

## Study on Supplementing Effects or Feeding Systems of Molasses and Urea on Methane and Microbial Nitrogen Production in the Rumen and Growth Performances of Bulls Fed a Straw Diet

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**ABSTRACT:** An experiment with growing bulls were conducted to determine the effect of supplementation of a straw (S) with 15% molasses and 3% urea as an intimate mix (UMS) on its dry matter (DM) intake and digestibility (DMD) and reduction of methane (CH<sub>4</sub>) production from fermentation *in vitro* of the straw. In the next experiment, the feeding of the UMS was compared with that of the feeding of molasses and urea in meals (DS) or in lick blocks (DSUMB) as supplements to straw. The UMS feeding increased daily intake of straw DM (89.5 g · kg W<sup>-0.75</sup>, p < 0.01) and digestible crude protein (DCP 333 g, p < 0.001) and nitrogen (N) balances (508 mg · kg W<sup>-0.75</sup> · d<sup>-1</sup>, p < 0.01) of the bulls than the feeding of 'S' (65 g · kgW<sup>-0.75</sup>, 55 g and 8.0 mg · kgW<sup>-0.75</sup> · d<sup>-1</sup>, respectively). It also increased the digestibility of DM (594 g · kg<sup>-1</sup>, p < 0.05), organic matter (OM, 641 g · kg<sup>-1</sup>, p < 0.05), CP (619 g · kg<sup>-1</sup>, p < 0.001) and acid detergent fibre (ADF, 773, p < 0.05). The CH<sub>4</sub> emitted per g of DOM apparently fermented in the rumen (DOMR) was 91.0 ml in the 'S' and reduced (p < 0.05) to 61.6 ml in the UMS. The feeding of the UMS when

compared with that of the DS or DSUMB also gave a higher straw intake (1.77% of live weight, LW, p < 0.01) and ADF digestibility (516 g · kg<sup>-1</sup>, p < 0.05) than the other diets (1.52% or 1.55% LW and 472 or 490 g · kg<sup>-1</sup>, respectively) in association with the increased microbial N yield in the rumen (14.1, 5.62 or 17.0 g · kg<sup>-1</sup> DOMR, respectively, p < 0.05), daily LW gains (233, 125 or 93 g, respectively, p < 0.05) and feed conversion ratios of the diets (26.0, 56.1 or 57.6 g feed/g LW gain, p > 0.05, respectively).

It can be concluded that molasses and urea feeding as an intimate mix with straw (UMS) increased its digestion and intake in association with a reduced methane emissions in the rumen. When compared with that of their feeding in meals or in lick blocks as supplements to straw, the UMS gave the highest straw intake and digestion and live weight gains of growing bulls concurring the finding that the UMS system may be the best way of molasses and urea feeding to ruminants fed straws.

**(Key Words:** Straw, Methane, Microbial Nitrogen, Growing Bulls)

### INTRODUCTION

Rice straw, a low quality roughage for ruminant livestock, is handicapped by its low digestion and slow passage of the refractory fibres in the rumen. It lacks the nutritional characteristics to optimize the rumen environment in terms of the microbial requirement of readily fermentable carbohydrates (RFC), rumen ammonia nitrogen (NH<sub>3</sub>-N) and some minerals. Moreover, when straw is fermented in the rumen about 15% of its digestible energy (DE) is lost as methane (CH<sub>4</sub>) (Leng, 1991). The increase of energy yields from straw fermentation in the rumen and reduction of CH<sub>4</sub> emission from it may be done either through supplementation of straw with a better quality feed and/or changing the

intrinsic properties of the straw by chemical treatments (Leng, 1991; Moss et al. (1994).

Urea treatment of straw was considered the best as it increases nitrogen content, cell wall digestibility and voluntary intake of the substrate (Greenhalgh, 1983). But the urea treatment of a straw, usually demonstrated at farm levels of Bangladesh, is often been considered by farmers as cumbersome (Huque et al., 1994). Nevertheless, supplementation of straw with molasses and/or urea has been found to increase its digestion and voluntary intake (Reyes, 1974; Huque and Talukder, 1995; Djajanegara and Doyle, 1989). But the method of feeding urea and molasses to ruminants is an important factor to consider as both the ingredients are highly fermentable in the rumen and bring changes to rumen fermentation of straw (Perdok, 1987, Preston and Leng, 1987, Sudana and Leng,

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1986). The continuous infusion of urea solution in the rumen increased straw digestion (Perdok, 1987) or spraying on a straw before feeding increased feed intake and N-balances of animals (Romero et al., 1976). Based on this principle, lick blocks of urea, molasses and minerals are developed for supplementation of a straw diet and found responsive to milk and meat production (Preston and Leng, 1987; Leng, 1991; Kunju, 1986). But blocking of molasses and urea with other feed ingredients incurs costs of manufacturing and its preservation in a hot and humid climate like Bangladesh needs the inclusion of preservatives. Moreover, farmers used to heat the molasses mixing with the urea at a boiling point to achieve a desired level of block hardness which results in the formation of 4-methyl imidazole (4-Ime), a compound which affects the nervous system of animals (Wiggins, 1956; Perdok and Leng, 1987). However, Huque and Talukder (1994) found that feeding of 15% molasses and 3.0% urea as a complete mix with rice straw (termed as the UMS) significantly increased growth rates of growing bulls.

The present research works were undertaken for the nutritional evaluation of the UMS in terms of its intake and digestibility in growing bulls of the effect of molasses and urea supplementation on the reduction of methane production *in vitro* of a straw diet. Further, the feeding of UMS was compared with that of the feeding of urea and molasses in meals or in lick blocks as supplement to a straw in growing bulls in terms of intake and digestibility of feed nutrients, the rumen environment (pH, ammonia

nitrogen and cellulolytic activities), live weight (LW) changes and microbial synthesis in the rumen.

## MATERIALS AND METHODS

Experiments described here include both animal feeding trials evaluating supplementing effects or feeding systems of molasses and urea with straw in growing bulls and fermentation *in vitro* of straw alone or being supplemented with molasses and the urea for determining the difference in CH<sub>4</sub> production.

