

**Comparison of feeding straw supplemented molasses,  
urea and rice gruel on the production performance of  
sheep**



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**A thesis submitted in the partial fulfillment of the requirements for the degree of  
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**DECEMBER 2016**

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**December 2016**

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***DEDICATED TO MY BELOVED  
PARENTS***

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## List of Abbreviations

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<b>Abbreviation</b>	<b>Elaboration</b>
<b>%</b>	Percentage
<b>***</b>	Significant At 0.1% Level Of Probability
<b>**</b>	Significant At 1% Level Of Probability
<b>*</b>	Significant At 5% Level Of Probability
<b>Ad Lib.</b>	Ad Libitum
<b>CP</b>	Crude Protein
<b>CF</b>	Crude Fibre
<b>DM</b>	Dry Matter
<b>DMB</b>	Dry Matter Basis
<b>OM</b>	Organic Matter
<b>NFE</b>	Nitrogen Free Extract
<b>EE</b>	Ether Extract
<b>HACCP</b>	Hazard Analysis And Critical Control Point
<b>Hb</b>	Hemoglobin
<b>TEC</b>	Total Erythrocyte Count
<b>TLC</b>	Total Leukocyte Count
<b>PCV</b>	Packed Cell Volume
<b>Kg</b>	Kilogram
<b>UMS</b>	Urea Molasses Straw
<b>URS</b>	Urea Rice Gruel Straw
<b>UMB</b>	Urea Molasses Block
<b>MUMB</b>	Medicated Urea Molasses Block
<b>SRL</b>	Stained Rumen Liquor
<b>SE</b>	Standard Error
<b>Sig.</b>	Significance
<b>Thou.</b>	Thousand
<b>mill.</b>	Million

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## Abstract

The experiment was conducted to study the comparison of feeding straw supplemented molasses, urea and rice gruel on the production performance of sheep. There were three dietary treatment groups such as, control diet (Concentrate feed and straw), 3% urea +15% molasses and 3% urea +15% rice gruel in diets of T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> respectively. Twelve indigenous sheep of about fourteen months of age with an average body weight of 15.14±0.07 kg were distributed into three treatment groups. The feeding trial continued for 10 weeks. Green grass and concentrate mixture was offered on the basis of dry matter requirement of the experimental sheep. From this experiment it was found that final body weight of different weeks differed significantly among treatment groups where highest value of body weight was found in T<sub>1</sub> (UMS) group. It was also found that 1<sup>st</sup> to 9<sup>th</sup> weeks of body weight gain differed significantly (P<0.001) among the treatment groups where highest value of weight gain was found in T<sub>1</sub> (UMS) group. Nutrient digestibility of DM and EE were found significantly increased in T<sub>1</sub> (UMS) group, while the highest value of CP, CF, Ash and NFE were found in T<sub>0</sub> (Control) group. Hematological parameters like Hb and PCV were significantly increased in T<sub>1</sub> (UMS) group. Glucose and cholesterol level in blood were significantly (P<0.01) increased in T<sub>0</sub> (Control) and T<sub>2</sub> (URS) groups respectively. It was found that blood level of total protein and albumin were significantly (P<0.05) differed among different treatment groups where highest value of total protein and albumin were found in T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups respectively and lowest was in T<sub>0</sub> (Control) group. K and P were significantly increased in T<sub>1</sub> (UMS) group whereas Ca, Mg, Cl and Na were significantly increased in T<sub>2</sub> (URS) and T<sub>0</sub> (Control) groups respectively. The pH of the rumen liquor peaked at 8h post feeding and lowest pH value was attained at 4h post-feeding for all the treatment groups. The bacterial count in 4h pre-feeding was significantly (p< 0.05) higher in T<sub>1</sub> (UMS) group. The protozoal count at different hours of post-feeding differed significantly (p<0.01) among all treatment groups and attained to peak at 4h of post-feeding for all and decreased to lowest value at 4h of pre-feeding for T<sub>1</sub>(UMS) and T<sub>2</sub> (URS) and 0h of post-feeding for T<sub>0</sub> (Control) group. The study showed that rice straw supplemented with urea and rice gruel could efficiently be added to sheep ration to increase the performance of the animals.

**Keywords:** Rice straw, rice gruel, molasses, urea, weight gain and blood metabolites.

## **Chapter-1: Introduction**

Livestock play a pivotal role in the economy of Bangladesh. Livestock are integral component of agriculture in Bangladesh and make multifaceted contributions to the growth and development in the agricultural sectors. The livestock resources of Bangladesh are mainly based on cattle, goat, sheep, buffalo and poultry. Rearing of small ruminants plays a very important role in the lives of households in developing countries. This is because small ruminants provide the easiest and most readily accessible source of credit available to meet immediate social and financial obligations. During the last twelve years sheep population increased 2.5 times, with annual growth rate of 5% (BBS, 2008). Although the growth of livestock production is the second highest among all other sub-sector of agriculture in Bangladesh, the production and consumption of livestock products is still much lower in comparison with other countries. The productivity of livestock is low even not adequate to meet demand of the people. The rearing of sheep and goats provides a small yet significant supply of animal protein in the form of milk and meat. In our country, rural women are involved in the raising or rearing of small ruminants – sheep and goats especially around homes by feeding them kitchen wastes or at most times leaving them to graze on surrounding herbs and shrubs. But the feed is the most expensive input within any livestock production system which accounts for 60-70% of the total production cost. Since there is scarcity of lands in Bangladesh, the production and availability of livestock feed is very less than the demand and therefore the price is high. So rice straw is mostly used of livestock feed due to its availability throughout the year as rice based agriculture of Bangladesh. Livestock feed provides the basic nutrients required for animal production, including energy, protein & amino acid as macro nutrients, as well as minerals, vitamins and other micro nutrients.

Moreover, rice straw is the main energy source for ruminants comprising over 60% of the dietary energy supply in Bangladesh (Jackson, 1981). But it is clearly demonstrated that the lower level of readily fermentable nitrogen and energy for the rumen and volatile fatty acids and amino acids for the animal provided by the rice straw are primary limitations to ruminant production in this country. It is common practice to add urea and molasses to rice straw based diet to up-grade the quality of the feed (Huque and Talukder, 1994; Chowdhury and Huque, 1998). This practice is

not always possible due to poor distribution channel and high cost of molasses. Furthermore, supplementation of other high energy source is impractical under Bangladesh condition. However, almost every household in the country produces considerable amounts of rice gruel, which is produced during the cooking of rice, containing considerable amounts of soluble starch materials. Traditionally, rice gruel is being used in the cattle diet as a drink with water. It could be a good source of fermentable energy when impregnated with straw for the rumen microbes (Chowdhury and Huque, 1998).

Considering the above discussions in mind, the current study was designed having aim to evaluate the effect of rice gruel as a source of readily fermentable energy for a urea supplemented straw with locally available ration sources in terms of nutrient digestibility and growth rate of native sheep.

**Objectives of the study:**

- a) To evaluate the effect of urea and rice gruel supplement with straw based diet on growth performance and nutrient digestibility in sheep.
- b) To identify the effect of urea and rice gruel supplement with straw based diet on hematological and biochemical parameters in sheep.
- c) To determine the effect of urea and rice gruel on rumen physiology and environmental of sheep.
- d) To investigate the possibility of using rice gruel compared to that of the molasses as a source of readily fermentable energy for a urea supplemented straw based diet in sheep.

## **Chapter-2: Review of literature**

Appropriate nutrition and feeding are the most important factors affecting current productivity of sheep. However, attentions to these particular factors do not appear to get the emphasis they deserve. This is reflected by the continuing low per animal performance. Inadequate attention to the nutrition of sheep is also compounded by problems concerned with the availability of feed resources. Research in this country on various production characteristics of this species is lacking. This part also focused about the previous works on effects of UMS and URS supplementation on different species. This chapter also discuss about the different research on performances of different species.

