Effect of Transinoculation of Goat Rumen Liquor on Degradation and Metabolism of Mimosine in Sheep Fed with *Leucaena leucocephala* Leaves

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ABSTRACT : The effect of transinoculation of goat rumen liquor into sheep rumen on mimosine toxicity was studied. One adult Kutchi male goat having higher mimosine degradation capacity than sheep was gradually adapted to *Leucaena leucocephala* (Leucaena) leaves by feeding increasing level of eucaena leaves supplementation for 1 month. Six Bharat Merino rams (12-18 months of age) were divided into two equal groups with (group I) or without (group II) infusion of 200 ml of goat rumen liquor per animal. The mimosine degradation in groups I and II were 3.04 and 2.31; 3.90 and 3.73 mg per day per 10 ml rumen liquor respectively after 1 and 2 weeks of leucaena feeding leaves. Total rumen bacterial population in RGCA medium and in a selective medium containing iron showed an increasing trend in both groups, while the bacterial population growing in the presence of cellulose showed a decreasing trend. Animal performance data did not show any adverse effect. Results revealed that transinoculation of rumen liquor from leucaena leaves adapted goat to sheep rumen did not help to improve mimosine degradation in the sheep. The sheep transinoculated with goat rumen liquor displayed no *in vivo* improvements in nutrient utilization vis-à-vis mimosine metabolism. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 332-339)***

Key Words : Mimosine, DHP, Transinoculation, Goat Rumen Liquor, Sheep

INTRODUCTION

Leucaena leucocephala (Leucaena) is a tropical legume that is native to Central America and is now widely distributed, including India. It is a multipurpose tree used as fuel wood and as forage (Pathak et al., 1983). Leucaena leaves are rich in crude protein (Tudsri and Kaewkunya, 2002; Bakshi and Wadhwa, 2003) but their utilization is limited due to the presence of a toxic amino acid mimosine, β-(3-hydroxy-4-oxopyridyl) α-amino-propionic acid, (Jones, 1979). Mimosine and its degraded product, 3-hydroxy-4-(1 H) pyridone (3,4-DHP) are implicated in the goitrogenic and alopecia effect in ruminants (Hammond, 1995). Recent evidence indicates that mimosine is an iron chelator, which affects folate metabolism in mammalian cells (Oppenheim et al., 2000), and the serine protease inhibitor isolated from the seeds of leucaena inhibits enzymes involved in blood clotting and fibrinolysis (Oliva et al., 2000).

In ruminants, the rumen microbial ecosystem shows varied mimosine degradation in different geographical regions leading to differences in the manifestations of toxic symptoms (Kumar and D'Mello, 1995). Adapted ruminants feeding on leucaena leaves are able to convert mimosine to 3,4-DHP and 2,3-dihydroxy pyridine (2,3-DHP) and to as yet unidentified non-toxic products (Jones, 1994). Successfully evolved animals in a region like Hawaii are able to convert mimosine and its degraded products (DHP) to non-toxic compounds. Animals in other regions do not possess the full bacterial complement to degrade DHP (Allison et al., 1990). Further considerable amelioration of adverse effects of mimosine in ruminants may be achieved either by controlled feeding or grazing of leucaena leaves (D'Mello, 1992). This can be exploited for improving/overcoming mimosine toxicity by transfer of rumen microbial population from one ruminant which can be adapted to a particular feed (Hegarty et al., 1964), to another ruminant having lesser mimosine degradation, or lacking the adaptation ability (Semenye, 1990). Recent attempts in transinoculating rumen liquor from adapted to non-adapted animals resulted in increased degradation of mimosine (Pratchett et al., 1991; Gupta and Atreja, 1998). Successful transfer of rumen microbial population has also been made from swamp buffalo to native cattle to improve feed digestibility (Wanapat et al., 2003). However, information on the effect of transinoculation of goat rumen liquor into sheep rumen is scanty. Further, in our conditions, sheep are grazed in the rangeland and are supplemented with lopped tree/shrub leaves, which often contain one or more toxic elements. The aim of the study was to assess the practicability of overcoming the problem of mimosine toxicity in mimosine susceptible sheep via transinoculation of goat rumen liquor into their rumen.