### Feeding trial (Experiment 1) :

Eight growing bulls (2.5 to 3.0 years of age and  $226 \pm 27.0$  kg LW), being divided into two equal groups, were fed either dry rice straw alone (S) or the 'S' intimately mixed with 3.0% urea and 15% molasses (DM basis) (UMS). The bulls were fed the two straws for a period of 21 days for an adjustment followed by a week of collection of feed, refusals, faeces and urine by transferring them to metabolic stalls. The straws were fed *ad lib* and the amount offered and refused were recorded daily. The samples of urine being mixed with a few drops of 1 M sulphuric acid (pH < 4.0) and faeces were stored in a refrigerator at  $-20^{\circ}\text{C}$ .

### *In vitro* fermentation trial :

Milling with 1.0 mm sieve, rice straw alone (S) or being supplemented with 3.0% urea and 15% molasses (UMS) was fermented with the rumen inoculum in an *in*

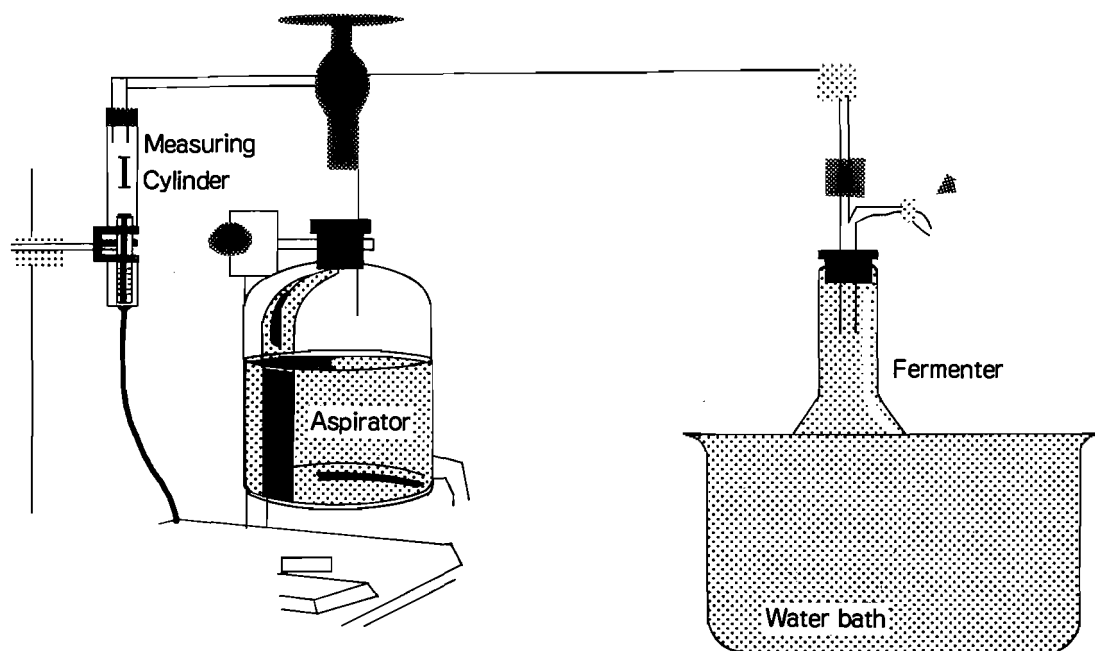


Figure 1. *In vitro* fermentation system for measuring methane production.

*in vitro* fermentation system shown in figure 1. The system was particularly designed to avoid air contaminations of gas produced during fermentation and/or collections. A 5 g sample of each diet was incubated with 100 ml strained rumen inoculum in glass bottles containing distilled water and McDougal's buffer at a ratio of 3:1 and kept in a water bath at 39°C. Two bottles, each of 1 L capacity were used to incubate the two diets in three separate days while a third bottle (control) was incubated without the substrate. Each incubation last for 24 hours and at the end total volumes of gas were recorded by replacing an equal volume of water contained in graduated gas collecting cylinders. A bottle was sealed by a rubber bung and was connected by a rubber tube to a three-way stopcock fitted on stoppers of an aspirator bottle completely filled with water. Thus, any gas produced in a fermentation bottle was transferred by pressure to the aspirator bottle connected with rubber tubes and water was pressed out to a reservoir through an out let at the bottom. The three-way stopcock was used for collecting and as well as transferring gas to the gas collecting cylinder. After collection the gas was transferred to a Orsat Fisher Gas Apparatus for the analyses of CH<sub>4</sub>. The gas produced by the inoculum itself (i.e. from the control) was measured and used for the correction of total gas production from the diet. The rumen inoculum was collected from three rumen cannulated animals fed a straw diet and distributed to the fermentation bottle using the system described by Huque (1991) to maintain anaerobiosis or temperature

required for the microbe. Before the incubation of the diet, the bottles containing water and buffer were flushed with CO<sub>2</sub> and warmed in the water bath at 39°C.

#### Feeding trial (Experiment 2) :

Twelve growing bulls of about 3.0 years of age and of 256 ± 44.0 kg LW were equally divided into three groups, two of them were fed dry straw *ad lib.* and the third group was fed with the UMS *ad lib.* A similar method to that described by Huque and Talukder (1994) was followed for the preparation of the UMS containing 82% straw, 15% molasses and 3.0% urea (DM basis). One of the straw groups (DSUMB) was given lick blocks of urea and molasses (Molasses 55.0%, urea 10.0%, rice bran 13.0 %, wheat bran 15.0%, calcium oxide 6.0% and Salt 1.0%) and the other (DS) was given the same amount of feed ingredients licked by the block group. The amount of calcium oxide and the urea was high enough for meal feeding. Hence daily 30 g/h of each of the ingredients was mixed thoroughly with the amount of molasses, brans and salt licked daily by the bulls of DSUMB group and fed in two meals in the morning and again in the evening. A similar amount of bran was given to the bulls fed the UMS in two meals. The daily amount of feeds fed to each animal is shown in table 1. The feeding trial was conducted for 93 days including a week for collecting feed, refusals, faeces and urine when the animals were transferred to metabolic stalls.

