one third of the protein of global basis (Bradford, 1999). The contribution of small ruminants is very helpful to agriculture economy through manure, wool, meat and milk production.. The contribution of total gross domestic products to livestock sector fluctuates between 6.5 to 4.8 and agriculture GDP significantly increased up to 22.6% (Bhat, 2002). The faster human population urbanization and high density animal population have squeezed the agricultural land. In traditional animal husbandry practices, about 80 to 95% Indian farmers utilize the local feedstuffs for regular supplementation to animals (Devendra, 1992). Sheep and goats are first domestic animal and well adapted to varying geographic and ecological variations. These two small ruminant species are being raised on grass based production system. In India, these two ruminants fulfill their food requirement by grazing on degraded natural rangeland with supplementation of top feed resources for growing animal production. Sheep population in India remained static whereas goat population increased steady (FAO, 2007), which is ascribed to their wider choice of vegetation (Malachek and Provenza, 1981), ability to survive on sparse vegetation (Gall, 1981) and better nutrient utilization efficiency on poor-quality feed (Singh and Bhatia, 1981). The inherent character of goat like wider choice of vegetation, resistance to dehydration and wide ranging feeding habits with preference for browse species, enable them to perform better than sheep in regions of scanty rainfall.

Livestock production in India is an integral part of traditional mixed farming (crop-livestock) and play important role in the agrarian economy. The importance of livestock increases with aridity of the environment since traditional crop production is a gamble in the region due to unfavourable agro-climatic condition. Sheep are predominantly grazers while the goats are browsers and in the process of grazing/browsing both the species resort to intensive selection on plant parts rich in nutrients and pick up a wide range of vegetation from the range land.

Both lambs and kids are mono-gastric at birth and by coming in contact with surrounding environment; microbes inhabit their rumino-reticulum, thereby transforming them to ruminants. Tanniferous trees and shrubs are of importance in small ruminants production in semiarid region of India, because they can provide significant protein supplements. As the demand for food rises, tanniferous plants and agro industrial by-products play an increasingly important part in the diet of animals (Makkar, 1999). Protein supplementation from plant and non protein nitrogen sources from plant are often used to improve animal performance. *Prosopis cineraria* leaves are most important feeding resources in Rajasthan state of India, especially during the dry season. The leaves are highly palatable and nutritious but contain 8-10% tannin (Bohra, 1980). Tanniferous feed are generally regarded as anti-nutritional, but certain tannins at low concentrations are known to alter rumen fermentation (Barry and Duncun, 1984; Bhatta *et al*., 2000) and microbial protein synthesis (Makkar *et al*.,1995; Bhatta *et al*., 2001) to the benefit of ruminants. The aim of this study was to investigate the effect of feeding *Prosopis cineraria* leaves on growth performance, nutrient utilization, blood biochemical constitute, rumen fermentation and methanogens in rumen content of Lambs and Kids.

Material and methods

Study Location

The experiment was conducted at the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India) located at 26 ° 17'N latitude and 75 ° 28'E longitude and 320 m above sea level. The climate is hot and semi-arid. The study initiated in the month of January, 2012 and ended in the month of June, 2012. During the experiment, minimum and maximum ambient temperature ranged from 5°C to 26 °C and 22°C to 40 °C, respectively while relative humidity varied from 11 to 96%. The animal care, handling and sampling procedures were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animal (CPCSEA), India.

Experimental animal housing, feeding regimen and live weight recording

Eight of each growing male lambs and kids (90 ± 5 day of age; 15.5 ± 0.89 kg) were maintained for 91 days on *ad-libitum* feeding of roughage (*Prosopis Cineraria*) and concentrate mixture constituted with different concentration of maize, barley, groundnut cake, til cake, mustard cake, salt and mineral mixture (Table 1) in individual system of feeding management. The diet contained essential constituents recommended for native growing lambs (ICAR, 1998). Fresh feed was offered daily at 09:00 AM after discarding the residue, in excess of 10 % of previous day's intake. Free choice water was available from 08:00AM to 16:00PM. Live weight was recorded at weekly interval in the morning using a dial balance before grazing and watering of the animals and these values were used to determine live weight gain. Patterns of performance were calculated on the basis of the 7-day periods.

Growth and metabolism experiment

The growth experiment lasted for 91 days during which lamb and kids body weight (BWs) were recorded for 2 consecutive days every 7^{th} day and these values were used to determine BW gain.

A metabolism experiment was conducted on eight representative animals of both lambs and kids, at 70^{th} day of experimental feeding. The metabolism experiment lasted for 12 day (*i.e.* 5 day adaptation followed by 7 days of sample collection).

Faeces and urine were collected into acidified containers containing 100 ml H_2SO_4 (100 ml concentrated H_2SO_4 diluted to 1000 ml with distilled water) using a total collection method. Urine pH was maintained below 3 by addition of diluted H_2SO_4 (10 ml/ 100 ml distilled water) and a representative sample was frozen and preserved. For chemical analysis samples of feeds, faeces and residues were dried to a constant weight in a forced air oven at 70 ^oC. Dried samples were pooled and ground to pass a 1 mm screen for chemical analysis.