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MATERIALS AND METHODS

Study area

The present study was conducted on a native range (grazing area of predominantly *Cenchrus ciliaris* pasture) as well as in the animal shed located at Central Sheep and Wool Research Institute, Avikanagar, India (75° 22' E longitude and 27° 17' N latitude, 326 m elevation). The

Table 1. Chemical composition (on % DM basis) of *Leucaena leucocephala* leaves and *Cenchrus ciliaris* hay

Composition	*Leucaena leucocephala* leaves	*Cenchrus ciliaris* hay
DM	96.2	94.5
CP (N×6.25)	20.26	9.52
NDF	50.05	6.86
ADF	19.98	51.76
Hemicellulose	30.07	15.10
Cellulose	4.44	16.07
ADL	15.52	35.69
GE (KJ/g DM)	22.38	18.45

climate is typically semiarid with annual rain fall of 673 mm. The study period was from December 1999 to February 2000.

Adaptation of goat to leucaena leaves

Our earlier study (Vaithiyanathan et al., 1998) showed that rumen microbes of goat had a higher mimosine degrading capacity than did sheep rumen microbes. So we planned to study inter species transinoculation of rumen liquor from goat to sheep to improve mimosine degradation. The goat was not exposed previously to leucaena leaves and was residing in the same shed during night hours only. The goat was fed increasing levels of 300, 600 and 800 g of leucaena leaves for 1 week, 1.5 weeks and 1.5 weeks respectively along with *Cenchrus ciliaris* (Cenchrus) hay. Rumen liquor was collected prior to feeding of leucaena leaves and then 2 weeks and 4 weeks after leucaena leaves feeding to determine mimosine degradation. Rumen liquor for single infusion into sheep rumen was taken after 4 weeks of leaves feeding.

Transinoculation

Six Bharat Merino (Rambouillet/Merino×Nali/Chokla, stabilized at 75% level of exotic inheritance) ram (12-18 months of age) previously maintained on cenchrus dominated pasture, were divided into two equal groups. Animals of group I animals were infused with 200 ml of goat rumen liquor (collected as above) per animal. Transinoculation was done only once before start of leucaena leaves feeding. They were grazed on cenchrus dominated pasture for 6 h daily. Animals of both groups were grazed together in the daytime but leucaena leaves were supplemented in individual feeding pens during night hours. All animals were supplemented with leucaena leaves at 200, 300 and 400 g animal^{-1} day^{-1} (mimosine concentration was 0.13, 0.19 and 0.25 g/kg body weight) for one week at each level and during metabolism trial. Rumen liquor was collected before the start of leucaena leaves feeding and on 1, 2 and 4 weeks after leucaena leaves feeding from all sheep to determine mimosine degradation and bacterial population.

Metabolism trial

A 5-day metabolism trial was conducted in the last week of leucaena feeding trial. Feeds, feces and urine samples were quantitatively collected and their suitable aliquots preserved for the estimation of mimosine, 3,4-DHP and 2,3-DHP. Samples were also analyzed for crude protein (CP) and dry matter (DM) in line with the AOAC (1984) and the cell wall constituents were assayed following the detergent system of analysis by the described methods of Van Soest et al. (1991). The gross energy of the samples was estimated using a Ballistic bomb calorimeter (Gallenkamp, UK).

Chemical composition of diet

The chemical composition of leucaena leaves and cenchrus hay are presented in Table 1. It indicated that the nutrients were sufficiently available in the feed materials. Dry matter, TDN and DCP requirements for maintenance in sheep of 25 kg bodyweight are 678 g/d, 305 g/d and 33 g/d respectively (Ranjahan, 1998).