**Table 1.** Composition of diets fed to the bull (Expt. 2)

Feed items/Diets	Diets		
	Control	Block	Urea molasses straw (UMS)
Dry straw	<i>Ad lib.</i>	<i>Ad lib.</i>	—
UMS	—	—	<i>Ad lib.</i>
Block	—	Licking	—
Molasses	425.0	—	—
Wheat bran	200.0	—	200.0
Rice bran	100.0	—	100.0
Urea	30.0	—	—
Calcium Oxide	30.0	—	30.0
Salt	10.0	—	10.0

Diets	Dry matter (g · kg <sup>-1</sup> )	Chemical components (g · kg <sup>-1</sup> DM)			
		Organic matter	Ash	Crude protein	Acid detergent fibre
Dry straw	875	882	118	53.7	430
UMS	734	865	135	100	406
Block	812	824	176	374	64.2

The bulls were weighed weekly and the cumulative LWs were regressed against the total days of experimentation to calculate the daily gains of LWs. The daily LW gains of the bulls were regressed against their respective daily intake of nitrogen (N) to calculate their tissue N requirement. The local ambient temperature and humidity during the experimental period were collected from the national record.

#### *In Sacco* degradability trial :

The three diets (DS, DSUMB and UMS) of Experiment 2 were fed to three cannulated bulls assigning the diets to the bulls in three separate periods. Each period had an adjustment to diets of three weeks followed by a week of collection when rumen samples were collected at 0, 2, 4, 6, 9, 12, 15, 18, 21 hours of morning feed for pH and  $\text{NH}_3$  analyses and dacorn bags containing a test straw (washed, dried and milled at 4.0 mm) were incubated at 0, 8, 16, 24, 48 and 72 hours following the method of Ørskov et al. (1980) for determining rumen cellulolytic activities. Immediately after collection of the rumen samples, pH was measured by a digital pH meter and the same rumen sample was stored at  $-20^\circ\text{C}$  mixing with a few drops of 6N HCl for analyzing  $\text{NH}_3\text{-N}$  contents. The data of DM degradability of the test straw incubated in three different rumen environments created by the three diets were analyzed by the NAWAY computer programme of the exponential model described by McDonald (1981).

#### Chemical analyses :

Chemical analyses of feeds, refusals and faeces for dry

matter (DM), ash, organic matter (OM) and crude protein (CP) were done following the methods described by AOAC (1987) and acid detergent fibre (ADF) following the method described by Goering and van Soest (1970). The urine samples were analyzed for determining purine derivatives (from allantoin excretion + 15% correction for uric acid) to quantify microbial nitrogen (MN) yields in the rumen following the method described by Chen and Gomes (1992).

#### Statistical analyses :

The data on feed intake, digestibility and  $\text{CH}_4$  production *in vitro* were analyzed for determining significant differences between the two diets of Experiment 1 using a 'Student t-test'.

The data of Experiment 2 on feed intake, digestibility, microbial protein production and LW changes were analyzed in an ANOVA of a completely randomized design (CRD) for the significant difference among the three diets while their response differences to the rumen environment in terms of straw degradation, pH and  $\text{NH}_3\text{-N}$  were analyzed by an ANOVA of a  $3 \times 3$  Latin square design.

## RESULTS

#### Effect of molasses and urea feeding :

The mixing of urea and molasses with the straw gave a higher CP ( $84.0 \text{ g} \cdot \text{kg}^{-1}\text{DM}$ ) and a lower ADF ( $406 \text{ g} \cdot \text{kg}^{-1}\text{DM}$ ) in the UMS than that of the DS ( $49.3$  and  $474 \text{ g} \cdot \text{kg}^{-1}\text{DM}$ , respectively, table 2).

**Table 2.** Chemical composition of dry or the urea molasses straw (UMS)

Straws	Dry matter ( $\text{g} \cdot \text{kg}^{-1}$ )	Chemical composition ( $\text{g} \cdot \text{kg}^{-1}\text{DM}$ )			
		Ash	Organic matter (OM)	Crude protein (CP)	Acid detergent fibre (ADF)
Dry straw	883	187	813	49.3	474
UMS	702	209	791	84.0	406

Table 3 shows that the daily DM intake expressed either total ( $6.41 \text{ kg}$ ,  $p < 0.001$ ) or in  $\text{kgW}^{-0.75}$  ( $89.5 \text{ g}$ ,  $p < 0.01$ ) and total digestible CP (DCP) ( $333 \text{ g}$ ,  $p < 0.001$ ) or  $\text{DCP kg}^{-0.75}$  ( $5.74 \text{ g}$ ,  $p < 0.001$ ) and N balances ( $508 \text{ mg} \cdot \text{kg}^{-0.75}$ ,  $p < 0.01$ ) of the bulls fed the UMS were higher than those fed the 'S' ( $3.73 \text{ kg}$  or  $65.0 \text{ g} \cdot \text{kg}^{-0.75}$ ,  $55.0 \text{ g}$  or  $0.96 \text{ g} \cdot \text{kg}^{-0.75}$  and  $8.0 \text{ mg} \cdot \text{kg}^{-0.75}$ , respectively). Similarly, digestibility of DM, OM, ADF ( $59.4$ ,  $64.1$ ,  $77.3 \text{ g} \cdot \text{kg}^{-1}$ , respectively,  $p < 0.05$ ) and CP

( $61.9 \text{ g} \cdot \text{kg}^{-1}$ ,  $p < 0.001$ ) were higher than those of the 'S' ( $45.4$ ,  $53.9$ ,  $71.0$  and  $29.8 \text{ g} \cdot \text{kg}^{-1}$ , respectively). The same table shows that the total  $\text{CH}_4$  production in 24 h of fermentation *in vitro* of the UMS and the 'S' had no significant difference ( $231$  and  $229 \text{ ml}$ ) but conversion of the total gas volume in to a unit production per g of digestible organic matter apparently fermented in the rumen (DOMR) gave a significant ( $61.6$  and  $91.0 \text{ ml}$ ) difference.