Rumen fermentation pattern and enumeration of protozoa count

Samples of rumen fluid (100 ml) were withdrawn from all intact animals of both groups using a stomach tube at 0 and 4 hr post feeding on last day of experimental metabolic feeding trial. The rumen liquor was then strained through four layers of muslin cloth. SRL samples were analysed for total nitrogen and TCA-ppt-N (total-N; Micro Kjeldahl), ammonia nitrogen (NH_3-N; Conway, 1962) and total volatile fatty acid (TVFA; Barnett and Reid, 1957). (Table 5) For protozoa count 1 ml of strained rumen liquor and 1 ml of formalized physiological saline was added into a scintillation vial. Thereafter two drops of brilliant green dye was added. Protozoa numbers in SRL was recorded according to (Kamra *et al.*, 1991).

Enzyme assay

Rumen hydrolytic enzymes were estimated in rumen fluid samples collected at 4 hr post feeding as per the procedure of Agarwal (2000) with slight modification (Raghuvansi, 2003). The α -amylase, carboxymethyl cellulase, xylanase and α -xylosidase were estimated. In brief, 10 ml of fresh SRL was centrifuged at 24, 000 g for 20 min. and the supernatant was used as source of enzyme for extracellular activity. The pellet containing microbial biomass (bacteria, fungi and protozoa) was suspended in 5 ml of 0.1 M phosphate buffer (pH 6.8), 2 ml CCl_4 and 2 ml lysozyme solution (4 g/l). The suspension was incubated for 3 h at 39°C and then centrifuged at 24, 000 g for 20 min. The supernatant was collected and used as an enzyme source for the cellular portion.

For the estimation of carboxymethyl cellulase (CMCase) and *α*-amylase, the reaction mixture contained 0.1 M phosphate buffer (pH 6.8) 1 ml, substrate 1 % carboxymethyl cellulose 0.5 ml and enzyme 0.5 ml for CMCase, substrate 1% starch solution and 0.25 ml enzyme for *α*-amylase, reaction mixture was incubated at 39 °C for 60 and 30 min, respectively. For xyalanse estimation the reaction mixture contained 0.1 M phosphate buffer (pH 6.8) 1 ml, substrate 0.25% xyalane 0.5 ml and enzyme 0.5 ml, was incubated at 39 °C for 30 min.

The reaction was stopped by the addition of dinitro-salicylic acid reagent. The glucose thus produced was estimated using the dinitro-salicylic acid method (Miller, 1959). A unit of enzyme activity was defined as the amount of enzyme which produced 1 µmol glucose per hour for CMCase and per minute for *α*-amylase. While µmol xylose released per min per ml was defined as a unit of xylanase enzyme. For *β*-xylosidase estimation, the reaction mixture contained 0.9 ml *p*-nitrophenol *β*-D-xylopyranoside 0.1% in 0.1 mol phosphate buffer (pH 6.8) and 0.1 ml enzyme. The reaction mixture was incubated for 10 min at 39 ˚C. The reaction was terminated by adding 1 ml of a solution of 2% Na_2CO_3. The amount of *p*-nitrophenol released during incubation of reaction mixture was estimated (Sewale and Sadana, 1978). The unit of enzyme activity was defined as the amount of enzyme that produced 1 µmol *p*-nitrophenol per minute per ml.

Haematological and serum biochemical profile

5 ml of blood was collected from five representative animals by Juglar vein poncture during morning before feeding. Few drops of blood were taken in heparinized micro centrifuge tube for hémoglobine estimation. Serum was separated after 3 hours of incubation at 37°C. Immediately after the serum collection, glucose was estimated by Glucose oxidase-glucose per-oxidase method (Trinder, 1969). Blood hemoglobin was estimated by cyanmethemoglobin method (Cook, 1985). Serum samples were preserved in polypropylene vials at -20°C temperature for protein, albumin, urea nitrogen, total cholesterol and HDL cholesterol content estimation using commercial kits of Span Diagnostics Ltd, Surat, India. Serum minerals like calcium, phosphorus, magnese, copper and zinc were estimated via atomic absorption spectrophotomètre (AAS).

PCR amplification, cloning and sequencing of most abundant methanogens from rumen content

Rumen liquor of lamb and kid was collected from rumen with the help of stomach tube following standard procedure and following ethical and safety measures.