Rumen bacterial counts

Anaerobic culture method was followed using roll tube techniques described by Hungate (1970). Total bacterial populations in a non-selective medium, Rumen fluid Glucose Cellobiose Agar (RGCA) medium, and in a selective medium, Fe-2 medium (containing mimosine) were determined as described by Holdeman et al. (1977) and Allison (1991) respectively. For the purpose of colony counting, agar was added (2%) in Fe-2 medium to solidify the medium. The cellulolytic bacteria were counted following the method of Stewart and Duncan (1985) in cellulose roll tubes. After the medium was prepared, it was heated to boiling to remove oxygen and the atmosphere above the medium was replaced by continuous flushing with oxygen free CO_2. The medium was cooled, dispensed (4.9 ml) into test tubes (size 18×100 mm), stoppered with butyl rubber stopper (Bellco Glass Inc, Vineland, NJ, USA) and autoclaved. Ruminal fluid to be used as ingredient was collected from a ram maintained on hay-concentrate diet and it was clarified (Holdeman et al., 1977) to remove most of the particulate matter. Prior to inoculation, agar tubes were melted and kept warm at 45°C. Then appropriate dilutions were inoculated and the roll tubes were made with the help of roll tube maker (Bellco Glass Inc. Vineland, NJ. USA). They were incubated at 39°C for 7 days before counting.

***In vitro* mimosine and DHP degradation**

Complex medium set up : i) Anaerobic culture methods were followed as mentioned above. The complex medium used was Fe-2 medium and its preparation was carried out as described above. Representative samples (0.1 ml SRL) from goat collected after 4 weeks of leucaena leaves feeding, and sheep collected after 2 and 4 weeks of

Table 2. Visual discolouration of medium and mimosine degradation by mixed rumen microbial populations of Malpura sheep and Kutchi goat at different proportions under *in vitro* conditions using complex medium (Fe-2 medium) up to four weeks

SRL (ml)		Mimosine (μg/ml)* and colour of the medium days after incubation						Mimosine
Sheep	Goat	0	4	8	12	22	28	degradation (%)
10^{-1}	-	250.0	227.5	217.5	212.0	212.0	206.5	17.2
		O	Y	Y	Y	B	B	
10^{-1}	10^{-2}	250.0	221.0	198.5	168.5	168.5	168.5	32.8
		O	Y	B	B	B	B	
10^{-1}	10^{-3}	250.0	223.5	208.5	200.0	205.0	200.0	20.0
		O	Y	Y	B	B	B	
10^{-1}	10^{-4}	250.0	224.5	212.5	201.0	208.0	201.0	19.6
		O	Y	Y	B	B	B	
10^{-1}	10^{-5}	250.0	225.5	207.5	200.0	207.5	200.5	20.0
		O	Y	Y	B	B	B	
10^{-1}	10^{-6}	250.0	227.0	210.5	207.5	207.5	199.5	20.0
		O	Y	Y	B	B	B	
10^{-1}	10^{-7}	250.0	228.0	210.0	200.5	206.5	200.5	20.0
		O	Y	Y	B	B	B	
10^{-1}	10^{-8}	250.0	227.0	214.5	204.5	206.0	203.0	18.8
		Y	Y	Y	B	B	B	
10^{-1}	10^{-9}	250.0	231.0	209.5	207.5	206.5	195.5	22.0
		Y	Y	Y	B	B	B	
10^{-1}	10^{-10}	250.0	222.5	215.5	210.0	210.0	199.0	20.4
		Y	Y	Y	B	B	B	
-	10^{-1}	250.0	220.5	190.5	175.0	180.5	171.5	31.2
		O	Y	B	B	B	B	

O: orange; Y: yellow; B: black.

* Average of two values. sheep and goat were maintained on hay-concentrate diet. They were not accessed to leucaena leaves previously.

leucaena leaves feeding were inoculated under anaerobic conditions and incubated at 39°C for 1 week. Representative controls were also kept. Aliquots of culture were filtered through 0.22 μm membrane and the filtrates were used for determination of mimosine and dihydroxypyridone (DHP) by the High Performance Liquid Chromatography (HPLC) method described in the next section. Mimosine, 3,4-DHP and 2,3-DHP were added for the purpose of total toxin determination. Then total toxin degradation was calculated in test culture in relation to control.