**Table 3.** Nutrients intake and digestibility of the dry straw and the UMS fed to bulls and their methane production *in vitro*

Items	Diets		Significance	
	Dry straw	UMS	SED	Levels
Initial live Wts (kg)	225	228	27.7	NS
Daily intake :				
Total DM (kg)	3.73	6.41	0.33	p < 0.001
Straw DM ( $g \cdot kg^{-0.75}$ )	65.0	89.5	10.4	p < 0.01
DCP (g)	55.0	333	19.9	p < 0.001
DCP ( $g \cdot kg^{-0.75}$ )	0.96	5.74	0.53	p < 0.001
N balance ( $mg \cdot kg^{-0.75} \cdot d^{-1}$ )	8.00	508	96.4	p < 0.01
Digestibility ( $g \cdot kg^{-1}$ ) :				
Dry matter	454	594	4.30	p < 0.05
Organic matter	539	641	3.63	p < 0.05
Crude protein	298	619	4.09	p < 0.001
Acid detergent fibre	710	773	2.42	p < 0.05
Methane (Total ml)	229	231	12.6	NS
Methane ( $ml \cdot g^{-1}$ DOMR*)	91.0	61.6	4.81	p < 0.05

\* Organic matter apparently fermented in the rumen (ARC, 1984).

#### Comparisons of molasses and urea feeding systems :

Molasses and urea feeding as a complete mix with straw (UMS) significantly ( $p < 0.05$ ) increased total ( $88.7 g \cdot kg^{-0.75} \cdot d^{-1}$ ) and straw DM ( $1.77\%$ ,  $p < 0.01$ ) intake than their feeding in meals (DS,  $71.6 g \cdot kgW^{-0.75} \cdot d^{-1}$  and  $1.52\%$ , respectively) or blocking with other feeds (DSUMB,  $71.9 g \cdot kgW^{-0.75} \cdot d^{-1}$  and  $1.55\%$ , respectively) (table 4). The intake of DOM ( $2.61 kg \cdot d^{-1}$ ), estimated

ME ( $41.2 MJ \cdot d^{-1}$ ) and DCP ( $341 g \cdot d^{-1}$ ) of the UMS was significantly ( $p < 0.05$ ) higher than those of the DS ( $2.05 kg \cdot d^{-1}$ ,  $32.4 MJ \cdot d^{-1}$  and  $163 g \cdot d^{-1}$ , respectively) but the difference with that of the DSUMB ( $2.16 kg \cdot d^{-1}$ ,  $34.1 MJ \cdot d^{-1}$  and  $279 g \cdot d^{-1}$ , respectively) were not significant. The UMS had a higher ADF digestibility ( $516 g \cdot kg^{-1}$ ,  $p < 0.05$ ) than that of the DS ( $472 g \cdot kg^{-1}$ ) or the DSUMB ( $490 g \cdot kg^{-1}$ ), but its DM, OM and CP digestibility had no significant

**Table 4.** Daily intakes of total (TDM) and straw dry matter (SDM), digestible organic matter (DOM), estimated metabolizable energy (ME), digestible crude protein (DCP) and digestibility of DM, OM, CP and acid detergent fibre (ADF) of the diets

Items	Diets			Significance	
	DS	DSUMB	UMS	SED	Level
Daily intake :					
Total dry matter (kg)	4.68	4.66	6.03	0.93	NS
Total DM ( $g \cdot kg^{-0.75}$ )	71.6	71.9	88.7	5.22	p < 0.05
Straw DM (kg)	4.01	3.95	4.65	0.18	p < 0.05
Straw DM (%LW)	1.52	1.55	1.77	0.03	p < 0.01
Digestible OM (kg)	2.05	2.16	2.61	0.21	p < 0.05
Estimated ME* (MJ)	32.4	34.1	41.2	3.32	p < 0.05
Digestible CP (g)	163	279	341	35.3	p < 0.05
Digestibility ( $g \cdot kg^{-0.75}$ ) :					
Dry matter	459	474	453	19.9	NS
Organic matter	513	530	502	34.5	NS
Crude protein	438	532	549	66.0	NS
Acid detergent fibre	472	490	516	12.9	p < 0.05

\* Estimated as  $1.0 kg$  of DOM =  $15.8 MJ$  ME (Kearl, 1982).

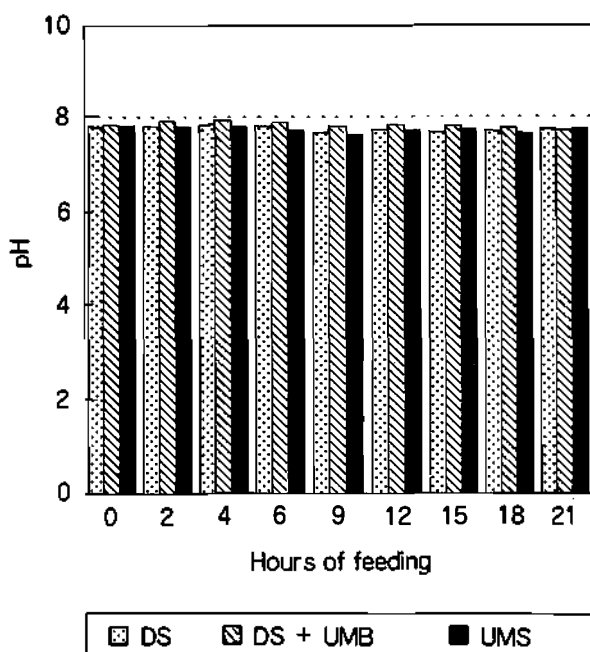
differences ( $p > 0.05$ ) with the other diets (table 4). The feeding of lick blocks of molasses and urea as supplement to straw had no significant difference with their feeding in meals (DS) in terms of total and straw DM intake and digestibilities of DM, OM and ADF. However, it gave a higher ( $p < 0.05$ ) intake and digestibility of CP than the DS ( $279 \text{ g} \cdot \text{d}^{-1}$  and  $532 \text{ g} \cdot \text{kg}^{-1}$ ).