Total DNA from rumen liquor was extracted by using DNA stool mini kit (Qiagen; Cat# 51504) followed by manufacturer instructions. The DNA was quantified by using UV spectrophotometer. The extracted DNA was examined for the presence ruminal methanogens using PCR with 16S ribosomal DNA-directed PCR primer designed for ruminal methanogens and identification of methanogens colonising young lambs and kids. PCR amplification was carried out in Eppendorf thermal cycler in a total volume of 25µl. Each reaction consist 2.5 µl 10X PCR buffer, 1 µl (100 ng) DNA sample, 0.5 µl dNTPs, 0.5 µl forwarded primer (5'CCTACGGGRBGCAGCAGG3'), 0.5µl reverse primer (5'GCGGTGTGTGCAAGGAGC3'), 0.5 µl (2.5 units) Taq Polymerase and 17.5 µl D/W. The reaction was performed on PCR program as initial denaturation at 95^{oc} for 5 min followed by 34 cycles of denaturation for 1 min at 94^{oc}; annealing for 45 sec at 58^{oc} followed by extension for 45 sec. at 72^{oc} and a final elongation at 72^{oc} for 5 min. Amplified PCR product was resolved on 1 % agarose gel electrophoresis stained with Ethidium bromide and illuminated under UV illuminator (Genetix, India). PCR bands were excised with help of clean B.P. blade and were purified by Gene-JET Gel extraction Kit (Thermo Scientific; Cat# K0692) followed by manufacturer instructions. PCR products (100 ng/0.15 pmol ends) were ligated with pJET 1.2/blunt cloning vector (50 ng) provided with Clone-JET PCR cloning kit (Thermo scientific, Cat#1232) followed by manufacturer instructions. Overnight culture of *Escherichia coli* (*DH5α*) were used for transformation of ligated products using Transform Aid Bacterial Transformation kit (Thermo Scientific, cat#K2711) followed by manufacturer instructions. Colony PCR using pJET F and pJET R primers were used for screening of true recombinants. Colonies (LB Agar) giving expected PCR band size were further grown overnight in LB broth selected on ampicillin. Further plasmids were isolated using Gene JET Plasmid mini prep kit (Thermo Scientific, Cat#K0503) and nucleotide sequencing was done at Sanger sequencing facility of Xcelris genomics, Ahmadabad.

Statistical analysis

Data were subjected to analysis of variance using SPSS Base14.0 The data for blood biochemical and rumen metabolites were analyzed using factorial design.

Results

Chemical composition of Diet

The DM of khejri was 45.09 while concentrate mixture having DM 96.09%.

Table 1. Constituents of concentrate mixture fed to lambs and kids.

Ingredient (g kg $^{-1}$)	
Maize	400
Barley	360
Groundnut cake	140
Til cake	40
Mustard cake	30
Salt	10.0
Mineral premix [a]	20.0

[a] Composition: Calcium 320 g / kg, phosphorus 62 g / kg, manganese 2.7 g / kg, zinc 2.6 g / kg, iron 1000 ppm, fluorine 900 ppm, iodine 100 ppm, copper 100 ppm.

The protein content (%) in khejri leaves was 17.89 and 14.5 was in concentrate mixture. Khejri leaves having 48.11 natural detergent fiber while concentrate mixture having 54.67%. Cellulose content in concentrate mixture was 9.43 while in khejri leaves it was 20.61 %.(Table 2).

Table 2. Chemical composition of Diet.

Chemical composition (% on DM basis)	Concentrate	Khejri
Dry Matter	96.09	45.09
Crude protein	14.5	17.89
Neutral detergent fibre	54.67	48.11
Acid detergent fibre	11.87	40.41
Cellulose	9.43	20.61
Hemi cellulose	41.84	7.7
Ether Extract	2.54	5.69

Dry matter intake, digestibility and performance

Weight (kg) of lambs was 23.21±1.24 and kids was 18.98±3.46, which showing significant difference between both species and metabolic body weight of lambs was 10.57±0.42 and kids was 9.07±1.24 which also significantly different between both species. Feed DM intake was 2.64 and 2.82 percent of body weight, which accounted 57.9 and 58.6 g per $kgW^{0.75}$ respectively in lamb and kids.

The DM intake through roughage, concentrate or in terms of g/day, percent of body weight and $g/kgW^{0.75}$ was similar between lambs and kids.

The roughage: concentrate (R: C) intake ratio was 0.10 in lambs and 0.11 in kids which also having significant difference between lambs and kids. Similarly intake of organic matter was 559.9g/d in lambs and 484.2g/d in kids, crude protein (CP) was 108.9g/d and 94.03g/d in lambs and kids respectively and the value of NDF was 195.5g/d in lambs and 170.2g/d in kids.

Digestibility of DM, OM and NDF was also similar but CP digestibility was higher in lambs by 2 percent compared to kids (Table 3).

Table 3. Effect of feeding on nutrient intake and digestibility (%) in lambs and kids.