### **2.1. Rice straw**

Rice is one of the major cereal crops of the world and it is produced mainly in South and South-East Asia. Rice production produces 330 million metric tons residues which are the largest crop residues among all the cereal crops (Van Soest, 2006).The digestibility and protein level of rice straw is low so the production of animals consuming rice straw as the main feed is also low.

Rice straw is unique relative to other cereal straws because it contains low lignin and high amount of silica (Jackson, 1977; Van Soest, 2006). Ammonia treatment does not remove silica, but greatly damages the cuticular layer allowing access by rumen bacteria (Ha et al., 1993) for their ultimate digestion. Straw quality in terms of lignin and silica contents, nutritive value and digestibility varies according to their varieties and location (Singh and Singh, 1995). Agbagla-Dohnami et al. (2001) observed that, in European varieties of rice straw, lignin is higher and silica is lower. So it would help to collect straw from South Asia to observe their digestibility by using novel supplement.

The level of phosphorus in rice straw is less than the level that the animals need for their growth and normal fertility (Jackson, 1977). Some sample of rice straw had shown positive balance for calcium (Nath et al., 1969) and some shown negative balance (Negi,1971).Rice straw collected from India had been reported for low cobalt content (Dube, 1964).

Phenolic compounds play an important role in ruminant nutrition. There is not that much data available for phenolic compounds in rice straw; however, (Vadiveloo and Fadel, 1992) reported 90g/kg ytterbium precipitable phenolics in rice straw. Van soest (2006) suggested that more study is needed to explore the soluble phenolic composition of rice straw.

## **2.2. Rice gruel and molasses**

Rice gruel feeding is common in East coast of India mainly Andhra Pradesh, Tamilnadu and West Bengal (Rao et al., 1995). In Bangladesh rice gruel has been used as a traditional supplement for milking dairy cattle and fattening beef cattle (FAO, 1999). A survey on rice gruel feeding practice to dairy cattle among farmers of North-Eastern agro climatic zone of Tamil Nadu indicated that majority of farmers are feeding gruel supplemented with rice bran (Suresh et al., 2016). Grazing or roughage feeding supplemented with rice gruel is a common practice among farmers who own low yielding dairy cattle, sheep, goat and buffalo etc. Das and Tripathi (2008) had reported that install fed cattle / buffaloes in Sundarbans delta, it is common to feed paddy straw along with rice gruel, rice washed water, rice bran and kitchen waste. Rao et al. (1995) also had reported feeding of rice washing, gruel, gram husk, rice bran, vegetable scraps, excess rice etc., in states of Andhra Pradesh, Tamil Nadu and West Bengal.

Suresh et al. (2016) had reported starch / energy content of the rice gruel was highly variable due to various factors such as dilution with water, addition of rice washings, addition of vegetable scraps, addition of excess rice and variability in the energy content of the gruel due to various cooking methods, which warrants the standardization of the energy content of the gruel. Creating an intervention in the existing feeding pattern (rice gruel) will be more useful rather than introducing a new method of feeding livestock.

Molasses is a term applied to a variety of by-product feeds derived from sugar-rich crops. The most appropriate role for small amounts of molasses in ruminant diets is as a vehicle for other nutrients (e.g. urea and minerals). A drought feeding strategy based on the use of liquid molasses supplements containing from 8 to 10 percent urea is now an established practice in Australia (Nicol et al., 1984) and has been introduced



successfully in Africa (Preston and Leng, 1986). The incorporation of urea and other nutrients in molasses-based (multi-nutritional) blocks promises to be an even more attractive technology, especially for smallholder-village farmers, for supplementation of locally available crop residues which are of low digestibility and also deficient in fermentable nitrogen (Leng and Preston, 1984; Sansoucy , 1986).

### **2.3. Urea treatment**

Urea treatment has been promoted in South and South-East Asia like India, Srilanka, Bangladesh, Indonesia etc. (Singh and Schiere, 1995). Most of the papers presented data on urea erroneously regard urea as a source of ammonia. Urea treatment also increases the CP level of animal feed. Urea treatment became popular for large farm levels in South and South-East Asia but its uptake at small farm level in village area is slow. Excess Urea can produce toxicity to animal. Completion of urea treatment requires 2 to 3 weeks in tropical area. Moreover it requires more labour, time and space which limit its farm scale application to upgrade low quality forages.

### **2.4. Animal feeding and growth performances**

Can et al. (2004) had evaluated the effect of different levels of urea and molasses liquid supplement on nutrient intake, digestibility and rumen parameters of wheat straw (WS) fed to 2 years old Awassi ram lambs. Result showed that urea and molasses supplementation produced significantly increased on DMI (Dry matter intake) and OMI (Organic matter intake). Dry matter and OM (Organic matter) digestibility of wheat straw control diet were found lower than urea and molasses supplemented treatment diets. They reported that control diet consuming animals had a lower CP digestibility than urea and molasses supplemented animals and increment of both urea and molasses increased CP digestibility of diets. Control (WS) diet had a lower NDF digestibility than urea and molasses supplemented treatment diets. While increasing molasses level did not affect NDF digestibility, increments of urea level improved NDF digestibility. ADF digestibility of control (WS) and treatment diets were found similar effect and increment of molasses levels and urea levels increased ADF digestibility of diets.

A feeding trial was conducted by Chowdhury and Huque (1998) using rice gruel compared to that of the cane molasses as a source of readily fermentable energy for a

urea supplemented straw diet fed to twelve native growing bulls. They reported that Organic matter (OM) intake was significantly higher in the UMS (64g/kg  $W^{0.75}$  /d) followed by UGS (53g/kg  $W^{0.75}$  /d) and US (49g/kg  $W^{0.75}$  /d) of growing bulls. Estimated (from digestible OM intake) metabolizable energy (ME) intake was 396, 348 and 301 kJ/kg  $W^{0.75}$ /d for UMS, UGS and US respectively. Urinary purine derivatives excretion was non-significantly higher in the UMS (51.73 mmol/d), followed by UGS (42.53 mmol/d) and US (35.26 mmol/d). The estimated microbial N (MN) yield was 21.10, 14.00 and 11.60g/d for UMS, UGS and US respectively. Observed live weight changes during the experimental period were 292, 125 and -19 g/d respectively for UMS, UGS and US. They were concluded that supplementation of readily fermentable N (urea) alone was not enough to optimize the rumen function and a source of readily fermentable energy required. Rice gruel was less effective than molasses as fermentable energy source to remove a restriction on voluntary intake and provide less amino acids of microbial origin for absorption from the small intestine, thus more substrate for protein synthesis and gluconeogenesis were available for growth in the molasses than the rice gruel supplemented animals.

Shiriyani et al. (2011) had evaluated the study of the effect of urea treated straw in a pelleted total mix ration on the carcass and growth characteristics of lambs. Results showed that feed conversion ratio (FCR) was  $7.95 \pm 0.31$ ,  $6.32 \pm 0.3$ ,  $6.15 \pm 0.62$  and  $6.52 \pm 0.27$  in 0, 10, 20 and 30 % treated straw groups respectively. The group which received 20% treated straw showed the highest mean value of lean meat,  $51.22 \pm 2.04$  compared to other treatments  $50.30 \pm 3.87$ ,  $51.02 \pm 3.89$ ,  $46.95 \pm 1.51$ , respectively. The total percent of carcass fat were  $15.27 \pm 0.25$ ,  $12.77 \pm 0.28$ ,  $14.2 \pm 3.38$  and  $14.55 \pm 1.1$  in experimental treatments and the lowest value obtained in treatment 10% treated straw. In conclusion supplementation urea treated wheat straw in a pelleted total mixed ration had positive effects on performance and carcass characteristics of Lori-Bakhtiari fattening lamb.