Table 5 shows that the feeding systems of molasses

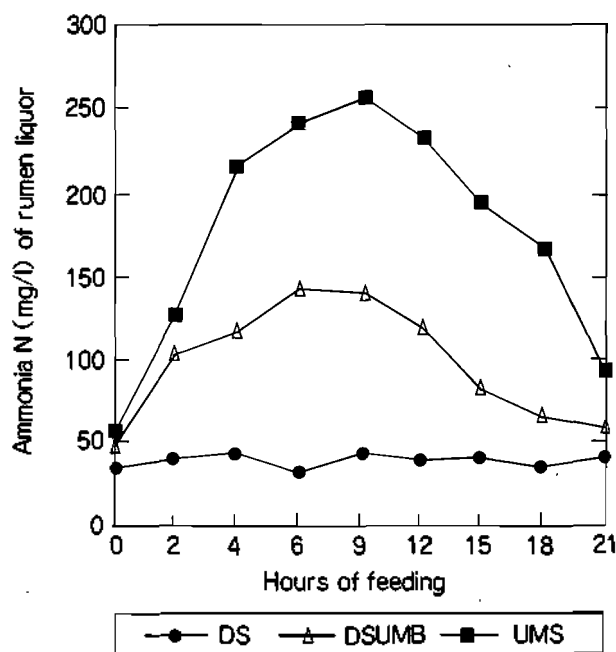
and urea as supplements to the straw had no significant ( $p > 0.05$ ) effects in the rumen environment in terms of straw digestion characteristics and pH but the rumen  $\text{NH}_3\text{-N}$  was higher ( $p < 0.001$ ) in the UMS ( $173 \text{ mg} \cdot \text{L}^{-1}$ ) followed by the DSUMB ( $101 \text{ mg} \cdot \text{L}^{-1}$ ) than the DS ( $38.2 \text{ mg} \cdot \text{L}^{-1}$ ). The diurnal changes of pH and the rumen  $\text{NH}_3\text{-N}$  are shown in figure 2a and 2b. The rumen pH of the diets varied from 7.49 to 8.13.

**Table 5.** Straw dry matter degradation characteristics, pH and  $\text{NH}_3\text{-N}$  concentrations in the rumen of the diets

Items	Diets			Significance	
	DS	DSUMB	UMS	SED	Level
Soluble fraction (A, $\text{g} \cdot \text{kg}^{-1}$ )	-16.0	-22.9	-37.7	23.9	NS
Digestion rate (C, $\% \cdot \text{h}^{-1}$ )	1.69	2.29	2.36	0.65	NS
Potential digestible fraction 'B' ( $\text{g} \cdot \text{kg}^{-1}$ )	624	516	633	80.7	NS
Extent of Digestion (A + B, $\text{g} \cdot \text{kg}^{-1}$ )	608	493	593	97.7	NS
DM degradation at 24 h ( $\text{g} \cdot \text{kg}^{-1}$ )	188	209	208	21.8	NS
DM degradation at 48 h ( $\text{g} \cdot \text{kg}^{-1}$ )	329	327	340	33.2	NS
Rumen pH	7.73	7.80	7.71	0.12	NS
Rumen $\text{NH}_3\text{-N}$ ( $\text{mg} \cdot \text{L}^{-1}$ )	38.2	101	173	22.1	$p < 0.001$



**Figure 2a.** Diurnal changes of the rumen pH in response to feeding a diet of straw supplemented with molasses and urea in meals (DS) or in blocks (DSUMB) or as a complete mix with straw (UMS).



**Figure 2b.** Diurnal changes of the rumen ammonia in response to feeding a diet of straw supplemented with molasses and urea in meals (DS) or in blocks (DSUMB) or as a complete mix with straw (UMS).

Table 6 shows that the UMS and DSUMB gave a significantly ( $p < 0.001$ ) higher total MN yield in the rumen (each gave  $23.8 \text{ g} \cdot \text{d}^{-1}$ ) or the yield per kilo

digestible organic matter apparently fermented in the rumen ( $14.1$  and  $17.0 \text{ g} \cdot \text{kg}^{-1}$  DOMR, respectively) than that of the DS ( $7.29 \text{ g} \cdot \text{d}^{-1}$  or  $5.62 \text{ g} \cdot \text{kg}^{-1}$  DOMR) in