Dry matter intake, nutrient digestibility and nitrogen retention in lamb and kids				
	Animal Species			
	Lambs	Kids	SEM	P
Body weight (kg)	23.21±1.24	18.98±3.46	0.832	0.006
BW $W^{0.75}$ (kg)	10.57±0.42	9.07±1.24	0.296	0.006
Dry matter intake (DMI)				
Roughage (g d^{-1})	54.76±19.42	53.74±19.42	4.69	0.917
Concentrate (g d^{-1})	559.58±79.83	478.03±111.80	25.67	0.115
R/C Ratio	0.10	0.11	0.18	0.01
Total DMI (g d^{-1})	614.28	531.77	30.36	1.032
DMI % BW	2.64±0.30	2.82±0.60	0.19	0.455
DMI g per kg$W^{0.75}$/d	57.95±7.16	58.65±12.18	2.41	0.890
Digestibility (%)	67.07±8.78	65.10±6.45	1.88	0.617
Organic matter				
Intake(g/d)	559.92±84.90	484.27±118.49	26.73	0.164
Digestibility (%)	68.60±8.72	66.71±6.88	1.91	0.640
Crude protein				
Intake(g/d)	108.96±16.30	94.03±22.81	5.16	0.154
Digestibility (%)	73.84±6.91	71.12±5.09	1.50	0.385
Neutral detergent fiber				
Intake(g/d)	195.52±30.90	170.22±42.61	9.56	0.195
Digestibility (%)	54.66±11.32	52.12±7.95	2.38	0.612
Acid detergent fiber				
Intake(g/d)	85.40±14.83	75.36±19.78	4.12	0.270
Digestibility (%)	40.81±13.27	38.84±12.89	3.16	0.766
Hemi-cellulose				
Intake(g/d)	110.13±16.30	94.86±22.86	5.18	0.146
Digestibility (%)	65.35±12.08	62.63±7.41	2.45	0.595
Cellulose				
Intake(g/d)	63.07±10.85	55.59±14.53	3.24	0.262
Digestibility (%)	58.78±10.01	51.96±7.17	2.28	0.140
Total Carbohydrate				
Intake(g/d)	431.49±65.60	373.35±91.44	20.63	0.166
Digestibility (%)	67.32±9.20	65.60±7.37	2.03	0.685
Ether Extract				
Intake(g/d)	19.47±3.01	16.90±4.17	0.94	0.178
Digestibility (%)	67.27±8.30	66.92±6.47	1.80	0.927
Digestible Crude Protein				
Intake(g/d)	79.93±10.28	65.96±12.25	3.27	0.027
ME intake (MJ/d)	5.70±0.80	4.75±0.81	0.23	0.033
Crude Protein in Diet	17.74±0.11	17.69±0.08	0.025	0.338
Digestible CP (%)	13.11±1.29	12.59±0.93	0.28	0.372
Metabolic Energy (mj/kgDM)	9.37±1.20	9.11±0.94	0.26	0.634
Total N Intake	17.43±2.61	15.04±3.65	0.83	0.154
Nitrogen Voided				
Faeces (g/d)	4.64±1.64	4.49±1.73	0.41	0.857
Urine (g/d)	6.31±1.22	4.76±1.36	0.37	0.031
Total (g/d)	10.10±2.69	9.25±2.22	0.63	0.187
Nitrogen Balance	6.48±1.90	5.80±2.30	0.51	0.529
Nitrogen balance % Intake	37.46±10.57	37.38±13.30	2.90	0.990

Mean value±standard error of eight replicates.

Nutritive value of diet, nutrient intake and nitrogen utilization

The diet fed to lambs having 79.93±10.28 DCP intake while kids having 65.96±12.25, which was significantly different between both species. ME intake of lambs was 5.70±0.80 and 4.75±0.81 of kids showing significant difference between lambs and kids. The N retention was 6.48 g/day, total N intake was 17.4% and 37.4 % of N absorbed in lambs, while respective retentions were 5.8 g, 15.04 and 37.3 % in kids (Table 3).

Table 4. Effect of feeding on rumen fermentation characteristics of Lamb and Kids.

Attributes	Animal Species			
	Lamb	Kids	SEM	T
Rumen pH	6.11	5.98	0.031	0.254
Total-N (mg/dl)	244.87	298.45	9.451	0.006
NH_3-N (mg/dl)	10.59	10.11	0.312	0.677
TCA-ppt-N(mg/dl)	166.99	204.86	7.999	0.021
TVFA (mmol/dl)	7.11	5.99	0.243	0.009

Table 5. Effect of feeding on rumen ciliates of Lamb and Kids.

Rumen Ciliates (X 10^4)				
	Lamb	Kid	SEM	Sig
Holotrichous	2.16	3.11	0.712	0.025
Spirotrichous	80.11	61.11	8.114	0.171

Lamb and kids under high plane of nutrition had similar total feed intake 49.84 kg and 44.03kg, average daily gain 60.16g and 73.16 g and feed conversion ratio (kg feed/ kg gain) 12.52 and 9.19 respectively. Final body weight of lambs was 23.12±1.23 and kids were 18.99±3.47 which was significantly different between both species.

Table 6. Blood biochemical characteristics of experimental lambs and kids.

Attributes	Lambs	Kids	SEM	Significance
Haemoglobin (g/dL)	11.99±0.58	12.11±1.19	0.26	0.611
Serum biochemical attributes				
Glucose (mg/dL)	39.78±8.98	32.44±5.23	1.99	0.111
Total Protein (g/dL)	5.11±0.39	5.66±0.51	0.14	0.122
Albumin (g/dL)	2.77±0.19	2.42±0.28	0.04	0.129
Globulin (g/dL)	2.71±0.32	3.11±0.49	0.12	0.019
Urea (mg/dL)	29.11±7.10	25.45±4.34	1.7	0.311
Total cholesterol(mg/dL)	86.91±9.89	114.54±19.78	5.41	0.007
High Density Lipoprotein (mg/dL)	23.84±8.69	20.99±6.59	2.01	0.432
SGPT (U/L)	24.99±4.12	22.41±3.13	1.12	0.129
Creatinine (mg/dL)	1.15±0.09	1.14±0.18	0.05	0.99
Alkaline Phosphates (mg/dL)	0.39±0.19	0.29±0.21	0.05	0.304
Serum minerals				
Calcium (%)	6.97±1.37	7.34±1.02	0.32	0.582
Phosphorous (%)	3.18±0.50	3.17±0.35	0.12	0.998
Manganese (ppm)	2.57±0.60	3.36±0.28	0.13	0.01
Copper (ppm)	0.86±0.45	0.89±0.34	0.11	0.871
Zinc (ppm)	1.17±0.74	0.72±0.46	0.17	0.207

Mean value±standard error of eight replicates

Table 7. Rumen enzyme activity.