ii) Anaerobic culture methods and preparation of Fe-2 medium were as described above. The inoculum (0.1 ml SRL) was from Malpura ram and Kutchi male goat or a mixture of both mixed at definite proportions (described in Table 2). Goat rumen liquor was serially diluted from 10^{-1} to 10^{-10} before inoculation. Serial dilutions were made in solution similar to the medium but it lacked mimosine and ferric chloride. These animals were permanently fitted with fistulae and were stall fed on hay-concentrate diet for maintenance. The culture medium inoculated with rumen microbes were incubated at 39°C. Representative controls were also kept. On every 4th day colour of the medium was observed and mimosine concentration (in an aliquot of 0.5 ml culture medium) was determined spectrophotometrically at 595 nm from a standard curve prepared from pure mimosine (0.1 to 1.0 mg) as described by Allison (1991) and Kudo et al. (1984), respectively. The principle behind the visual test is the appearance of orange colour, yellow colour and black colour. Orange colour of the medium is due to the formation of complex between mimosine and Fe^{3-}. The yellow colour of the medium is due to the formation of complex between 3,4-DHP and Fe^{3+}. The black colour is due the formation of compound between Fe^{3-} and sulfide of H_2S produced by rumen microbes. When mimosine or 3,4-DHP is utilized by rumen microbes, the medium becomes black. Mimosine degradation was calculated in test culture in relation to control.

In vitro dry matter disappeance set up : Rumen liquor was strained through 2 layers of muslin cloth (strained rumen liquor (SRL)) and mixed separately with Mc Dougall's saliva (Mc Dougall, 1948) in the ratio of 1:4 to make a final volume of 50 ml each and incubated with 0.5 g leucaena leaves (1mm particle size) under anaerobic condition at 39°C for 24 h (Tilley and Terry, 1963). Representative control flasks were also kept. After 24 h, the microbial activity was stopped using 2.0 ml of 6 N HCl, filtered in Gooch crucible No.1 under suction and the dry matter disappearance was determined. Then the mimosine, 3,4-DHP and 2,3-DHP were determined in the incubation fluid (IF) and residue (undigested feed) by HPLC described in the next section. Mimosine, 3,4-DHP and 2,3-DHP were added separately in IF, residue and feed for the purpose of total toxin determination. Then total toxin degradation was calculated in test flask in relation to control.

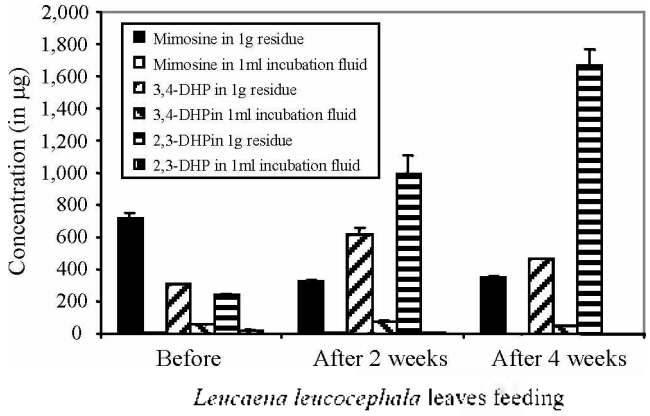

Figure 1. Concentration of mimosine and dihydroxypyridone in *in vitro* dry matter disappearance set up using goat rumen microbes after 24 h of incubation.

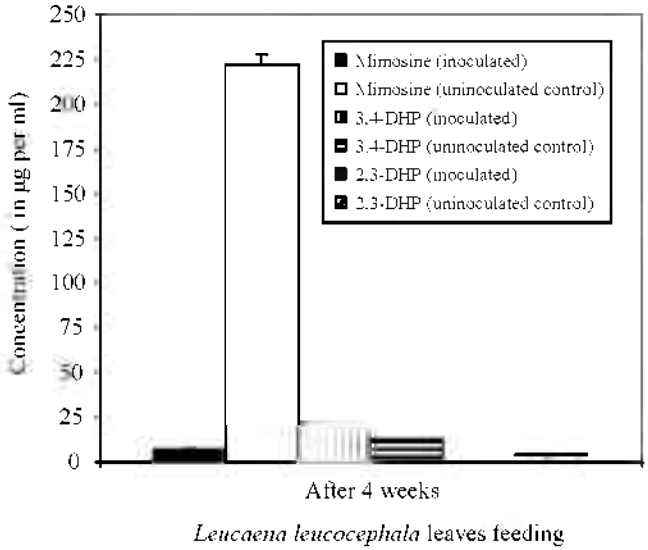

Figure 2. Bioconversion of mimosine into dihydroxypyridone in complex medium set up by Kutchi goat rumen microbes in 7 days of incubation.