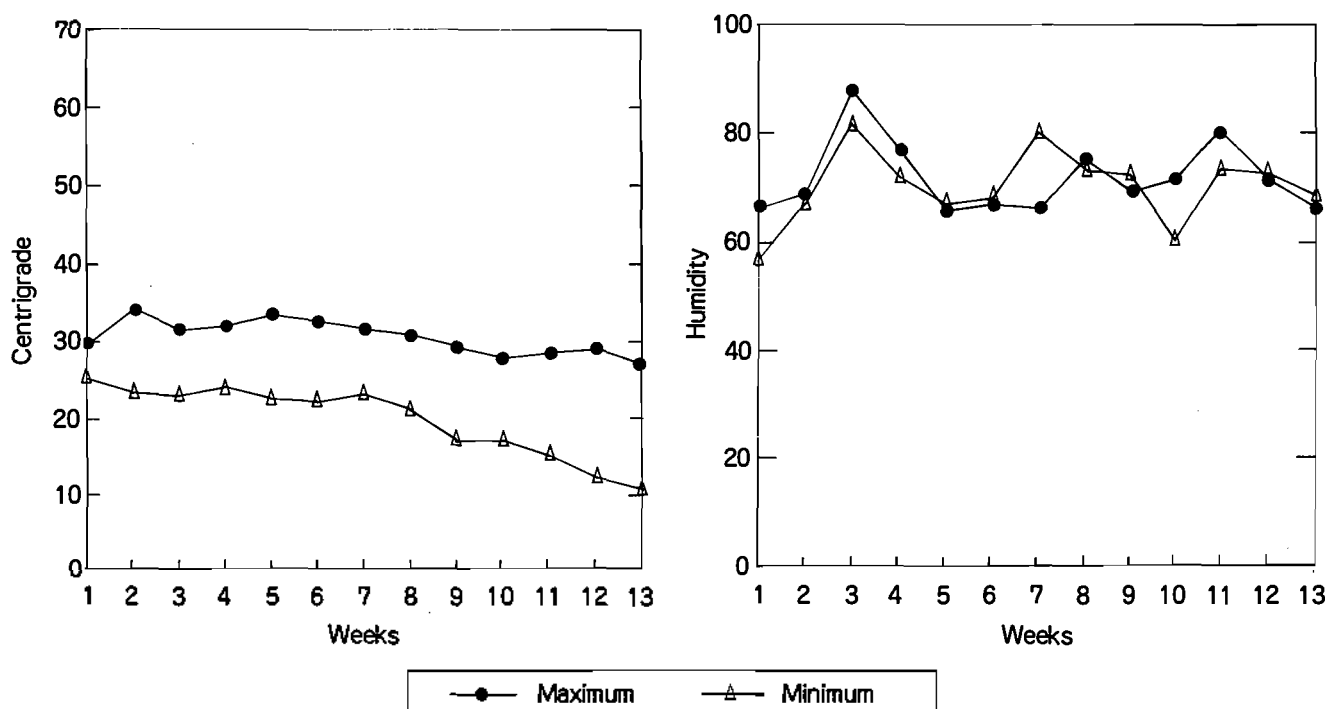
the experimental period of 93 days. The bulls fed the UMS gave the highest LW gains ( $233 \text{ g} \cdot \text{d}^{-1}$ ) followed by the DS ( $125 \text{ g} \cdot \text{d}^{-1}$ ) and the former differed significantly ( $p < 0.05$ ) from that of the DSUMB ( $93.3 \text{ g} \cdot \text{d}^{-1}$ ). The LW gains of the bulls were related to their daily N intakes ( $r = 0.48$ ) and the relationship may be expressed by the equation of  $Y = -56.5 + 0.171X$ , where  $Y = \text{LW gains (g} \cdot \text{d}^{-1}\text{)}$  and  $X = \text{N intake (mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}\text{)}$ . Therefore, N intake of zero LW gain calculated to be  $330 \text{ mg} \cdot \text{kgW}^{-0.75} \cdot \text{d}^{-1}$  which is essentially the tissue maintenance requirement of the

experimental bulls. The feed conversion ratio of the UMS (26.0) was higher ( $p > 0.05$ ) than that of the DS (56.1) or the DSUMB (57.6). Table 6 shows that during the first 45 days of the experimental period, the bulls fed the three diets (DS, DSUMB and UMS) had LW gains higher (279, 146 and  $458 \text{ g} \cdot \text{d}^{-1}$ , respectively) than that found at the last 48 days (201, 110 and  $232 \text{ g} \cdot \text{d}^{-1}$ , respectively). It was observed that the ambient temperature and humidity in the first half ranged from  $35^\circ\text{C}$  to  $20^\circ\text{C}$  and 88% to 66%, respectively and in the last half  $29^\circ\text{C}$  to  $10^\circ\text{C}$  and 79% to 60%, respectively (figure 3).

**Table 6.** Microbial nitrogen (MN) yields in the rumen and Liveweight (LW) gains and feed conversion ratios (FCR) of the bulls

Items	Diets			Significance	
	DS	DSUMB	UMS	SED	Level
Initial LWs (kg)	248	259	263	43.5	NS
LW Changes ( $\text{g} \cdot \text{d}^{-1}$ ):					
Overall for 93 days	125	93.3	233	51.8	$p < 0.05$
First 45 days	279	146	458	98.0	$p < 0.05$
Last 48 days	201	110	232	158	NS
MN yield ( $\text{g} \cdot \text{kg}^{-1}$ )	7.27	23.8	23.8	3.20	$p < 0.001$
MN yield ( $\text{g} \cdot \text{kg}^{-1} \text{ DOMR}^*$ )	5.62	17.0	14.1	2.03	$p < 0.05$
Feed conversion ratio (FCR, g feed/g LW gain)	56.1	57.6	26.0	22.8	NS

DOMR means DOM apparently fermented in the rumen which is  $\text{DOM} \times 0.65$  (ARC, 1984).



**Figure 3.** Weekly maximum and minimum temperature and humidity.

## DISCUSSIONS

The mixing of straw with urea increased the CP level in it (84.0 and 100 g · kg<sup>-1</sup> DM for the UMS of Experiment 1 and Experiment 2, respectively) but the level was lower than the expected CP content (131 and 135 g · kg<sup>-1</sup>DM, respectively). This might have resulted in due to the loss of NH<sub>3</sub>-N during the storage time which did not exceed three days. The replacement of straw by molasses decreased the ADF content of the UMS (table 1 and 2).

### Experiment 1 :

#### Straw intake, digestibility and the rumen environment :

Feeding of molasses and urea as an intimate mix with straw (UMS) increased its ADF digestibility in both Experiment 1 and Experiment 2, which confirm our earlier observations (Huque and Talukder, 1994). Usually, readily fermentable carbohydrate supplementation at a higher rate to roughage replaces fibre intake or depresses its digestibility (Mould, 1982, Fahmy et al., 1984). The factors responsible for them were described as the lower rumen pH than a critical level (< 6.2) for fibre digestion, termed as pH effect and/or the presence of RFC in the rumen, termed as carbohydrate effect (Mould 1982). In addition, the rumen fill effect of supplementary feeds can not be ruled out. The complete mixing of molasses and urea with the straw did not show any such building up pH or carbohydrate effect in the rumen, instead, it increased straw intake and its digestibility in the whole gut. How molasses and urea solution as a mix with straw gave the above nutritional effects to the animals may be explained by the fact that it increased nitrogen and energy availability to the micro colony of bacterial cells attached to fibres or in the fluid. Their availability in the rumen of a dry straw is limited. The UMS prepared was fed from the day of preparation and never kept reserve for more than 3 days. During this time period ammonification from the urea in a moisture content of 25 to 30% may have brought some chemical changes in the straw. Waiss et al. (1972) stated that optimal effect of ammonia treatment was obtained at a moisture content of about 30%. Bergner et al. (1994) found that the *in vitro* digestibility of wheat straw after treatment with urea-saccharose mixture was found highest with a water content of 30 to 40% in a storage period of 3 to 7 days. The higher fibre digestibility in the whole gut in the present study was an important factor to consider the increased intake of straw but the presence of molasses in it may have resulted in a greater outflow rate of the rumen contents. Reyes (1974)