Hossain et al. (1995) was evaluated the growth performance of sheep fed supplementary urea molasses block lick with rice straw based diet using six indigenous sheep of about two years of age with an average body weight of 12.88 kg. The study revealed that supplemented urea molasses block with rice straw based diet produced significant effect on weight gain of the sheep and required low DM (Dry

matter) intake to increased live weigh gain. Their study suggested that feeding feeding rice straw with urea molasses block lick able to utilize more crop- residues efficiently.

A study was conducted by Misra et al. (2000) to compare the feeding value of urea treated and untreated mustard straw (MS) for sheep using a total of six empty Avikaline ewes in two groups were fed untreated (UTMS) and treated (TMS) mustard straw along with 200 g concentrate per head daily for 90 days. Dry matter intake of TMS was consistently higher than that of UTMS. Digestibility of DM, OM and fibre fractions of MS improved by the urea treatment. Ewes in both groups were in positive N balance while % N retention was lower in UTMS (26.30%) than in TMS (52.14%). The TMS fed group on average consumed 30.2g DM, 2.9 g digestible crude protein and 0.2 MJ DE per kg BW /day and maintained their weight whereas, the UTMS fed ewes lost weight. It is concluded that urea treatment of MS improved N value of MS from 0.41% to 1.58% along with sizable improvement in nutritive value and in conjunction with 200g concentrate; it can serve as maintenance ration for sheep.

## **2.5. Animal feeding and rumen ecology**

Hasanuzzaman et al. (2014) had evaluated to observe the possibility of using rice gruel as a source of readily fermentable energy and to see its effect on rumen pH as well as microbial population in cattle. Six growing cattle were divided into two groups fed on two different concentrate mixtures at the point of molasses and rice gruel. G- I was fed with rice gruel where molasses were offered to G- II, in addition, three hours of grazing and ad-lib. water was offered to all the experimental animals. The feeding trial was continued for 60 days. Live weight changes during the experimental period for Group I and Group II were observed as  $303.33 \pm 14.53$  and  $406.67 \pm 14.53$  gm, respectively. The pH of the rumen liquor varied from  $5.4 \pm 0.35$  to  $7.3 \pm 0.46$  in Group I and  $6.3 \pm 0.90$  to  $7.87 \pm 0.42$  in Group II with highest value at 12 h in both groups and lowest value at 20h and 16h of post feeding in G-I and G-II, respectively. The bacterial population ( $\text{cell} \times 10^{10}$ ) per ml of SRL ranged from  $7.33 \pm 0.50$  to  $9.67 \pm 0.15$  in G-I and  $5.23 \pm 0.25$  to  $8.47 \pm 0.15$  in G-II with peak level at 20h and 12h in G-I and G-II diets, respectively and lowest value found at 4h and 8h of post feeding in G-I & G-II diets, respectively. The rumen protozoal population ( $\text{cell} \times 10^6$ ) per ml of SRL ranged from  $4.53 \pm 0.50$  to  $7.33 \pm 0.50$  in G-I and  $3.30 \pm 1.0$  to  $6.57 \pm 1.70$  in G-II being highest at 20h of post feeding in both G-I & G-II diets and

lowest at 4h and 24h of post feeding in G-I & G-II diets, respectively. It can be concluded that rice gruel was less effective than molasses as fermentable energy source, however in situation where molasses is not available or costly; rice gruel does appear to have a place as readily fermentable energy source.

A rumen fermentation study was conducted by Thakur et al. (2006) where rumen fistulated male buffalo calves (9; 2.0-3.0 yr old of  $238.36 \pm 13.97$  kg BW) were divided into three equal groups and fed total mixed rations (TMR) containing concentrate: green maize fodder: wheat straw in 50:25:25 proportions on DM basis for 120 days. Concentrate of TMR<sub>1</sub> comprised of traditional feed ingredients whereas that of TMR<sub>2</sub> and TMR<sub>3</sub> contained concentrates in which maize and barley grains were replaced with wheat, groundnut cake with mustard cake and urea, mineral mixture reduced by 0.5 per cent and common salt increased by 0.5 per cent. The type of TMR did not have any significant effect on rumen metabolites or microbial counts. The N, Ca and P retention was statistically similar in all groups. The average daily gain was significantly ( $P < 0.01$ ) higher in calves fed TMR<sub>3</sub> as compared to those fed TMR<sub>1</sub> and TMR<sub>2</sub>. Feed cost (Rs/kg BW gain) was significantly ( $P < 0.01$ ) lower in calves fed TMR<sub>2</sub> and TMR<sub>3</sub> than that of TMR<sub>1</sub>. It was concluded that the feeding of TMR based on locally available cheap feed ingredients improved the growth rate and reduced the cost of feeding in buffalo calves.

## **2.6. Effect of hematological & biochemical changes**

Muralidharan et al. (2011) were evaluated the blood biochemical profile of Mecheri lambs fed concentrate and urea molasses mineral block (UMMB) supplemented diets using forty Mecheri male lambs (3-4 months old) over a period of 150 days. Result showed that serum protein increased in concentrate and UMMB supplemented group alone with grazing whereas, serum albumin remained similar in all the groups. Serum calcium values were differed significant among the treatment groups and highest value was found in UMMB supplemented group. Serum phosphorus had significantly ( $P < 0.05$ ) higher in concentrate supplemented group. Serum cholesterol had significantly ( $P < 0.05$ ) higher in grazing and concentrate supplemented group than control group and stall feeding group. UMMB supplemented group had significantly ( $P < 0.01$ ) higher blood urea nitrogen values compared to other groups. It was

concluded that blood biochemical parameters were influenced by supplementation of concentrate and UMMB.

A research study was conducted by Kioumars et al. (2011) to evaluate the effect of molasses/mineral feed blocks along with the use of medicated blocks on hematological and biochemical blood parameters in Boer goats. A total number of twenty four male Boer goats with an average age of 7-8 months were used. Goats were divided into four groups: (1) a control group; (2) an experimental group fed with a ratio of molasses/mineral feed blocks (UMB); (3) an experimental group fed with a ratio of medicated blocks (MUMB) and (4) an experimental group fed with a ratio of UMB+MUMB. The result was showed that a combination of molasses/ mineral feed blocks and medicated blocks has significant effects ( $p < 0.05$ ) on blood factors includes calcium, creatinine, urea nitrogen and Packed Cell Volume (PCV) and has no negative effects on body function. The result for PCV (%) shows that goats fed with the ratio contain UMB+MUMB have highest percentages which are  $94.90 \pm 14.70$  and  $27.25 \pm 2.50$ , respectively. The goats fed with MUMB have highest amount of calcium in their blood which is  $3.70 \pm 0.37$ . The highest amount of urea nitrogen is  $5.48 \pm 2.15$  which belongs to the goats fed with UMB. According to the results, it can be concluded that the molasses were uses in the ratios of the goats had positive effects on body function.

## **Chapter 3: Materials and Methods**

### **3.1. Location and climatic condition**

The experiment was conducted at Sheep Farm Unit of Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh during the period of 10 weeks from February to April 2016. The weather of Chittagong is characterized by tropical monsoon climate. Chittagong is located at 22°22'0"N 91°48'0"E on the banks of the Karnaphuli River. The dry and cool season is from November to March; pre-monsoon season is from February to April which is very hot. The sunny and the monsoon season are from June to October, which is warm, cloudy and wet.

### **3.2. Preparation of experimental house**

The experiment was conducted in a pre-prepared house. The experimental house for lamb rearing had a wooden floor of about 2.5 feet high from the ground. The surrounding wall was made of metal wire arranged in a wooden frame. There were three separate pens for three treatment groups. The experimental house was properly washed and cleaned by using tap water initially then bleaching powder was sprinkled on floor and waited for 24 hours then brushing with steel brush along with clean water was done. After that Ceiling, walls and floor were thoroughly cleaned and disinfected by spraying diluted disinfectant. After proper drying, the house was prepared for experiment. Proper ventilation was provided by natural ventilation.