Mimosine, 3,4-DHP and 2,3-DHP estimation

One hundred milligram of dried leucaena leaves, faeces and residue was extracted separately with 25 ml of 0.1 N HCl for 24 h and filtered through 0.22 µm filter paper. Aliquots of urine samples were hydrolyzed with equal amounts of 10 N HCl at 110°C for 4 h and the pH was adjusted to 3.0 by NaOH solution followed by filtration as above. Similarly, aliquots of IF and complex medium were also filtered as above for estimation. The filtrate (1 to 5 µl) was injected into C18 CLC ODS column and eluted with 0.2% w/v Orthophosphoric acid at flow rate of 2 ml/min at 254 nm in HPLC (Shimadzu, Japan) (Tangendjaja and Willis 1980; Gupta and Atreja 1998). The mimosine (molecular weight 198.98) for using in the culture medium and as standard was obtained from Sisco Research Lab, India. The 2,3-DHP was procured from Aldrich Chemicals (USA) and the 3,4-DHP was prepared as described by Hart et al. (1977).

Statistical analysis

Means for intake, digestibility and balances were compared by using 't' tests as described by Gomez and Gomez (1976). Mimosine, 3,4-DHP, 2,3-DHP, bacterial population and mimosine degradation variables were analyzed for treatment and sampling time as main effects and treatment by sampling time interaction using the following mathematical model in a two way analysis of variance procedure of SPSS Base 10.0 (SPSS Inc., USA):

$$Y_{ijk} = \mu + T_i + P_j + (TP)_{ij} + e_{ijk}$$

Where, μ = general mean,
T_i = effect of $_i^{th}$ treatment,
P_j = effect of $_j^{th}$ period,
$(TP)_{ij}$ = interaction effect of i^{th} treatment with j^{th} period,
e_{ijk} = random error

RESULTS AND DISCUSSION

Mimosine degradation by mixed microbial population of sheep and goat in a complex medium

Mimosine degradation by rumen microbes differs between animal species. Vaithiyanathan et al. (1998) reported that mimosine degradation by rumen microbes of sheep and goat were 19% and 26% respectively. So, prior to the transinoculation of rumen liquor from goat into sheep rumen, one in vitro experiment was conducted to assess the ability of mixed rumen microbial populations of sheep and goat in various ratios to degrade mimosine. Results of this experiment are presented in Table 2. It showed that mixing of sheep rumen liquor with goat rumen liquor in the ratio of 10:1 had improved the mimosine degradation (32.8%) over the mimosine degradation by sheep rumen bacterial population alone (17.2%). Further the addition of goat rumen liquor at lesser ratio helped in only slight improvement in the mimosine degradation. In an another study, by using varying proportions of the strain 78-1 (3,4-DHP degrader) and rumen fluid of sheep, Allison (1991) reported the bacterial population (78-1 strain) at 10^{-7} ml was able to degrade 3,4-DHP and to change the colour of the medium from orange to black. Our results with respect to the colour were similar to the reports of Allison (1991). However, the black colour of the medium did not match the concentration of mimosine (colorimetric tests) in the medium which was contrary to the report of Allison (1991). Based on these observations, we conducted the transinoculation of goat rumen liquor into sheep rumen to study the mimosine degradation and mimosine metabolism in sheep.

Table 3. Levels of mimosine (µg) and dihydroxypyridone (µg) in residue, incubation fluid, culture medium and total toxin degradation by rumen microflora of sheep with (group I) or without (group II) infusion of goat rumen liquor