found that the rumen retention time of digesta was decreased linearly with the increase of molasses levels in the diet. Huque (1992) and Gomes et al. (1994) found that the rumen outflow rates of the liquid or solid digesta were increased as a result of starch supplementation to straws. Both molasses and the urea are highly digestible in the rumen which is resulted in the increased intake and digestibility of CP and the total DM. The higher urea intake (192 g · d<sup>-1</sup>), but of course in a slower rate as it was mixed with straw, increased the DCP intake or the N balance of the bulls fed the UMS. Romero et al. (1976) found that the spraying of 50 g urea significantly increased DOM intake and N balances of sheep than the control (without urea) or the feeding of 100 g urea in every second day or the 50 g urea once in a day or the 25 g urea twice a day.

#### Methane production *in vitro* :

A lower CH<sub>4</sub> production by the fermentation *in vitro* of the UMS than the 'S' may be a result of a combination of biochemical factors. The UMS gave a higher MN yield in the rumen (table 6) and this may provide an evidence of a similar response to the yield of MN due to fermentation *in vitro* of the diets. As the efficiency of cell syntheses increases methane production and heat of fermentation decreases (Leng, 1982). Reyes (1974) found that molasses feeding favoured propionate and butyrate production. They are conducive to lower CH<sub>4</sub> production (Demeyer and van Nevel, 1975). However, the volume of CH<sub>4</sub> produced in the 'S' and the UMS (91.0 and 61.6 L · kg<sup>-1</sup> DOMR) was much higher than that found by Moss et al. (1994) from feeding urea treated oat, wheat, or barley straw (46.1, 37.0 or 50.0 L · kg<sup>-1</sup> DOMR, respectively) to sheep. In addition to straw difference with the present experiment (rice straw was used), fermentation *in vitro* of the diet in a closed system may have added some CH<sub>4</sub> from the accumulated end products, specially, acetate (CH<sub>3</sub>CO<sub>2</sub>H ⇒ CH<sub>4</sub> + CO<sub>2</sub>) and methanol (4CH<sub>3</sub>OH ⇒ 3CH<sub>4</sub> + CO<sub>2</sub> + H<sub>2</sub>O) to the main stream of CH<sub>4</sub> production from CO<sub>2</sub> and H<sub>2</sub> (CO<sub>2</sub> + 4H<sub>2</sub> ⇒ CH<sub>4</sub> + 2H<sub>2</sub>O, Hungate, 1967). On the contrary, the rumen is a dynamic system and its outflow of digesta to some extent restrict CH<sub>4</sub> production in it. As methanogens are slow growing bacteria, the reduced rumen retention time may influence their growths (Preston, 1972).

#### Feeding systems of molasses and urea

The response of feeding the UMS to a higher total DM and straw intake and fibre digestibility confirmed the previous result. Nevertheless, the UMS feeding did not show any changes in the rumen pH or straw degradation



characteristics from that of the DS or DSUMB. The calculated daily urea intake of the bulls fed the UMS was about 180 g and thus the diet maintained diurnal changes of rumen  $\text{NH}_3\text{-N}$  higher than the others (figure 2b). The rumen  $\text{NH}_3\text{-N}$  concentration of the UMS (53.0 to 256 mg  $\text{N.L}^{-1}$ ) was in the optimal range for maximum straw degradation (see Perdok, 1987; Hume et al., 1970, Mehreze et al., 1977; Kang-Meznarich and Broderick, 1981). However, despite of the increased whole gut fibre digestibility of the UMS no positive response on the rumen straw degradation was found. The dacron bag technique used to measure rumen cellulolytic activities in response to feeding different diets may not be sensitive enough to detect the differences. Mgheni et al. (1994) also raised a similar question on the sensitivities of the rumen parameters, such as, straw degradation characteristics to detect the difference in rumen environments in response to feeding different diets, specially, the rumen cellulolytic activity.

The bulls fed the DS or DSUMB had a similar intake of nutrients except the urea, which was restricted to 30 g in two meals daily for the former. The latter had a daily urea intake of about 75 g, but of course, through lick blocks and the difference of the urea intake is been reflected on the  $\text{NH}_3\text{-N}$  levels found in the rumen (figure 2b). Schiere et al. (1989) or Badrudeen et al. (1994) found no positive response of feeding lick blocks to straw intake and digestibility. Nevertheless, the absence of positive effect of lick block feeding on straw intake and digestibility does not agree with the result of Sudana and Leng (1986) or Preston and Leng (1987), who fed lick blocks on the top of a basal diet of straw alone or straw and other feeds. Thus, a difference resulted in the supply of nutrients to their animals.