### **3.3. Selection of experimental animals**

The animals were selected in healthy condition having shiny body coat, active and alert movement, normal feeding, rumination, eructation, defecation, urination with other physical parameters ( Rectal temperature, heart rate, pulse rate, respiration rate etc.) normal. A total number of twelve (12) sheep (09 males and 03 females) of approximately same age and size were selected for the experimental trial from sheep farm unit of Chittagong Veterinary and Animal Sciences University (CVASU). The animals were divided into three groups, T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> with 04 animals in each group having 03 males and 01 female and their age was fourteen months.

### 3.4. Tagging of experimental animals

For proper identification of the animal for experimentation, they were marked using plastic numbering tags that were attached to the neck of lamb with cotton thread. Each and every tag had the unique numeric number.

### 3.5. Vaccination and deworming of experimental animals

Before beginning of the experiments, all animals were vaccinated properly with the PPR vaccine at the rate of 1 ml per animal through intramuscular route. Feces of each sheep was examined initially for checking internal parasitic infestation and all animals were de-wormed with suitable anthelmintic immediately before starting of the experiment.

### 3.6. Layout of the experiment

On the basis of availability and uniformity of animals, a total of 12 sheep (9 males and 3 females) with average body weight  $15.14 \pm 0.07$  were allocated in three dietary treatment groups following CRD (Completely Randomized Design). Males and females were randomly distributed in different treatment groups as each group contains 3 males and 1 female sheep.

**Table 1: Layout of the experiment showing the distribution of sheep to treatment**

Dietary treatment groups	Total no. of sheep per treatment
T <sub>0</sub> (Control/ basal diet)	4
T <sub>1</sub> (With 3% urea and 15% molasses)	4
T <sub>2</sub> (With 3% urea and 15% rice gruel)	4
<b>Grand total</b>	<b>12</b>

### 3.7. Collection of feedstuffs

Rice gruel was collected from M A Hannan Hall Dinning of Chittagong Veterinary and Animal Sciences University (CVASU). Green grasses were collected from the

fodder plot and rice straw was collected from large animal feed store of Chittagong Veterinary and Animal Sciences University. Urea, Molasses, Grass pea (Khesari), Red Lentil (Mushari), Wheat bran, Broken Maize, common salt, DCP and Vitamin mineral premix were purchased from local market.

**Table 2: Percentages of feed ingredients in concentrate feed mixture**

Sl. No.	Name of the ingredients	Amount (Kg)
1	Grass pea (Khesari)	45
2	Red Lentil (Mushari)	30
3	Wheat Bran	10
4	Broken Maize	14
5	DCP	0.500
6	Vitamin Mineral Premix	0.250
7	Salt	0.250
	<b>Total</b>	<b>100</b>

**Table 3: Proximate composition of concentrate feed, UMS and URS (DMB)**

Parameters (%)	DM	CP	CF	Ash	EE	NFE
Concentrate Feed	90.83	14.6	24.54	4.98	2.32	53.55
UMS	57.65	9.72	29.32	14.85	1.63	44.48
URS	46.24	7.84	33.5	15.54	1.42	41.3

**Table 4: Proximate composition of green grass (DMB)**

Parameters	DM	CP	CF	Ash	EE	NFE
Percentages (%)	25.13	11.38	35.44	10.24	1.44	41.5



### **3.8. Formulation of (UMS) urea molasses straw (DMB)**

**Composition:** Straw 82%, molasses 15% and urea 3%.

**Procedure:** Firstly straw was weighted and chopped. Molasses and urea were weighted separately. Then urea was mixed thoroughly with required amount of fresh water and then molasses was mixed homogenously. The straw was kept on the polythene then urea molasses mixture was spread over the straw thoroughly. The straw layer was then pressed thoroughly by trampling with hands, in order to make the stack more compact and to drive out unwanted air from the stack. At last the stack should preferably be covered by polythene sheet.

### **3.9. Formulation of (URS) urea rice gruel straw (DMB)**

**Composition:** Straw 82%, Rice gruel 15% and urea 3%

**Procedure:** Firstly straw was weighted, and chopped. Rice gruel and urea was weighted separately. Then urea was mixed thoroughly with adequate amount of fresh water and then rice gruel was mixed homogenously. The straw was kept on the polythene then urea rice gruel mixture was spread over the straw thoroughly. The straw layer was then pressed thoroughly by trampling with hands, in order to make the stack more compact and to drive out unwanted air from the stack. At last the stack was covered by polythene sheet.

### **3.10. Feeding of experimental animals**

Fresh, clean and safe drinking water was supplied to the sheep at all the times. Accurate amount of Concentrate and green grass were offered in each group. Green grass was collected every morning and afternoon followed by cleaning, chopped, weighted and supplied to the sheep. Concentrate mixture and green grass was offered two times daily, once in the morning at 8.00 A.M. and another in the afternoon at 4.00 P.M to offer UMS, URS and straw.

### **3.11. Record keeping**

Animals were weighed initially before feeding trial and then at 7 day interval throughout the experimental period. After completion of 10 weeks experimental

period, final live weight of each animal was recorded. In each time the animals were weighed at early morning prior to grazing. Daily feed intake of the animal was also recorded on a regular basis. The animals were observed for any abnormalities every morning.

### **3.12. Blood collection and sample preparation**

At the end of the feeding trial, 2 sheep was selected from each group, blood samples were collected through jugular vein about 4ml from each sheep. Blood samples were taken into two separate vials. One containing EDTA (anticoagulant) for hematology and another do not contain anticoagulant which was used for serum preparation for biochemical analysis. The blood samples with anticoagulant were analyzed for HB, PCV, TEC and TLC within 24 hours of collection. The separated serum samples were preserved into deep freeze at -18 °C and biochemical analysis were done within 7 days.

### **3.13. Sanitation and bio-security**

Strict sanitary measures were taken during the experimental period. Disinfectant was used to disinfect the feeders and waterers. The animal shed was cleaned and disinfected on a regular basis with appropriate disinfectant. Bush was cleaned and burnt surrounding the animal shed. All other bio-security program was properly maintained according to HACCP.

### **3.14. Collection and preservation of rumen liquor**

Rumen liquor samples were drawn from the sheep by 19 G needle with syringe and kept in a thermos where temperature was maintained at 39<sup>0</sup>C. Then the samples were processed immediately after collection for different parameter studies.

### **3.15. Determination of pH of the rumen liquor**

The pH of rumen liquor was determined using pH paper. After collection, pH paper was inserted inside the vial of rumen liquor and matching the pH color and pH was determined.

### **3.16. Protozoal motility assessment**

The protozoa motility gives a tentative idea about the digestion of food in the rumen; therefore it was studied for the protozoal motility in rumen liquor to know the feed effect.

#### **3.16.1. Procedure**

2-3 drops of fresh rumen liquor was transferred on a clean glass slide and was covered with cover slip. The movement of protozoa was examined under low power of microscope immediately.

The movement of protozoa was rated as follows:

++++ = very rapid movement, whole mass is moving

+++ = rapid movement, very large population of protozoa showing their motility

++ = moderate movement, less number of protozoa is moving moderately

+ = slow movement, very few protozoa showing their slow movement

0 = No movement, all protozoa are dead.

### **3.17. Estimation of protozoa concentration in rumen liquor**

1ml of rumen liquor was placed into a test tube through a wide bore pipette. Exact volume of 9 ml of Lugol's Iodine solution was added and mixed gently. Then 0.1 ml of sample was transferred swiftly to a dry clean slide and spread under a glass cover of known area (24 × 60 mm). Counting of protozoa was done under low power of microscope in a zig zag manner. Thirty fields were counted per slide both for ease and accuracy. After that the average count per field was calculated. Total protozoal count per ml was calculated by following formula:

Total protozoa/ml rumen liquor

= {(Average number of protozoa counted per field) × Microscopic factor × dilution factor}.

