EFFECT OF SUPPLEMENTING UREA MOLASSES MINERAL BLOCK LICKS ON BACTERIAL PRODUCTION RATE IN THE RUMEN OF CROSSBRED CALVES

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Summary

Sixteen crossbred (Sahiwal × Holstein) male rumen fistulated calves of 18 to 24 months of age were randomly divided into four groups of four animal, each. Animals in all the groups were fed wheat straw *ad lib* as basal roughage. However, the animals in group I were fed concentrate mixture at maintenance level, whereas, the animals in groups II, III and IV had free access to existing, modified (A) and modified (B) urea molasses mineral block licks respectively.

Daily wheat straw intake (kg) was significantly ($p < 0.01$) higher in groups II (4.20 ± 0.13), III (4.07 ± 0.16) and IV (4.22 ± 0.20) as compared to group I (3.21 ± 0.14). Total N and TCA precipitable-N were non-significantly different in different groups. However, ammonia-N level (mg/100 ml strained rumen liquor) was significantly higher in groups II (22.36 ± 0.25), III ($21.63 + 0.25$) and IV (21.77 ± 0.50) as compared to group I (18.31 ± 0.41).

Bacterial production rate (g/day and g/kg digestible organic matter intake) were non-significantly different amongst groups I (214.4 ± 13.28; 85.38 ± 3.69); II (198.7 ± 5.70; 86.17 ± 3.53); III (214.4 ± 8.19; 96.15 ± 2.16) and IV (218.2 ± 10.62; 94.44 ± 5.52). Similarly, percent efficiency of N incorporation into bacterial protein was not found significantly different amongst groups I, II, III and IV.

These studies indicate that when concentrate mixture (upto maintenance level) in the diet of ruminants was replaced with UMMB licks, various N fraction in SRI, and efficiency of bacterial production rates in the rumen were not affected.

(**Key Words**: Urea Molasses Mineral Block, TCA Precipitable-N, Ammonia-N, Bacterial Production Rate)

Introduction

Cereal straws and other agro industrial by-products have been used as basal feeds for ruminants for many years in South-East Asia and other developing countries. Such feed resources are poor in fermentable nitrogen, energy and minerals and thus, can not support even body maintenance needs of the ruminant animals.

Of the various methods, the supplementation of deficient nutrients in the form of fermentable N, energy and minerals through urea, molasses and mineral mixture respectively is important for increasing the utilization of cereal straws. Several workers have shown the increase in intake and/or digestibility of straws when supplemented with urea, molasses and minerals as liquid feed supplement (Pathak and Ranjhan, 1976; Church and Santos, 1981 and Daniel et al., 1986a,b) or urea-molasses impregnated straws (Putnam et al., 1964; Gupta et al., 1968, 1970; Sharma et al., 1972a,b and Mclennan et al., 1981) or in the form of 'Uromol' (Chopra et al., 1974, Malik et al., 1978a and Ahuja et al., 1982).

To circumvent the problem of mixing urea, molasses and mineral mixture properly and in the right proportions to avoid risk of urea toxicity under field conditions, National Dairy Development Board (NDDB), Anand, has introduced urea molasses mineral block lick in India (Kunju, 1986). Although, some basic trials using urea molasses mineral block lick as feed supplement were conducted at NDDB, Anand, yet more extensive rumen fermentation studies were considered necessary using this product. For the present investigations, the ingredient composition of the urea molasses mineral block lick was also modified to study the effect of existing and modified urea molasses mineral block licks on rumen fermentation pattern and straw utilization.

Materials and Methods

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Received August 14, 1991
Accepted April 27, 1992

Selection, feeding and management of experimental animals

Sixteen male crossbred (sahiwal × Holstein Friesian) rumen fistulated calves of 18 to 24 months of age were selected for these studies and divided into four groups of four animals each using randomised block design.

The animals were kept individually in a well ventilated shed where there was separate arrangement for individual feeding and the quantitative collection of feces and urine. Animals were fed wheat straw *ad lib* as the only basal roughage in all the groups. However, animals in group I were given concentrate mixture for maintenance as per ARC (1980) and the animals in groups II, III and IV were allowed to lick at free choice urea molasses mineral block I, II and III respectively.