Attributes	Lamb	Kids	SEM	Significance
Alpha amylase (enzyme activity U/h/ml)				
Extra Cellular	1.05±0.27	0.44±0.26	0.12	0.002
Cellular	1.23±0.34	1.57±0.09	0.08	0.027
Total	2.28±0.49	2.02±0.32	0.12	0.304
Specific activity (U/ml)				
Extra Cellular	72.34±42.65	18.45±16.92	12.07	0.016
Cellular	40.88±23.37	52.09±17.08	5.60	0.339
Total	113.22±59.05	68.05±28.01	14.43	0.121
Beta-xylosidase Enzyme activity (U/h/ml)				
Extra Cellular	2.06±0.44	1.64±0.52	0.15	0.162
Cellular	5.55±3.28	9.57±0.27	0.16	0.239
Total	7.08±2.23	11.69±7.96	1.78	0.272
Specific activity(U/ml)				
Extra Cellular	2.29±1.15	1.04±0.70	0.32	0.046
Cellular	2.96±1.90	4.72±2.81	0.70	0.223
Total	5.25±2.80	5.71±2.77	0.77	0.784
Xylanase Enzyme activity (U/h/ml)				
Extra Cellular	11.29±2.20	9.14±3.99	0.94	0.275
Cellular	11.25±2.92	11.18±2.62	0.73	0.965
Total	22.54±4.39	19.68±5.82	1.48	0.360
Specific activity(U/ml)				
Extra Cellular	12.32±5.97	6.15±4.81	1.76	0.770
Cellular	6.16±3.22	6.35±2.89	0.81	0.910
Total	18.48±7.54	11.68±6.03	2.14	0.115
CMCase Enzyme activity (U/h/ml)				
Extra Cellular	3.42±0.44	2.36±0.71	0.23	0.011
Cellular	4.14±1.14	4.78±2.13	0.47	0.531
Total	7.57±1.40	7.30±2.37	0.54	0.820
Specific activity(U/ml)				
Extra Cellular	3.73±1.76	1.48±0.84	0.51	0.018
Cellular	2.35±1.56	2.65±1.32	0.38	0.709
Total	6.08±2.71	4.10±2.14	0.74	0.190

Mean value±standard error of eight replicates.

Rumen fermentation

Rumen fluid pH was 5.98 in kids and 6.11 in lambs, Rumen NH_3-N (mg/dl) concentration was 10.59 in lambs and 10.11 in kids. While total nitrogen was 244.87 in lambs and 298.45 in kids which was showing significant (p=0.006) difference between both species.

The TCA ppt-N (mg/dl) was 166.9 in lambs and 204.8 in kids was also significantly different between both species. The TVFA (mmol/dl) content was 7.11 in lambs and 5.99 in kids which is also significantly (p=0.009) different between lambs and kids. Holotrichous ciliates were higher in kids, while spirotrichous ciliates were high in lambs(Table 5 & 6).

Serum biochemistry and mineral content

Blood haemoglobin, serum glucose, protein, albumin, urea, high density lipoprotein, alkaline phosphatase and SGPT activity were near about similar in lambs and kids while globulin and total cholesterol content were higher in kids compared to lambs. Globulin (g/dl) was 2.71±0.32 in lambs and 3.11±0.49 in kids which is showing significant (p=0.019) difference between both species. Serum minerals *viz.* Ca, P, Zn and Cu were similar but Mn content was higher in kids compared to lambs and between lambs and kids on this particular diet serum manganese was showing significant(p=0.01) difference (Table 7).

Rumen microbial hydrolytic enzymes

The extracellular activity of α- amylase was 1.05±0.27 in lambs and 0.44±0.26 in kids which is significantly different between lambs and kids, and CMCase extra cellular activity was 3.42±0.44 in lambs and 2.36±0.71 in kids which is also significantly different between both species. Although cellular activity of β-xylosidase and CMCase were higher in kids were did not attain statistical significance, but extracellular activity was higher in kids attained statistical significance (Table 7).

In silico analysis of nucleotide sequences

Amplified PCR sequences of methanogen ribosomal RNAs were analyzed through Bioedit v7.2.5 tool (http://www.mbio.ncsu.edu/bioedit). A total four different sequences *viz.* two from lamb (Accession numbers- KP752400, KP752401) and two from kids (accession numbers-KP752398, KP752399) were submitted to Gene Bank.