### **3.18. Estimation of bacteria concentration in the rumen liquor**

Collected and filtered rumen liquor was centrifuged 3000 rpm for 5 minutes. A volume of 5 ml of centrifuged content was taken in a test tube and 5 ml of 10 percent

formalin was added to kill the bacteria. Then 2 ml of formalin mixed with rumen liquor was transferred in a test tube and 8 ml of distilled water was added to give  $1 \times 10^{-1}$  dilution and serial dilutions up to  $1 \times 10^{-4}$  were made. Exactly 0.01 ml of sample from  $1 \times 10^{-4}$  dilution was placed on a clean glass slide on a marked area  $2 \times 2$  cm and loopful of saturated solution of nigrosine was taken on glass slide. Finally both were mixed thoroughly and stained with the help of loop wire, spreaded on slide as thin as possible. The slide was kept on hot plate for 2 seconds to dry the smear and counting was done under oil immersion lens where bacteria appear colorless against black background. The bacteria were counted in 10 different in zig zag manner and the average number of bacteria per field was calculated by following formula:

Ruminal bacteria per ml of rumen liquor

$$= \{(\text{Average number of bacteria per field} \times \text{microscopic factor (1000)} \times \text{dilution factor (10}^6)\}$$

### **3.19. Measurement of live weight and body weight gain**

The sheep were weighed individually at the beginning of the experiment and the average weight was taken as the initial body weight. There after the sheep were weighed individually in every week by using an electric digital weighing balance before morning feeding. The weekly live weight gain was calculated by subtracting the weight at starting of the week from end of the week.

### **3.20. Digestibility trial**

A digestibility trial was conducted in the last 7 days of the experimental period to evaluate the effect of URS and UMS in digestibility of nutrients. Feed supply and feces collection was performed two times daily. During digestibility trial, the quantity of feed supplied and faeces collected were recorded carefully. After collection of feces it was immediately stored in a freezer. Both the feed and feces were subjected to proximate analysis following the standard procedure (AOAC, 2004) to determine nutrient contents of feed and feces. The digestibility of each nutrient was estimated by the following formula.

$$\% \text{ Digestibility of nutrient} = \frac{\text{Nutrient intake through feed (gm)} - \text{Nutrient in feces (gm)}}{\text{Nutrient intake through feed (gm)}} \times 100$$

### **3.21. Total erythrocyte count (TEC)**

The number of RBC was estimated by using Neubaur Hemocytometer. The blood was diluted 200 times with Hayem's solution. Red blood cells were then counted into Neubaur Haemocytometer under microscope in diluted blood. The TEC in undiluted blood was calculated by multiplying volume correction factor and dilution factor. The results were expressed as number of RBC per ml of blood.

### **3.22. Estimation of hemoglobin (Acid-Hematin method)**

Hemoglobin (Hb) was determined by acid hematin method. Hb is converted to acid hematin by dilute HCL which in solution brown in colour. The intensity of this colour depends in the amount of acid hematin in solution which in turn depends on Hb concentration. The colour of the solution is matched against brown titled glass filter by direct vision and result were expressed as gm/100ml blood (gm %).

### **3.23. Total leukocyte count (TLC)**

The blood was diluted with 0.1N HCl which destroys the red cells and stains the nuclei of WBC. White blood cells (WBC) were then counted into a Haemocytometer under microscope in diluted blood. The TLC in undiluted blood was calculated by multiplying volume correction factor and dilution factor. The results were expressed as number of WBC per ml of blood.

### **3.24. Packed cell volume (%)**

Blood samples were centrifuged in a hematocrit tube. The RBC (Sp. gr. =1.09) being heavier than plasma (Sp. gr. =1.03) get pack towards the bottom of the tube by centrifugal force. The reading of percentage of blood that is red cells was then noted.

### **3.25. Biochemical analysis**

The biochemical analysis was performed from the preserved serum sample. The samples were allowed to be in room temperature before starting the analysis. The serum total protein (TP), Albumin, Cholesterol, Uric acid and blood metabolites (minerals, glucose) level were estimated by using biochemical analyzer (Humalyzer-3000 chemistry analyzer, semi automated Benchtop chemistry photometer) in

Biochemistry Laboratory of CVASU. For each parameters the commercial kit of RANDOX company (<http://www.randox.com/reagent>) were used and followed the manufacturer's procedure.

### **3.26. Statistical analysis**

All collected data and sample evaluated values were imported in Microsoft office excel-2007 and transferred to SPSS-16 (Statistical Package for the Social Sciences) software for analysis. Descriptive statistics of some parameters were done. Quantitative performance parameters from different groups of dietary treatment, values of digestibility trial and hematological parameter were compared by one way ANOVA by using SPSS-16. The differences of different parameters were considered significant when the p-value was  $< 0.05$  and highly significant when p-value was  $< 0.01$  and  $< 0.001$ .



Figure1: Different activities in feeding trial



Figure 2: Processing of UMS and URS



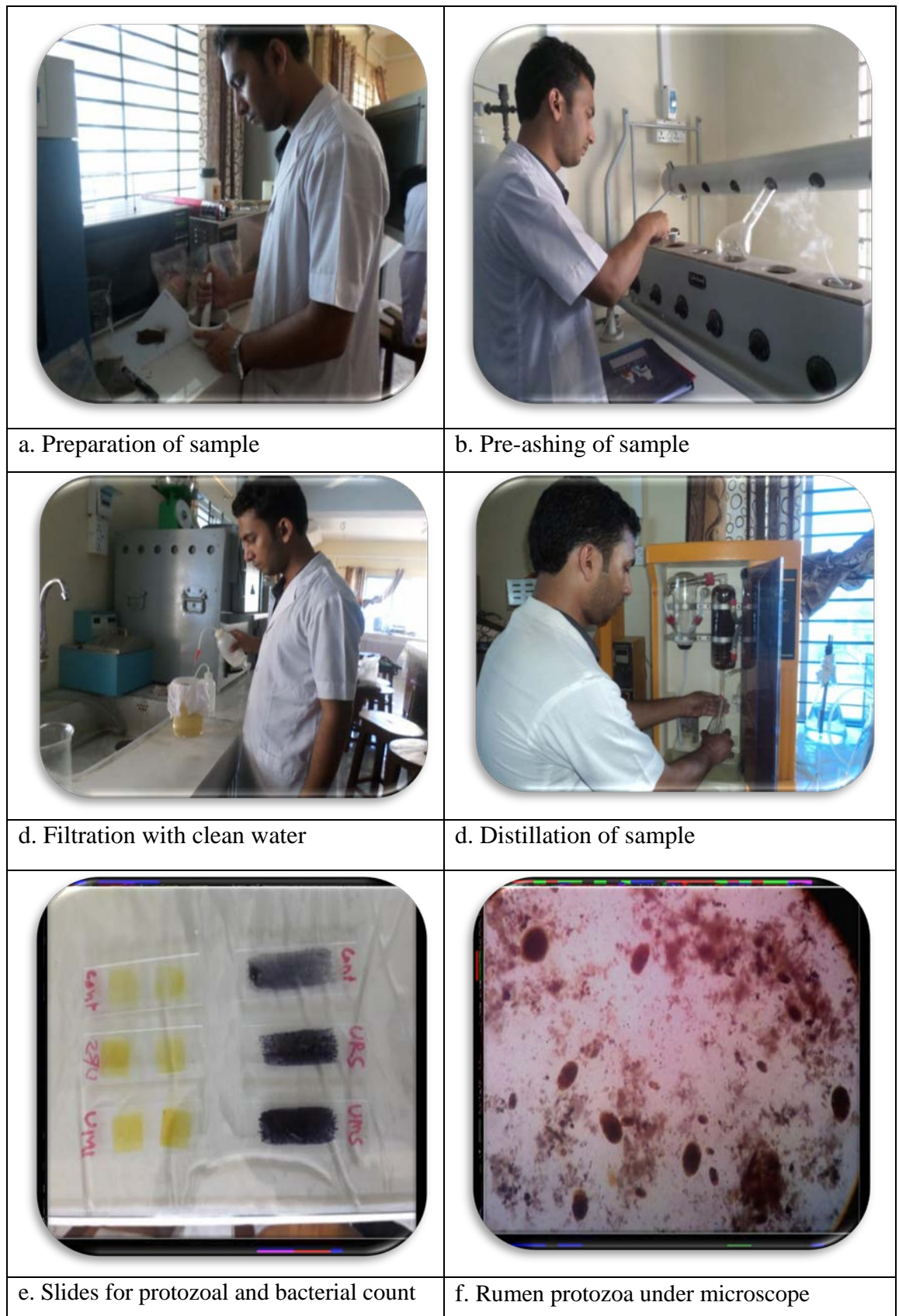


Figure 3: Different activities in Animal Nutrition and Microbiology Lab.