After a preliminary feeding period of 40 days, the rumen liquor samples were taken for various estimations. However, two days before the sampling of rumen liquor, the total ration was divided into 12 equal parts and fed at 2 hourly interval with the objective of reaching a near steady state in the rumen.

Method of manufacturing urea molasses mineral block licks

The urea molasses mineral block licks were being manufactured (Kunju, 1986) by hot process at NDDB, Anand and supplied for the experiment. The hot process of manufacture involved first heating of molasses to 70°C to dissolve urea. Later, other ingredients were mixed together along with the binder i.e. sodium bentonite. The moisture of the mixture was squeezed out by agitating and scrapped heating. On completion of the process, the batch was discharged into specially fabricated moulds having non sticky surface. After cooling, the solid blocks were demoulded and packed. The urea molasses mineral block licks were of the size 245 × 150 × 65 mm with a weight of 3 kg.

Sampling of rumen liquor

Samples of rumen liquor were collected for the estimation of various N fractions and bacterial production rate. Samples were collected through cannula with the help of specially made stainless steel probes having large number of holes drilled in them and covered with fine nylon cloth. These probes were kept at four different sites in the rumen so as to get a representative sample of rumen liquor. About 100 ml of rumen liquor was collected at different time intervals in small plastic bottles. Immediately after collection, 0.2 ml of 10 N sulphuric acid was added to terminate the microbial activity.

Analytical procedures

Total N in strained rumen liquor was estimated with the usual Kjeldahl method. For TCA percipitable-N, 5 ml strained rumen liquor was precipitated with 5 ml of 30 per cent trichloroacetic acid (TCA). After 3 to 4 hours, the contents were centrifuged at 3000 rpm for 15 minutes. Two ml supernatent was taken for estimation of nitrogen by Kjeldahl method which was designated non-protein nitrogen. This value was subtracted from the total-N and was designated as TCA precipitable-N. Ammonia-N in strained rumen liquor was also estimated using Kjeldahl method.

The bacterial production rate was estimated by the method described by Singh et al. (1974). Bacterial cells from the rumen of the cattle used for *in vivo* experiment were first labelled with 35s Sodium sulphate. For this, 0.5 g of powdered concentrate mixture or urea molasses mineral block lick actually fed to animals, 50 ml of freshly drawn Strained rumen liquor were taken in a flask alongwith 0.5 m Ci of sodium sulphate and incubated at 39 + 1°C under an atmosphere of CO_2 for 12 hours in a metabolic shaker. At the end of the incubation period, the bacteria were separated from the coarse feed particles by centrifuging at 200 g for 2 minutes. The supernatent was again centrifuged at 15,000 g for 15 minutes to separate out the labelled bacterial pellet. It was washed twice with centrifuged rumen liquor to remove free radioactivity. The sediment was suspended in centrifuged rumen liquor and an aliquot was kept separately to estimate the amount of radio-activity present. This bacterial suspension was infused into the rumen through cannula in a single dose for one animal. Simultaneously, the contents of the rumen were mixed manually as indicated earlier. The samples of rumen liquor were drawn at different time intervals (30, 60, 90, 120, 150, 180, 240, 300 and 360 minutes) and the specific radio activity of the bacterial cells was measured which was exp-

ressed as dpm/mg (dry weight) of bacterial cells. The exponential decline in specific radio activity with time was calculated and fitted to the equation :

$$SR_t = A_e^{-mt}$$

Where,

SR_t = Specific activity (dpm/mg) at time 't' minutes,

A = Intercept of SR at zero time (dpm/mg),

m = Rate constant of best fit by least square analysis

Pool size (mg) of bacteria was calculated as under :

$$\text{Pool size} = \frac{\text{Dose injected (dpm)}}{\text{SR at zero time (dpm/mg of bacterial cells)}}$$

Bacterial production rate (mg/minutes) = P × m

Where,

P = Pool size,

m = Rate constant (slope)

Statistical analysis

The data were analysed statistically using randomised block design as per Snedecor and Cochran (1967).