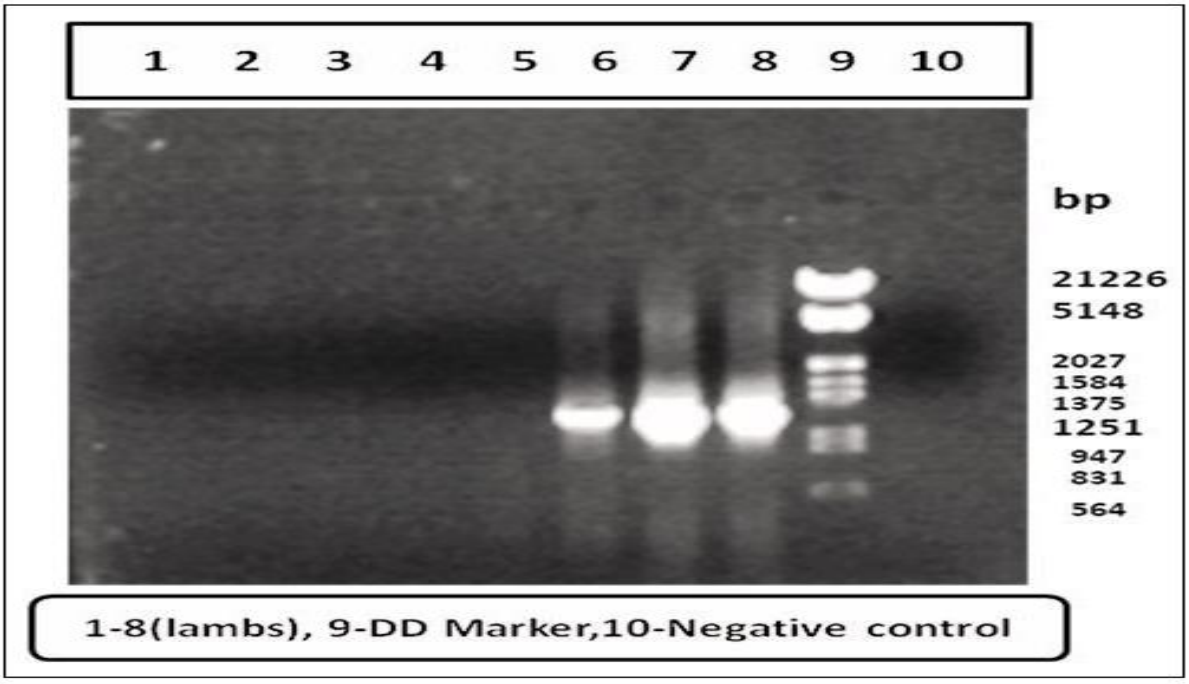

Fig. 1. Sheep rumen liquor with Double digested marker and negative control.

These sequences were BLAST aligned from available sequences in NCBI. BLAST search indicated 99% similarity with other sequences of sheep, cattle, water buffalo, bactrian camel, Sika deer, Alpaca, etc with exception of one sequences (Accession number-KP752400), which has 96% of maximum identity with any sequences available in NCBI database. This may be unique to this geographical location.

Discussion

In identical feeding regimens, lambs generally shows better growth performance compared to kids. However under draught and harsh conditions kids perform better (Karim *et al.*, 2008). The differences in intake between of the two species is due to high sensitivity of kids to the effect of cell wall on rumen fill than lambs (Woodward and Reed, 1995) and

the selective browsing by kids provide better nutrient intake ascribed for better performance under grass based animal production system. Kids have also known to harbour special rumen microbes that promote rumen fermentation of poor quality food and significant difference between animal species for apparent protein digestibility as well as the variable difference between true and apparent N digestibility across diets have been reported (Woodward and Reed, 1995).

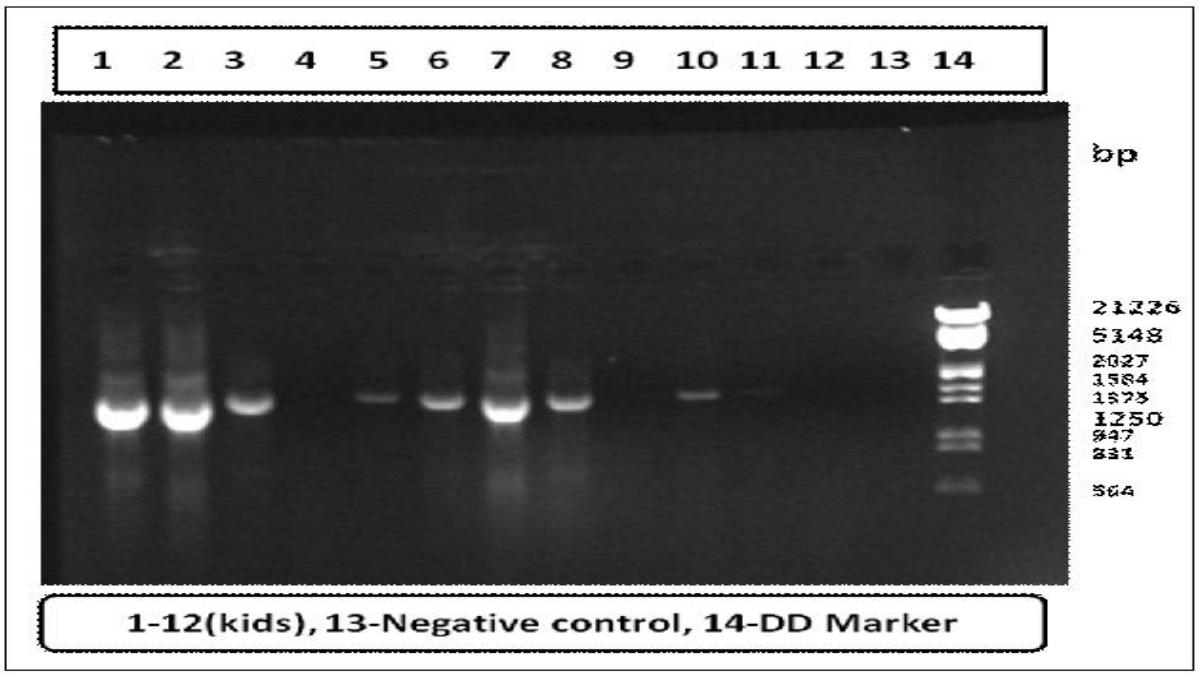

Fig. 2. Sheep rumen liquor with Double digested marker and negative control.