## Chapter 4: Results

The findings of the study on supplementation of urea and rice gruel with straw on growth performance, nutrient digestibility, some hematological and sero-biochemical profile and rumen ecology of sheep is discussed in this chapter underneath headings.

### 4.1. Live weight of experimental sheep

The live weight of experimental sheep was measured on a weekly basis and the data were analyzed and incorporated in table below.

**Table 4.1:** Effect of UMS and URS supplementation on body weight (kg) of sheep

Age (Week)	Body weight (Mean $\pm$ SE)			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
Initial	15.16 $\pm$ 0.11	15.16 $\pm$ 0.16	15.10 $\pm$ 0.12	NS
1 <sup>st</sup>	15.37 $\pm$ 0.11	15.67 $\pm$ 0.17	15.41 $\pm$ 0.14	NS
2 <sup>nd</sup>	15.59 <sup>a</sup> $\pm$ 0.12	16.19 <sup>b</sup> $\pm$ 0.17	15.74 <sup>ab</sup> $\pm$ 0.14	*
3 <sup>rd</sup>	15.85 <sup>a</sup> $\pm$ 0.13	16.74 <sup>b</sup> $\pm$ 0.17	16.08 <sup>a</sup> $\pm$ 0.15	**
4 <sup>th</sup>	16.10 <sup>a</sup> $\pm$ 0.14	17.27 <sup>b</sup> $\pm$ 0.17	16.44 <sup>a</sup> $\pm$ 0.16	**
5 <sup>th</sup>	16.45 <sup>a</sup> $\pm$ 0.17	17.80 <sup>b</sup> $\pm$ 0.17	16.82 <sup>a</sup> $\pm$ 0.17	**
6 <sup>th</sup>	16.59 <sup>a</sup> $\pm$ 0.20	18.34 <sup>c</sup> $\pm$ 0.18	17.22 <sup>a</sup> $\pm$ 0.18	***
7 <sup>th</sup>	16.81 <sup>a</sup> $\pm$ 0.21	18.90 <sup>c</sup> $\pm$ 0.20	17.62 <sup>b</sup> $\pm$ 0.20	***
8 <sup>th</sup>	17.02 <sup>a</sup> $\pm$ 0.23	19.44 <sup>c</sup> $\pm$ 0.19	18.06 <sup>b</sup> $\pm$ 0.21	***
9 <sup>th</sup>	17.24 <sup>a</sup> $\pm$ 0.25	19.98 <sup>c</sup> $\pm$ 0.21	18.50 <sup>b</sup> $\pm$ 0.22	***

(N=12); SE=Standard Error; NS=Non-Significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001); T<sub>0</sub>=Control group (Concentrate feed, straw and green Grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). UMS = Urea molasses straw, URS = Urea rice gruel straw. Means with different superscripts in the same row differ significantly.

From above data in table 4.1, it was found that initial and 1<sup>st</sup> week of body weight did not differ significantly among the treatment groups. At the 2<sup>nd</sup> week the body weight of the experimental sheep differed significantly (P<0.05) among the treatment groups and the same trend was observed in case of 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> weeks of body weight where it observed highly significant (P<0.01) variations among the treatment groups. The body weight in 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> weeks differed significantly (P<0.001) among the treatment groups. The highest mean of body weight was observed in T<sub>1</sub> (UMS) group throughout the experimental period followed by T<sub>2</sub> (URS) and lastly in T<sub>0</sub> (Control) group in most of the times (weeks).

## 4.2. Live weight gain of experimental sheep

Average live weight of sheep of each group were measured weekly and the live weight gain was measured by subtracting the weight of current week from that of previous week and analyzed result was incorporated in the table below.

**Table 4.2:** Live weight gain (kg) in different weeks among various treatment groups

Age (Week)	Live weight gain among the different treatment groups (Mean $\pm$ SE)			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
1 <sup>st</sup>	0.20 <sup>a</sup> $\pm$ 0.01	0.51 <sup>c</sup> $\pm$ 0.01	0.31 <sup>b</sup> $\pm$ 0.01	***
2 <sup>nd</sup>	0.22 <sup>a</sup> $\pm$ 0.02	0.52 <sup>c</sup> $\pm$ 0.01	0.32 <sup>b</sup> $\pm$ 0.01	***
3 <sup>rd</sup>	0.25 <sup>a</sup> $\pm$ 0.03	0.54 <sup>c</sup> $\pm$ 0.02	0.34 <sup>b</sup> $\pm$ 0.02	***
4 <sup>th</sup>	0.25 <sup>a</sup> $\pm$ 0.03	0.53 <sup>c</sup> $\pm$ 0.02	0.36 <sup>b</sup> $\pm$ 0.02	***
5 <sup>th</sup>	0.24 <sup>a</sup> $\pm$ 0.05	0.53 <sup>c</sup> $\pm$ 0.01	0.37 <sup>b</sup> $\pm$ 0.02	***
6 <sup>th</sup>	0.23 <sup>a</sup> $\pm$ 0.04	0.53 <sup>c</sup> $\pm$ 0.01	0.39 <sup>b</sup> $\pm$ 0.02	***
7 <sup>th</sup>	0.22 <sup>a</sup> $\pm$ 0.03	0.55 <sup>c</sup> $\pm$ 0.02	0.40 <sup>b</sup> $\pm$ 0.02	***
8 <sup>th</sup>	0.20 <sup>a</sup> $\pm$ 0.02	0.53 <sup>c</sup> $\pm$ 0.01	0.43 <sup>b</sup> $\pm$ 0.02	***
9 <sup>th</sup>	0.21 <sup>a</sup> $\pm$ 0.03	0.54 <sup>c</sup> $\pm$ 0.01	0.44 <sup>b</sup> $\pm$ 0.01	***

N= 12; SE=Standard Error; \*\*\*=Significant (P<0.001); T<sub>0</sub>=Control group (Concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly.

From the above data in table 4.2 it was found that 1<sup>st</sup> to 9<sup>th</sup> weeks of body weight gain differed significantly (P<0.001) among the treatment groups . At 9<sup>th</sup> week, the highest body weight gain (0.547 kg) was observed in T<sub>1</sub> (UMS) group followed by T<sub>2</sub> (URS) and T<sub>0</sub> (Control) groups . The lowest body weight gain was observed in T<sub>0</sub> (Control) group.

## 4.3. Digestibility of different proximate component

A digestibility trial was conducted and the result on the digestibility of nutrients as affected by supplementation of UMS and URS is shown in table 4.3.