Group	IVDMD set up													Factorial analysis	G	P	G×P
	Period[1]							Period[2]									
	Residue (g)			IF (ml)			Toxin degradation[1]	Residue (g)			IF (ml)			Toxin degradation[2]			
	Mimosine	3,4-DHP	2,3-DHP	Mimosine	3,4-DHP	2,3-DHP		Mimosine	3,4-DHP	2,3-DHP	Mimosine	3,4-DHP	2,3-DHP				
I	346.15 ±46.00	1,154.88 ±69.59	632.90 ±41.73	21.55 ±9.35	63.46 ±9.34	ND	3.04 ±0.36	257.63 ±61.73	670.49 ±106.21	329.23 ±50.77	17.21 ±6.57	56.72 ±2.38	ND	3.90 ±0.38	Mimosine (Residue)	**	***	*
II	595.86 ±14.68	548.46 ±16.99	970.27 ±35.59	72.30 ±8.46	28.11 ±5.49	ND	2.31 ±0.63	333.60 ±89.13	793.25 ±25.09	302.56 ±11.06	29.93 ±10.02	45.94 ±6.50	ND	3.73 ±0.38	Mimosine (IF)	**	-	*
															3,4-DHP (Residue)	**	***	***
															3,4-DHP (IF)	**	NS	NS
															2,3-DHP (Residue)	**	***	***
															Toxin degradation	NS	***	NS

	Complex medium set up								Factorial analysis	G	P	G×P
	Period[2]				Period[3]							
	Culture medium (ml)				Culture medium (ml)							
	Mimosine	3,4-DHP	2,3-DHP	Toxin degradation[2]	Mimosine	3,4-DHP	2,3-DHP	Toxin degradation[2]				
I	45.98 ±1.37	7.26 ±0.29	ND	1.32 ±0.01	11.16 ±0.74	58.11 ±7.63	22.53 ±4.36	1.05 ±0.08	Mimosine	NS	***	**
									3,4-DHP	***	NS	***
									2,3-DHP	NS	**	NS
II	32.50 ±2.29	148.99 ±8.30	1.30 ±0.43	0.41 ±0.05	24.24 ±6.80	80.55 ±13.77	26.59 ±10.87	0.77 ±0.16	Toxin degradation	***	NS	**

IVDMD: *In vitro* dry matter disappearance. Complex medium: Fe-2 medium.

Period[1]: after 1 week of leucaena leaves feeding. Period[2]: after 2 weeks of leucaena leaves feeding.

Period[3]: after 2 weeks of leucaena leaves feeding; Period[3]: after 4 weeks of leucaena leaves feeding

Toxin degradation[1]: toxin degradation mg per day per 10 ml rumen liquor. Toxin degradation[2]: toxin degradation mg per day per ml rumen liquor.

3,4-DHP: 3-Hydroxy-4 (1H) pyridone; 2,3-DHP: 2,3-dihydroxypyridone; G: group; P: period

* p<0.05, ** p<0.01, *** p<0.001.

Adaptation of goat to leucaena leaves

Total toxin (including mimosine and DHP) degradation was increased by 18 units in the IVDMD set up using goat rumen liquor during the period of leucaena leaves feeding. However, it increased by 58 units in the complex medium set up. The differences in toxin degradation might be due to two different media. The amount of mimosine, 3,4-DHP and 2,3-DHP (Figure 1) in residue and IF in IVDMD set up showed extensive mimosine conversion. Similarly, the concentration of mimosine, 3,4-DHP and 2,3-DHP in the complex medium (Figure 2) also showed extensive mimosine conversion. Moreover, it did not show any clinical signs i.e. loss of hair and other toxicity signs. Aregheore (2002) also reported similar findings in goats fed leucaena leaves at 40% of diet for about 2 months. The absence of adverse effects may be attributed to enhanced ruminal detoxification of mimosine and its metabolic products. The rumen microbes of goats, which was not exposed previously to leucaena leaves showed lesser degradation and the subsequent controlled feeding and gradual increase of leucaena leaves showed enhanced toxin degradation. Similar results have been reported for cattle (Gupta and Atreja, 1998). These results suggested that goats may have adapted to leucaena leaves after 4 weeks of leucaena leaves feeding.