Results

The per cent ingredient composition of different urea molasses mineral block licks and concentrate mixture fed in different groups is shown in table 1. In urea molasses mineral block lick II, cottonseed extraction was replaced with groundnut extraction, whereas, in urea molasses mineral block lick III, salt and mineral mixture were reduced but cottonseed and groundnut extractions were put together @ 21 per cent. The groundnut extraction was added in place of or in addition to cotton seed extraction to study its effect on bacterial protein synthesis as former is comparatively soluble in rumen and a better source of performed amino acids.

TABLE 1. PERCENT INGREDIENT COMPOSITION OF DIFFERENT UMMB LICKS AND CONCENTRATE MIXTURE FED IN DIFFERENT GROUPS

Particular	UMMB licks			Particular	Percent of ingredients in concentrate mixture
	Existing	Modified (A)	Modified (B)		
Urea	15	15	15	Maize grain (crushed)	40
Molasses	45	45	45	Wheat bran	40
Salt	8	8	2	Groundnut extraction	17
Mineral mixture	15	15	10	Mineral mixture	2
Sodium bentonite	3	3	3	Common salt	1
Calcite powder	4	4	4		
Cottonseed extraction	10	–	10		
G.N. extraction		10	11		

The per cent chemical composition of different feed ingredients has been shown in table 2.

Daily intake of wheat straw, concentrate mixture and urea molasses mineral block licks under different groups is shown in table 3. The straw intake (kg/day) was significantly ($p < 0.01$) higher in groups II, III and IV as compared to group I. There was no significant difference in urea molasses mineral block lick consumption (kg/day) amongst groups II, III and IV. Total DM intake (per cent body weight) was non-significantly different amongst different groups.

Total-N and TCA precipitable-N (mg/100 ml strained rumen liquor) were not significantly different in groups I, II, III and IV. However, ammonia-N (mg/100 ml strained rumen liquor), as shown in table 4, was significantly ($p < 0.01$) higher in groups II, III and IV as compared to group I.

Bacterial production rate (g/day and g/kg digestible organic matter intake) were non-signi-

TABLE 2. PERCENT CHEMICAL COMPOSITION OF DIFFERENT UMMB LICKS, WHEAT STRAW AND CONCENTRATE MIXTURE FED UNDER DIFFERENT GROUPS (DM BASIS)

Particular	UMMB licks			Wheat straw	Concentrate mixture
	Existing	Modified (A)	Modified (B)		
Organic matter	63.65	63.34	69.04	87.50	91.85
Crude protein (N × 6.25)	9.64*	9.74*	10.06*	3.25	20.82
Ether extract	0.62	0.65	0.60	0.82	3.96
Crude fibre	1.77	1.80	1.88	37.55	7.82
Total ash	36.35	36.66	30.96	12.50	8.15
NFE	51.62	51.15	56.50	45.88	59.25

* N Content.

TABLE 3. ACCOUNT OF DAILY FEED INTAKE IN DIFFFRENT GROUPS

Treatment	Body weight (kg)	Straw** intake (kg)	Conc. mix. intake (kg)	UMMB intake (kg)	DM intake kg/100 kg body weight	DM intake ($g/W_{kg}^{0.75}$)
I.	191.75 ±8.20	3.21[a] ±0.14	1.41	–	2.41 ±0.02	90 ±0.48
II.	190.75 ±5.41	4.20[b] ±0.13	–	0.475 ±0.03	2.45 ±0.03	91 ±0.92
III.	188.50 ±11.02	4.07[b] +0.16	–	0.493 +0.09	2.43 +0.06	90 ±1.08
IV.	192.50 ±8.81	4.22[b] ±0.20		0.478 ±0.06	2.44 ±0.02	91 +0.82

[a,b] Figures with different superscripts in a column differ significantly; $p < 0.01$.
Note: Mean of four calves ± SEM.