**Table 4.3:** Digestibility of different nutrients in different treatment groups

Parameter	Digestibility of different nutrients in different treatment groups (Mean $\pm$ SE)			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
DM%	70.92 <sup>a</sup> $\pm$ 0.57	72.11 <sup>b</sup> $\pm$ 0.94	66.68 <sup>b</sup> $\pm$ 0.68	***
CP%	84.24 <sup>c</sup> $\pm$ 0.20	80.62 <sup>b</sup> $\pm$ 0.39	77.93 <sup>a</sup> $\pm$ 0.23	***
CF%	79.05 <sup>b</sup> $\pm$ 0.31	77.13 <sup>a</sup> $\pm$ 0.48	76.37 <sup>a</sup> $\pm$ 0.49	**
EE%	75.09 <sup>a</sup> $\pm$ 0.67	77.94 <sup>b</sup> $\pm$ 0.35	77.79 <sup>b</sup> $\pm$ 1.19	*
Ash%	35.91 <sup>c</sup> $\pm$ 1.57	23.98 <sup>b</sup> $\pm$ 1.32	19.39 <sup>a</sup> $\pm$ 1.43	***
NFE%	72.72 <sup>c</sup> $\pm$ 1.01	68.46 <sup>b</sup> $\pm$ 0.77	64.75 <sup>a</sup> $\pm$ 0.63	***

(N=12); SE=Standard Error; \*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001); T<sub>0</sub>= Control group (Concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly.

Digestibility of DM, CP, Ash and NFE differed significantly (p<0.001) among different treatment groups where highest value of DM was found in T<sub>1</sub> (UMS) group followed by T<sub>0</sub> (Control) and T<sub>2</sub> (URS) groups. On the other hand highest value of digestibility of CP, Ash and NFE were found in T<sub>0</sub> (Control) group followed by T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups. Digestibility of CF differed significantly (P<0.01) among treatment groups where highest value was found in T<sub>0</sub> (Control) group and lowest in T<sub>2</sub> (URS) group. Digestibility of EE differed significantly (P<0.05) among treatment groups where highest value was found in T<sub>1</sub> (UMS) group and lowest in T<sub>0</sub> (Control) group.

#### 4.4. Changes of hematological parameters of the experimental sheep

Table 4.4: Effects of UMS and URS supplement on hematological parameter of sheep

Parameter	Hematological parameter of sheep (Mean $\pm$ SE)			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
Hb (gm/dl)	5.90 <sup>a</sup> $\pm$ 0.10	7.10 <sup>b</sup> $\pm$ 0.10	6.10 <sup>a</sup> $\pm$ 0.10	**
PCV (%)	29.50 <sup>b</sup> $\pm$ 0.50	32 <sup>b</sup> $\pm$ 1.00	25 <sup>a</sup> $\pm$ 1.00	*
TEC (mill./mm <sup>3</sup> )	8.55 $\pm$ 0.05	9.36 $\pm$ 0.44	8.95 $\pm$ 0.43	NS
TLC (thou./mm <sup>3</sup> )	11.67 $\pm$ 0.23	12.18 $\pm$ 0.22	11.64 $\pm$ 0.08	NS
Lymphocyte (%)	47 <sup>b</sup> $\pm$ 1.00	36.5 <sup>a</sup> $\pm$ 1.50	41 <sup>a</sup> $\pm$ 1.00	*
Monocyte (%)	4.5 $\pm$ 0.50	5.5 $\pm$ 0.50	3 $\pm$ 1.00	NS
Neutrophil (%)	41 $\pm$ 1.00	50.50 $\pm$ 4.5	47 $\pm$ 3.00	NS
Eosinophil (%)	6.5 $\pm$ 0.50	7.0 $\pm$ 3.00	8.5 $\pm$ 0.50	NS
Basophil (%)	1.0 $\pm$ 1.00	0.50 $\pm$ 0.50	0.50 $\pm$ 0.50	NS

N=12; SE=Standard Error; NS=Non-Significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01); T<sub>0</sub>=Control group (concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly.

From the above data in table 4.4 it was found that Hb concentration in blood of sheep differed significantly (P<0.01) among treatment groups where highest value was found in T<sub>1</sub> (UMS) group followed by T<sub>2</sub> (URS) and T<sub>0</sub> (Control) groups. Further, the percentage of PCV and lymphocyte were differed significantly (P<0.05) among treatment groups where highest value of PCV and lymphocyte were found in T<sub>1</sub> (UMS) and T<sub>0</sub> (Control) groups respectively. TEC , TLC and others blood cell were found insignificant (P>0.05) among various treatment groups.

#### 4.5. Changes of biochemical parameters of the experimental sheep

Table 4.5: Effect of UMS and URS supplementation on serum biochemical parameter of sheep

Parameter	Biochemical parameter of sheep (Mean ± SE)			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
Glucose (mg/dl)	53.65 <sup>c</sup> ±0.85	32.9 <sup>a</sup> ±0.30	46.41 <sup>b</sup> ±0.78	**
Total Protein (g/dl)	8.20 <sup>a</sup> ±0.53	10.58 <sup>b</sup> ±0.02	9.01 <sup>a</sup> ±0.90	*
Albumin (g/dl)	2.04 <sup>a</sup> ±0.02	2.3 <sup>ab</sup> ±0.04	2.62 <sup>b</sup> ±0.15	*
Uric Acid (mg/dl)	1.67±0.62	1.36±0.16	0.83±0.15	NS
Cholesterol (mg/dl)	53.26 <sup>a</sup> ±3.24	52.30 <sup>a</sup> ±2.70	81 <sup>b</sup> ±2.20	**
Calcium (mg/dl)	13.30 <sup>b</sup> ±0.40	10.77 <sup>a</sup> ±0.21	14.13 <sup>b</sup> ±0.26	**
Phosphorus (mg/dl)	4.67 <sup>a</sup> ±0.19	6.87 <sup>b</sup> ±0.31	4.66 <sup>a</sup> ±0.15	*
Magnesium (mg/dl)	1.80 <sup>a</sup> ±0.05	2.02 <sup>b</sup> ±0.02	2.1 <sup>b</sup> ±0.02	*
Sodium (mmol/l)	139.15 <sup>c</sup> ±0.80	130.45 <sup>b</sup> ±0.80	107.75 <sup>a</sup> ±0.75	***
Potassium (mmol/l)	5.36 <sup>b</sup> ±0.13	6.1 <sup>a</sup> ±0.20	4.3 <sup>c</sup> ±0.10	**
Chlorine (mmol/l)	136.45 <sup>b</sup> ±1.50	112.10 <sup>a</sup> ±1.10	138.22 <sup>b</sup> ±1.78	**

N=12; S.E=Standard Error; NS=Non-Significant (P>0.05); \*=Significant (P<0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001). T<sub>0</sub>=Control group (concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly.

From the above table 4.5 it was revealed that glucose level in blood was significantly higher (P < 0.01) in T<sub>0</sub> (53.65 mg/dl) and lower in T<sub>1</sub> (32.9 mg/dl) group. It was found that blood level of total protein and albumin were significantly (P<0.05) differed among different treatment groups where highest value of total protein and albumin

were found in T<sub>1</sub> (10.58g/dl) and T<sub>2</sub> (2.625g/dl) groups respectively and lowest was in T<sub>0</sub> (Control) group. It was also found that blood cholesterol level was significantly (P<0.01) differed among different treatment groups where highest value was found in T<sub>2</sub> (81mg/dl) group and lowest was in T<sub>1</sub> (52.30 mg/dl) group. However, it was found that the blood uric acid level differences were insignificant of T<sub>1</sub>(UMS) and T<sub>2</sub> (URS) supplemented group compared to T<sub>0</sub> (Control) group. Blood Sodium (Na) level was significantly (P<0.001) differed among different treatment group where highest value was found in T<sub>0</sub> (139.15 mmol/l) group and lowest value was found in T<sub>2</sub> (107.75 mmol/l) group. The blood level of Calcium (Ca), Potassium (K) and Chlorine (Cl) were found significantly higher (P<0.01) variations among the treatment groups. The increment of Phosphorus (P) and Magnesium (Mg) were differed significantly (P<0.05) among the treatment groups where highest value was found in T<sub>1</sub> (6.87mg/dl) and T<sub>2</sub> (2.1mg/dl) groups respectively and lowest value were found in T<sub>2</sub> (4.66mg/dl) and T<sub>0</sub> (1.80mg/dl) groups respectively.