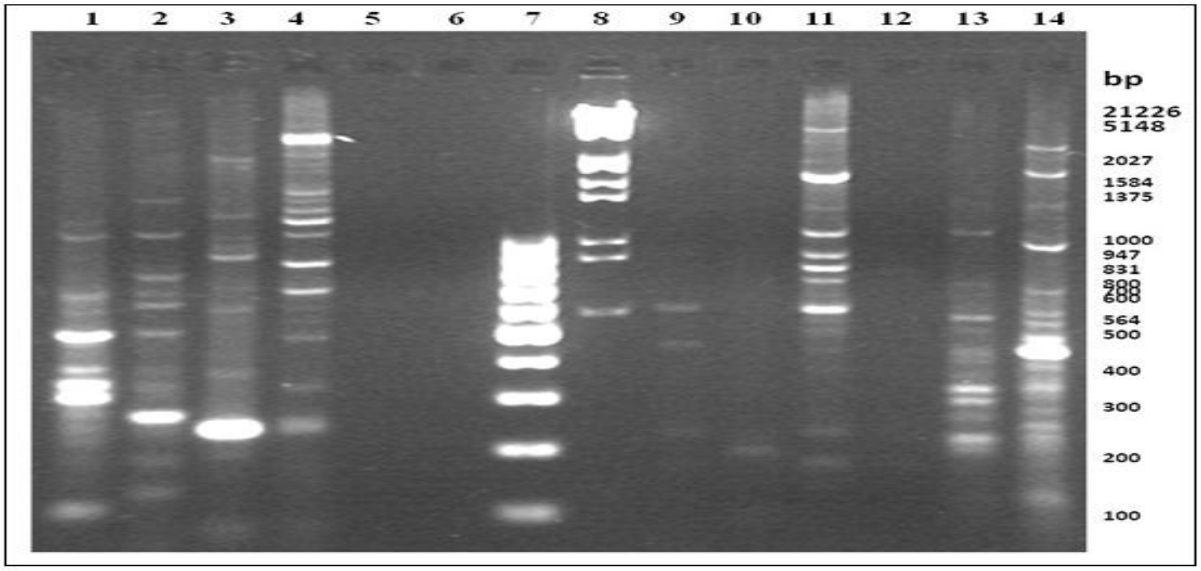

Fig. 3. PCR of DNA isolated from whole rumen liquor of lambs and kids run with DD Marker.

The present study aim was to compare the performance, nutrient utilization, rumen fermentation and blood biochemical profile of lambs and kids feeding *Prosoposis cineraria* leaves. When the lambs and kids fed concentrate and roughage in the cafeteria system of feeding management had similar level of feed intake and nutrient digestibility, but lower CP digestibility in kids may be the consequence of higher protein intake in kids or better proteolytic activity in lamb rumen.

According to Tripathi *et al.* (2007) and Santra *et al.* (1998) lambs and kids under tropical climates shows normal feed intake, nutrient utilization, nutrient digestibility, nutritive value and growth performance. It is proposed that kids might have superior DMIs on medium quality forages than sheep, but on high or very poor quality forages, the differences between species could be small (Tolkamp *et al.*, 1994).

Fig. 4. Animals (Sheep & Goat) during metabolic trial in different metabolic cages.

The digestibility coefficients is a function of intake for all the components of the diets, although several studies have presented that kids digested fibre fractions more efficiently than lambs, especially those from low quality forages of tropical origin.

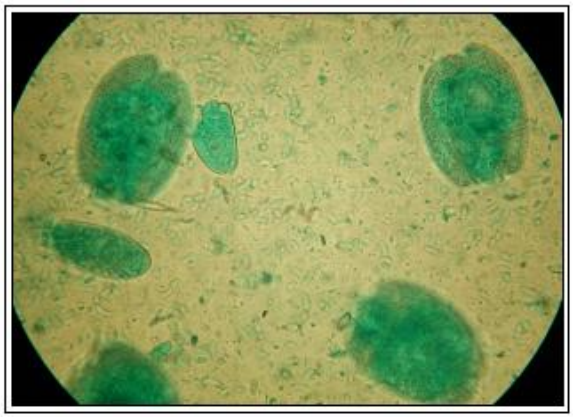

Fig. 5. *Entodinium* Sp. of protozoa screened in rumen liquor of Lambs and Kid).

Under control feeding on high roughage diets, due to higher number of holotrichous and spirotrichous protozoa in the rumen medium, dry-matter intake is lower while fibre digestibility is higher in kids compared to lambs, (Santra *et al.*, 1998).

Tolkamp and Brouwer (1993) concluded that there is no overall interspecies difference in feed digestibility with a possible exception for diets low in crude protein content. In this experiment, animals were given free choice to ingest forage and concentrate hence inter species variations in intake and digestibility were not observed. The higher crude protein intake in goats due to their selection toward proteinaceous foods may in part explain the depression in their digestion of protein in comparison to sheep. Voluntary intake and digestibility of diets can be considered to be determined by the regulation of the removal of undigested residues from the alimentary tract.