TABLE 4. DIFFERENT N FRACTIONS IN STRAINED RUMEN LIQUOR (SRL) IN ANIMALS FED UNDER DIFFERENT GROUPS

Treatment	Total-N (mg/100 ml SRL)	NH_3-N** (mg/100 ml SRL)	TCA precipitable N (mg/100 ml SRL)
I.	111.30 ± 4.06	18.31[a] ± 0.41	64.04 ± 1.45
II.	112.35 ± 3.25	22.36[b] ± 0.25	63.00 ± 3.02
III.	112.70 ± 3.86	21.63[b] ± 0.25	65.45 ± 2.32
IV.	112.70 ± 4.90	21.77[b] ± 0.50	65.45 ± 2.45

[a,b] Figures with different superscripts in a column differ significantly;
** $p < 0.01$.
Note: Mean of four calves ± SEM.

ficantly different amongst groups I (214.4 ± 13.3; 85.38 ± 3.69), II (198.7 ± 5.7; 86.17 ± 3.53), III (214.4 ± 8.2; 96.15 ± 2.16) and IV (218.2 ± 10.6; 94.44 ± 5.52). Similarly, bacterial production rate g/mole of ATP (Y_{ATP}) and per cent efficiency of N incorporation into bacterial protein were non-significantly different amongst groups I, II, III and IV (table 5).

TABLE 5. BACTERIAL PRODUCTION RATE IN THE RUMEN OF ANIMALS FED UNDER DIFFERENT GROUPS

Treatment	Bacterial production rate		ATP production	Y_{ATP}	Percent efficiency
	g/day	g/kg DOMI	(moles/day)		of N incorporation
I.	214.4 ± 13.28	85.38 + 3.69	22.55 ± 1.62	9.53 ± 0.16	30.83 ± 1.28
II.	198.7 ± 5.70	86.17 ± 3.53	20.12 ± 0.36	9.87 ± 0.19	27.98 ± 0.63
III.	214.4 ± 8.19	96.15 ± 2.16	22.85 ± 1.16	9.40 + 0.14	29.54 + 0.51
IV.	218.2 + 10.62	94.44 ± 5.52	23.03 ± 1.40	9.50 ± 0.16	29.63 ± 1.35

Discussion

The urea molasses mineral block lick A had 10 percent groundnut extration whereas, urea molasses mineral block lick B had 11 percent groundnut and 10 percent cottonseed extractions. The objective of these modifications was to provide higher quantity of preformed amino acids and peptides in the rumen which could increase bacterial production rate as CP from groundnut extraction has been shown to be highly soluble in the rumen (Gupta and Manget Ram, 1988).

The different urea molasses mineral block licks had different N content, obviously, due to the incorporation of different amounts of cotton seed or groundnut extraction in addition to 15 percent of urea. The higher total ash content was due to the presence of mineral mixture calcite powder, salt and sodium bentonite. The ash content in urea molasses mineral block lick B was on the lower side due to the reduction in the proportion of mineral mixture and salt. Supplementation of urea molasses mineral block lick to wheat straw based diet increased voluntary consumption of straw (Sudana and Leng, 1986). This increase in straw intake was mainly due to the fact that straws which are deficient in nitrogen, energy and minerals, are consumed in such amounts that these deficiencies were corrected. However, in the present studies, increase in straw intake in groups II, III and IV as compared to group I might not be due to above mentioned reason. When different diets fulfil the nitrogen, energy and mineral requirements of the ruminants for maintenance, irrespective of the source, the total DM intake is expected to be more or less similar for specific breed and age group of animals as it was in the present experiment. In this experiment, 30 percent of the total DM intake was met through concentrate mixture in group I whereas, urea molasses mineral block lick (a substitute of concentrate mixture) constituted only 10 percent of total DM intake in groups II, III and IV. Thus, in groups II, III and IV when fermentable N and energy were not a limiting factor (supplied by urea molasses mineral block licks), animals might have met their DM requirements through higher consumption of wheat straw.