***In vitro* mimosine and DHP degradation in sheep**

Results of total toxin degradation by ruminal microbes of sheep and the levels of mimosine and DHP in residue, IF and culture medium in two set up are presented in Table 3. The mimosine, 3,4-DHP and 2,3-DHP in residue and in incubation fluid showed that in group I animals' rumen microbes converted mimosine to 3,4-DHP and 2,3-DHP to greater extent than in group II animals. This was reflected in higher toxin degradation. Subsequently, 2,3-DHP may possibly have converted to as yet unidentified products. Thus 2,3-DHP was not detected. But, the toxin degradation did not attain significantly higher levels in group I animals. However, the differences observed in toxin degrading activity among periods were significant ($p<0.001$) in both groups independently of transinoculation. Therefore transinoculation of goat rumen liquor may have helped only to some extent in mimosine degradation. Others have reported complete or enhanced mimosine degradation in goat and cattle by drenching the animals orally or ruminaly with pure cultures of mimosine degrading bacteria or rumen liquor collected from adapted animals (Jones and Lowry, 1984; Jones and Megarrity, 1986; Quirk et al., 1988; Gupta and Atreja, 1998). Besides, the gradual increase in the supplementation of leaves have also contributed to the enhanced mimosine degradation. The increased mimosine degradation over the period of time in group II animals may be attributed to gradual adaptation. Hegarty et al. (1964) have reported that by gradually replacing other feed with leucaena leaves, the enhanced mimosine detoxification ability can be introduced into the non-adapted animals.

The toxin degradation in a complex medium set up was also higher but significant in group I than in group II animals through out the feeding period. The differences in the mimosine degradation between periods was not significant in complex medium. But the differences between the groups and the interaction between groups versus period

Table 4. Bacterial population (log_{10}/ml) in rumen liquor of Bharat Merino sheep with (group I) or without (group II) infusion of goat rumen liquor

Period[a]	Group I			Group II				Factorial analysis		
	Culture medium			Culture medium						
	RGC	Fe-2	Cellu	RGC	Fe-2	Cellu		G	P	G×P
2	8.76±0.20	8.50±0.10	3.59±0.11	8.67±0.04	8.34±0.23	3.67±0.12	RGC	NS	*	NS
4	8.87±0.02	8.28±0.08	3.34±0.04	8.78±0.07	8.19±0.08	3.41±0.12	Fe-2	NS	***	NS
							Cellu	NS	***	NS

[a] Leucaena leaves feeding in weeks.
G: group; P: period: RGC: Rumen fluid glucose cellobiose agar medium: Fe-2: medium containing mimosine: cellu: medium containing cellulose.
*** $p<0.001$; * $p<0.05$, means of triplicate analysis.

Table 5. Live weight, dry matter intake and nutrient digestibility in Bharat Merino sheep with (group I) or without (group II) infusion of goat rumen liquor

Parameters	Group I	Group II	SEM	P
Initial body weight (kg)	23.50	25.5	2.483	NS
Final body weight (kg)	24.25	24.67	2.459	NS
DMI g/kg body weight	32.79	32.21	1.890	NS
DMI g/kg $W^{0.75}$	72.34	71.85	3.375	NS
DCPI g/kg $W^{0.75}$	6.89	6.76	0.479	NS
DEI MJ/kg $W^{0.75}$	0.787	0.899	0.033	NS
DCP of the ration (%)	9.488	9.229	0.577	NS
DE MJ/kg DM	10.82	12.28	0.421	NS
Digestibility coefficient				
DM	54.80	56.95	1.820	NS
CP	63.51	64.32	2.213	NS
NDF	50.84	54.29	2.258	NS
ADF	26.87	33.09	3.645	NS
N retention (g/d)	5.85	7.09	0.762	NS

SEM: standard error of mean: P: statistical significance.
NS: non-significant.

were significant at $p<0.01$ and $p<0.001$ respectively (Table 3). Further, the concentration of mimosine, 3,4-DHP and 2,3-DHP in the complex medium after the growth of rumen microbes demonstrated that enhanced toxin conversion to 3,4-DHP and then to 2,3-DHP occurred in group I animals.

In this study there was no improvement in toxin degradation due to transinoculation in IVDMD set up which simulates a true rumen environment. Thus introduction of goat rumen microbes did not provide improvement of toxin degradation in IVDMD set up. But the toxin degradation showed significant improvement due to transinoculation in group I animals in complex medium set up. But the complex medium defines only the possibility of pure mimosine degradation.