Similar rumen pH in kids and lambs showed that both species has equal level of fermentation of readily fermentative dietary carbohydrates, which is also supported by similar rumen activity of α-amylase enzyme. The higher total N in rumen fluid of kids show the selectivity of kids toward higher protein feed, which might have caused

reduction in protein digestibility and evident with similar NH_3-N concentration. The higher TCA-ppt-N in kids might be due to better microbial biomass synthesis in the rumen, which is further supported by reduced rumen NH_3-N and TVFA levels.

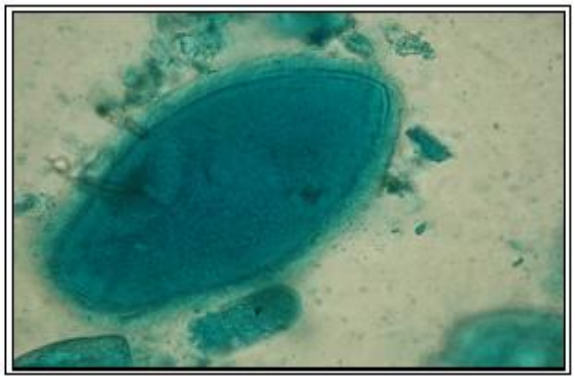

Fig. 6. *Dasitricha* Sp. of protozoa of rumen liquor (Lambs & Kids) strained with Brilliant Green dye.

It has been reported that higher amount of N recycled to the rumen in goats than sheep (Wahed and Owen, 1986; Domingue *et al.*, 1991) and goats are able to use N more efficiently than the sheep (Molina-Alcaide *et al.*, 2003). Further, rumen NH_3-N and TVFA reduced upon microbial biomass synthesis in the rumen because rumen microbes use NH_3-N as source of N and TVFA as energy (Van Soest, 1982). Nitrogen of bacterial cell walls produced in the rumen is expressed as a fraction of N intake because it represents an alternative metabolic fate for N other than growth and excretion. For variable crude protein digestibility two explanations are suggested, first one is that level of microbial N is influenced by intake of fiber and the relative importance of the reticula-rumen as sites of fermentation, that microbial-N decrease with decreasing dietary fiber because rapidly fermentable feed deprives the energy, reducing bacterial biomass synthesis. The second explanation applies to an enhancement of rumen microbial production due to higher levels of recycled N (Reed and Soller, 1987).

Under high plane of nutrition, total enzyme activity between Lambs and Kids was not much different while higher extracellular activity of α-amylase, β-xylosidase and CMCase in lambs and higher cellular activity of β-xylosidase and CMCase in kids may be different due to variations in the pattern of rumen microbes that produce hydrolytic enzymes. El-Meccawi *et al.* (2009) also reported similar level of dry matter intake and nutrient digestibility between sheep and goats even fed a straw based diet which is also similar to our study.

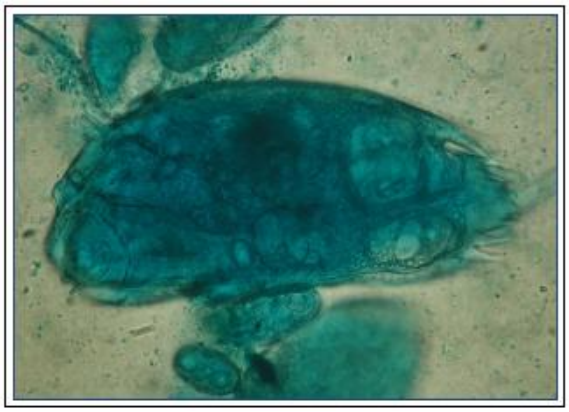

Fig. 7. *Entodinium* Sp. of protozoa of rumen liquor (Lambs & Kids) strained with Brilliant Green dye.

Due to large rumen microbial population, better utilization of nutrients has been reported in goats than in sheep on low quality roughage (Domingue *et al.*, 1991). In serum biochemical and mineral contents only globulin and cholesterol show some inter species variations, which were higher in kids may be due to differences in metabolism of fat and Mn.

Conclusion

Under high level of feeding lamb and kids had similar feed intake, growth and feed conversion ratio. Lambs had higher crude protein digestibility, while dry matter, organic matter and neutral detergent fiber digestibility did not vary. Ruminal fluid pH remained similar while higher TCA-ppt-N and lower NH_3-N and TVFA content show a better microbial protein synthesis in kids rumen. Inter species variations in cumulative specific and total enzyme activities of α-amylase, β-xylosidase and CMCase were not evident between lamb and kids, however minor variations in cellular and extra cellular microbial hydrolytic enzymes exist. Therefore, lamb and kids did not differ in digestive capabilities and growth under high level of feeding. Amplified methanogens ribosomal RNAs sequence were showed similarity with other sequence of sheep, cattle, sika deer with exception of one

sequence having accession no KP752400, which was 96% of maximum identity with any sequence available in NCBI database may be unique for this geographical region hence a detailed characterization of different methanogens would be necessary to find out different methanogens inhabiting lamb and kids in this geographical region.

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