Non-significant differences in total-N and TCA precipitable N amongst various groups could be due to the reason that rumen fermentable N requirements of the animals were fully met by way of either feeding required quantity of concentrate mixture (maintenance level) or allowing the animals free access to urea molasses mineral block licks. Thus, neither the animals were having a deficient nor excess supply of the ruminal fermentable N due to which the various N fractions, except ammonia-N were similar and remained largely unaffected amongst different groups. However, in the animals having access to urea molasses mineral block licks, most of the fermentable N was in the form of urea which was completely hydrolysed in the rumen. Thus, significantly higher level of ammonia-N in the rumen of animals with an access to urea molasses mineral block lick was mainly due to complete hydrolysis of urea in the rumen. Various workers have shown that when urea was incorporated in the diet of the animals in one or the other form, it led to an increase in the level of ammonia-N in the rumen (Horton, 1978; Manget Ram and Kunju, 1986; Sudana and Leng, 1986 and Maeng et al., 1986).

It has been reported that the ruminal ammonia-N levels should be held higher than 50 mg/l strained rumen liquor to support maximum microbial growth in the rumen (Leng and Nolan, 1984). The actual levels of ammonia required for

optimum activity of rumen micro-organisms depend on diet and feeding regime (Leng and Nolan, 1984). The level of ammonia required to support maximum microbial growth in the rumen was reported to be 50 to 80 mg N/l rumen fluid (Satter and Slyter, 1974) but others recommended higher levels for maximum rate of fermention (Mehrez et al., 1977 and Miller, 1973). Krebs and Leng (1984) reproted that a level of 210 mg ammonia-N/l rumen fluid led to maximum rate of digestion of cotton wool in the rumen. In the present study, ruminal ammonia-N levels were in the range of 180 to 220 mg/l rumen fluid in groups I to IV which clearly indicated that ammonia-N in the rumen was not a limiting factor to bacterial production rate in any of the groups.

With the modifications in the ingredient composition of the urea molasses mineral block lick, there was an apparent increase in the bacterial production rate in the rumen but not to the level of statistical significance. Thus, although the present modifications did not show significant increase in the bacterial production rates, there was an indication of non-significant enhancement in bacterial production rates. Bacterial production rate (g/kg digestible organic matter intake) ranged from 85 to 96 in the present studies. When leguminous hay diet was fed to crossbred calves, bacterial production rate (g/kg digestible organic matter intake) in the rumen ranged from 81 to 92 (Manget Ram and Gupta, 1988). However, Sharma and Gupta reported a value of 67 when buffalo calves were fed wheat straw impregnated with 2 percent and 15 percent molasses. Thus, the bacterial production rates per unit of digestible organic matter intake were quite optimum on wheat straw based diets supplemented with urea molasses mineral block licks.

Considering that 2.2 moles of ATP are produced in the rumen per mole of VFA (Leng, 1973), ATP production (moles/day) was calculated. Based on ATP production, bacterial production rates (g/mole ATP; Y_{ATP}) were calculated. The value of Y_{ATP} reported by Forrest and Walker (1971) was 10.5 which was considered constant but later on Owens and Issacson (1977) reported that this value could approach 19. The Y_{ATP} values are largely dependent upon the diet and feeding regime. The value of Y_{ATP} ranged from 9.4 to 9.8 in the present studies. Lower value of Y_{ATP} in all the groups might be due to the fact that the animals were fed at maintenance level and the energy could be a limiting factor for higher bacterial production rates.

Considering that the bacteria contain 10.5 percent N (Moustafa and Collins, 1968), the percent efficiency of N incorporation into bacterial protein was determined. Efficiency of N incorporation into bacterial protein was similar in different groups, which indicated that N from urea molasses mineral block licks on a wheat straw based diet was utilised for bacterial production with similar efficiency as that from concentrate mixture.

Acknowledgements

The authors wish to thank National Dairy Development Board, Anand, for manufacture and supply of various urea molasses blocks and financial assistance for this project. Guidance for the project work rendered by Dr. MPG Kurup, Executive Director and Dr. PJ George Kunju, Ex. Scientist-IV (ANFT), NDDB, Anand is gratefully acknowledged.

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