Rumen bacterial counts

The results on rumen bacterial population are presented in Table 4. The differences between the groups were not significant but the differences between the periods were significant ($p<0.001$). Rumen bacteria growing in the presence of mimosine were higher in number in group I than in group II animals. This bacterial population constituted more than 90% of the total bacteria population after 2 weeks of leucaena leaves feeding in both groups. However there was no significant difference between groups. The amount of goat rumen liquor transinoculated was 200 ml per animal and the difference in the potent mimosine degraders in the selective medium between two groups should be not less than 5% beyond which it becomes significant. This non-significant difference in potent mimosine degraders has reflected the absence of improvement in toxin degradation. The number of potent mimosine degraders transinoculated from goat to sheep may not be sufficient enough to increase its population so as to influence significantly upon toxin degradation. Moreover, the rumen bacterial population appeared to change significantly in both groups over the period of leucaena leaves feeding. Further, the significant ($p<0.05$) increase in the bacteria number over the period of time was also in accordance with the increased mimosine degradation. Conversely, the cellulolytic bacteria population was significantly ($p<0.05$) decreasing over the period of time. Dominguez-Bello and Stewart (1990) reported reverse trend but insignificant results in total bacteria population and cellulolytic bacteria population of rumen of Venezuela sheep fed with or without leucaena leaves.

Live weight changes, nutrient digestibility and mimosine metabolism

The results of body weight changes and digestibility coefficients are presented in Table 5. The performance of animals did not indicate any significant difference in the change in body weight. The digestibility coefficients, N balance and digestible energy were higher but insignificant in group II animals. The intake of digestible CP and of digestible energy were sufficient for maintenance (Ranjhan, 1998). The results of the mimosine metabolism during metabolism trial are presented in Table 6. It was observed that the intake of mimosine, 3,4-DHP and 2,3-DHP were higher in group I then in II animals. The intake of mimosine at 0.2 g/kg body weight for two weeks did not produce any sign of wool shedding in either group. Hegarty et al. (1964) reported wool shedding at this level of mimosine intake in sheep. Singh et al. (2003) have reported no adverse effect on DMI, nutrient utilization and growth performance of lambs fed on diet with leucaena seeds containing mimosine

Table 6. Intake and output of mimosine and its degradation products (mg animal^{-1}day^{-1}) in Bharat Merino sheep with (group I) or without (group II) infusion of goat rumen liquor

Group/intake/qut put	Mimosine/3,4-DHP/2,3-DHP	Group I	Group II	SEM	P
Intake	Mimosine	5,496.55	5,377.58	55.29	NS
	3,4-DHP	306.17	299.55	3.08	NS
	2,3-DHP	18.48	18.08	0.19	NS
Urine out put	Mimosine	158.14	157.38	28.01	NS
	3,4-DHP	355.79	310.62	49.95	NS
	2,3-DHP	1,263.52	1,000.48	206.45	NS
Feces out put	Mimosine	102.53	105.62	5.67	NS
	3,4-DHP	880.47	858.62	65.39	NS
	2,3-DHP	543.65	25.31	162.60	NS

SEM: standard error of mean; P: statistical significance; NS: non-significant.

at 0.06 g/kg body weight for 120 days. Excretion of mimosine and its metabolites showed that large amount of mimosine were converted into DHP in the rumen of sheep and they were subsequently excreted in urine and feces. However, the differences in the amount excreted between the two groups were insignificant. The increased excretion of 3,4-DHP in urine and feces in group I animals may be due to increased intake of mimosine and DHP. Jones and Hegarty (1984) also reported similar results. The detection of lower amount of 2,3-DHP in urine and higher amount of 2,3-DHP in feces of group I animals may be due to further and higher degradation of 2,3-DHP and 3,4-DHP into yet unidentified product and 2,3-DHP respectively. The higher degradation of 3,4-DHP may be from the fact that rumen microbes of group I animals had higher toxin degrading capacity. But the absence of beneficial effects on nutrient utilization in group I over the group II animals may possibly be due to lack of *in vivo* improvements from transinoculation. The reason could be insufficient number of potent mimosine degraders transinoculated into sheep rumen.

CONCLUSION

The rumen of Kutchi goat in India was found to harbour higher proportion of bacterial population capable of degrading mimosine to 3,4-DHP and 2,3-DHP. Co-culturing of goat and sheep rumen microbes in the same environment enhanced mimosine degrading capacity. Transinoculation of goat rumen liquor (200 ml per animal) into sheep rumen did not induce *in vivo* improvements in nutrient utilization vis-à-vis mimosine degradation.

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