#### Microbial nitrogen (MN) production :

The diet DS failed to give an optimum rumen  $\text{NH}_3\text{-N}$  (50 to 100 mg  $\cdot \text{L}^{-1}$ , Kang-Meznarich and Broderick, 1981; Pisulewski et al., 1981) for the maximum MN production in the rumen due to the restricted amount of urea feeding (30 g  $\cdot \text{d}^{-1}$ ). The urea is highly fermentable in the rumen and the peaks resulted from the meal feeding of it with the DS possibly could not be detected (figure 2b). Similarly, as a highly fermentable carbohydrate, molasses supply was not regular in the DS like the DSUMB or the UMS. This interrupted supply of molasses and urea gave a lower MN production in the DS. The MN production in the rumen depends on the availability of  $\text{NH}_3\text{-N}$ , readily fermentable carbohydrate (Maeng and Baldwin, 1976) and some minerals (Preston and Leng, 1987). The MN production in the rumen of the DSUMB

or the UMS was lower than the figure adopted by ARC (1984) which is 30 g  $\text{N} \cdot \text{kg}^{-1}$  DOMR. However, the OM of microbial origin was not considered to correct the total OM digestibility calculation by the ARC (1984). The MN production in the rumen affect OM digestibility i.e., the higher the MN production the lower the calculation of OM digestibility in the gut. The lower OM digestibility calculation will give a higher value of MN expressed in per kilo DOMR. The factor 0.65 used for calculating the fraction of OM apparently digested in the rumen may not be correct in case of the present study, as most of the urea and molasses fraction of the diet may have been fermented in the rumen.

#### Liveweight gains of bulls :

The bulls fed the DS had a higher ( $p > 0.05$ ) LW gains (125 g  $\cdot \text{d}^{-1}$ ) than those fed the DSUMB (93.3 g  $\cdot \text{d}^{-1}$ ). The former was deficient in the supply of ME, DCP or rumen degradable nitrogen (RDN) (calculated as 32.4 MJ, 163 g  $\cdot \text{d}^{-1}$  or 31.2 g  $\cdot \text{d}^{-1}$ , respectively) than their respective requirements for maintenance and growth (35.0 MJ, 180 g  $\cdot \text{d}^{-1}$  or 51.8 g  $\cdot \text{d}^{-1}$ , respectively; ME and RDN were calculated according to ARC, 1984 and DCP was calculated according to Kearn, 1982). The bulls fed the DSUMB daily ate about 34.1 MJ ME, 279 g DCP or 51.8 g RDN, and except ME the supply of others were higher than their requirements [calculated daily ME and RDN requirements were 35.0 MJ and 44.0 g, ARC (1984) and DCP was 180 g, Kearn, (1982)]. The lower LW gain of the bulls fed the DSUMB can not be explained by the difference of nutrients intake between the two diets, as their actual requirement is not known at the present condition. However, during the block preparation, molasses was heated above 70°C in the presence of urea and thus, 4-methyl imidazole (4Me-I) may form in blocks which caused hyperexcitability in cattle (Tillman et al., 1957; Mogan and Edwards, 1986). The bulls daily ate about 600 to 750 g of block which did not cause any hyperexcitation in them but could have an effect on the availability of minerals in the body, specially Ca and Mg, which form chelates with the 4-MeI. Vosloo (1985) stated that Ca and Mg may form chelates with the 4-MeI formed during heating molasses and urea. Moreover, in the present study lime was added at the time of heating. Farmers used to heat molasses in the presence of urea and lime to get an optimum hardness of blocks, especially, in a humid condition. A similar method of preparation of blocks was followed by Ghebrehwet et al. (1994) in Bhutan. Thus, heating of molasses with urea need to be avoided for block preparation.

The continuous and slow eating of the UMS supplied

a higher amount of molasses and urea ( $\text{NH}_3\text{-N}$  levels, figure 2b) than the other diets to both the rumen microbes (increased MN yield,  $23.8 \text{ g.d}^{-1}$ ) and the host without having any nonadditivity of feeds in the rumen (straw degradation characteristics were not affected). Thus, the continuous eating of molasses and urea mixed straw (UMS) may have synchronized the supply of energy and amino acids of microbial cells digested in and absorbed from the lower gut to tissue levels and might have helped increased growth efficiencies and FCR of the host animal. The daily requirement (maintenance and growth) of ME and RDN according to ARC (1984) and DCP according to Kears (1982) of the experimental bulls was 40.0 MJ, 329 g and 50.0 g, respectively. But, their supply was 41.2 MJ, 341 and 100 g, respectively. It shows that the supply of ME from the diet met its requirements by the bulls but the supply of RDN or the DCP was higher. However, the calculation of N requirement in terms of DCP does not tell anything about its requirement in the rumen for microbial synthesis or undegradable protein requirement in the lower gut. The ARC (1984) system of nitrogen requirement calculation, on the other hand, is done according to its requirement in the rumen or in the post rumen and is based on the supply of energy to animals. Factors related to maximization of microbial synthesis or cellulolytic activities in the rumen need to be taken in to consideration while calculating nitrogen and energy requirements of ruminant animals, especially, in a poor quality diet. However, the N required for tissue maintenance of the bulls of the present experiment was calculated as  $330 \text{ mg N.kg}^{-0.75}.\text{d}^{-1}$ . The mean value of tissue maintenance requirement of N adopted by ARC (1984) was  $350 \text{ mg N.kg}^{-0.75}.\text{d}^{-1}$ .

#### Observations on live weight changes as affected by the weather :

The animals were kept in an open house and the wind flow (not recorded) in addition to a low temperature at the last half of the experimental period affected them with an intense cold. The slower LW gain with the decrease of ambient temperature may be explained by the fact that the animals used a part of their available nutrients to maintain their body temperature. Blaxter (1962) showed that ruminants oxidize acetate, butyrate and long chain fatty acids (fats) for heat production in cold-stressed. This means, under the present experimental condition, more oxidizable substrates were available for tissue accretion during the first half (warm weather) than the last half (cold weather) of the trial. This may be the possible reason for the difference in LW gains of the bulls between the two halves. Nevertheless, the low temperature found in

the present condition may not be a factor for the animal of temperate zones.

It can be concluded that molasses and urea feeding as an intimate mix with straw (UMS) increased its digestion and intake in association with a reduced methane emissions in the rumen. When compared with that of their feeding in meals or in lick blocks as supplements to straw, the UMS gave the highest straw intake and digestion and live weight gains of growing bulls concurring the finding that the UMS system may be the best way of molasses and urea feeding to ruminants fed straws.

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