#### 4.6. Examination of rumen liquor

The physical and chemical parameters of rumen liquor as well as the microbial count were conducted after collection of rumen liquor.

##### 4.6.1. Physical characters

**Table 4.6:** Effect of diet and time on various physical parameters of rumen liquor.

Parameters	Group	Pre-Feeding		Post-Feeding	
		4 h	0 h	4 h	8 h
Color	T <sub>0</sub>	Grey	Grey	Greenish	Grey
	T <sub>1</sub>	Grey	Grey	Grey	Greenish
	T <sub>2</sub>	Grey	Grey	Grey	Grey
Odor	T <sub>0</sub>	Aromatic	Aromatic	Aromatic	Aromatic
	T <sub>1</sub>	Aromatic	Aromatic	Aromatic	Aromatic
	T <sub>2</sub>	Aromatic	Aromatic	Aromatic	Aromatic
Consistency	T <sub>0</sub>	Viscous	Viscous	Viscous	Viscous
	T <sub>1</sub>	Viscous	Viscous	Viscous	Viscous
	T <sub>2</sub>	Viscous	Viscous	Viscous	Viscous
Protozoal	T <sub>0</sub>	++++	++++	+++	++++
Motility	T <sub>1</sub>	++++	++++	+++	++++
	T <sub>2</sub>	++++	++++	+++	++++

++++ = very rapid, +++ = rapid, ++ = moderate movement of rumen flora. T<sub>0</sub>=Control group (Concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass).

### Color:

The color of rumen liquor was found almost mostly grey in both groups in all groups except 4h of post-feeding in T<sub>0</sub> and 8h of post-feeding in T<sub>1</sub> as greenish.

### Odor:

There were no dissimilarities in odor of rumen liquor of all groups and was found as aromatic.

### Consistency:

The consistency of ruminal fluid was found viscous in all groups.

### Motility:

The protozoal motility of almost all groups was very rapid where moderate movement was present in all groups of 4h of post-feeding.

#### 4.6.1.1. Rumen pH

**Table 4.7:** Effect of feeding time and feed on pH of rumen liquor (Mean ± SE)

Time/ Treatment	pH			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
4 hr Pre-Feeding	6.3±0.00	6.45±0.07	6.35±0.07	NS
0 hr Post-Feeding	6.15±0.05	6.35±0.15	6.25±0.05	NS
4 hr Post-Feeding	5.95±0.05	6.15±0.15	5.90±0.10	NS
8 hr Post-Feeding	6.40±0.10	6.50±0.10	6.52±0.15	NS

N=12; SE=Standard Error; NS=Non-Significant (P>0.05). T<sub>0</sub>=Control group (Concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). 0 hr Post Feeding = Just after feeding.

From the above data in table 4.7 it was found that 4h pre-feeding and 0, 4, 8h post-feeding pH value of rumen liquor did not differ significantly among the treatment groups. The pH of the rumen liquor peaked at 8h post-feeding and lowest pH value was attained at 4h post-feeding for all the treatment groups. The pH of the rumen

liquor varied from  $5.95 \pm 0.05$  to  $6.40 \pm 0.10$ ,  $6.15 \pm 0.15$  to  $6.50 \pm 0.10$  and  $5.90 \pm 0.10$  to  $6.52 \pm 0.15$  in  $T_0$  (Control),  $T_1$  (UMS) and  $T_2$  (URS) groups respectively.

#### 4.6.1.2. Bacterial count

Bacterial population was counted from the SRL (Stained rumen liquor) after collecting at 4h of pre-feeding and 0, 4, 8h of post-feeding from each treatment. The values are given in the following table where the number is expressed as (cell x  $10^{10}$ ).

**Table 4.8:** Effect of diet & time on bacterial count/ml of SRL (Mean  $\pm$  SE).

Time/ Treatment	Bacteria (cell x $10^{10}$ )			Sig.
	$T_0$	$T_1$	$T_2$	
4 hr Pre-Feeding	$4.95^a \pm 0.05$	$5.35^b \pm 0.05$	$5.0^a \pm 0.10$	*
0 hr Post-Feeding	$5.05 \pm 0.05$	$4.45 \pm 0.25$	$4.95 \pm 0.05$	NS
4 hr Post-Feeding	$5.05 \pm 0.10$	$5.70 \pm 0.10$	$5.20 \pm 0.20$	NS
8 hr Post-Feeding	$6.10 \pm 0.20$	$6.35 \pm 0.15$	$5.70 \pm 0.20$	NS

N=12; SE=Standard Error; NS=Non-Significant ( $P > 0.05$ ); \*=Significant ( $P < 0.05$ ).  $T_0$ =Control group (Concentrate feed, straw and green grass);  $T_1$ = (UMS, concentrate feed and green grass);  $T_2$ = (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly. 0 hr Post Feeding = Just after feeding.

From the above data in table 4.8 it was found that bacterial count in 0,4 and 8h post feeding did not had significant difference among the treatment groups except bacterial count at 4h pre-feeding was significantly ( $p < 0.05$ ) differed among the treatment groups. The microbial population (cell x  $10^{10}$ ) in case of rumen bacteria ranged from  $4.95 \pm 0.05$  to  $6.10 \pm 0.20$ ,  $4.45 \pm 0.25$  to  $6.35 \pm 0.15$  and  $4.95 \pm 0.05$  to  $5.7 \pm 0.20$  in  $T_0$  (Control),  $T_1$  (UMS) and  $T_2$  (URS) groups respectively. From the above table it was found that the peak value of bacterial count observed at 8h of post-feeding for all groups and decreased to lowest value at 0h of post-feeding for  $T_1$  (UMS) and  $T_2$  (URS) groups and 4h of pre-feeding for  $T_0$  (Control) group.

#### 4.6.1.3. Protozoal count

Both ciliated and non-ciliated protozoal population was counted from the SRL (Stained rumen liquor) after collecting SRL at 4h of pre-feeding and 0, 4, 8h of post-feeding from each treatment.



**Table 4.9:** Effect of diet and time on protozoal count/ml of SRL (Mean  $\pm$  SE).

Time/ Treatment	Protozoa (cell x 10 <sup>6</sup> )			Sig.
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	
4 hr Pre-Feeding	2.51 $\pm$ 0.39	3.12 $\pm$ 0.07	2.31 $\pm$ 0.03	NS
0 hr Post-Feeding	2.08 <sup>a</sup> $\pm$ 0.08	3.24 <sup>c</sup> $\pm$ 0.02	2.90 <sup>b</sup> $\pm$ 0.04	**
4 hr Post-Feeding	2.84 <sup>a</sup> $\pm$ 0.04	4.28 <sup>b</sup> $\pm$ 0.02	3.42 <sup>c</sup> $\pm$ 0.04	***
8 hr Post-Feeding	2.12 <sup>a</sup> $\pm$ 0.02	3.84 <sup>c</sup> $\pm$ 0.05	3.10 <sup>b</sup> $\pm$ 0.02	***

N=12; S.E=Standard Error; NS=Non-Significant (P>0.05); \*\*=Significant (P<0.01); \*\*\*=Significant (P<0.001); T<sub>0</sub>=control group (Concentrate feed, straw and green grass); T<sub>1</sub>= (UMS, concentrate feed and green grass); T<sub>2</sub>= (URS, concentrate feed and green grass). Means with different superscripts in the same row differ significantly. 0 hr Post Feeding = Just after feeding.

From the above data in table 4.9 it was showed that protozoal count in 0h post feeding differed significantly (p< 0.01) among treatment groups and incase of 4 and 8h post-feeding their observed highly significant (p<0.001) variations among treatment groups. The rumen mixed protozoal population (cell x 10<sup>6</sup>) ranged from 2.08 $\pm$ 0.085 to 2.84 $\pm$ 0.045, 3.12 $\pm$ 0.075 to 4.28 $\pm$ 0.02 and 2.31 $\pm$ 0.03 to 3.42 $\pm$ 0.04 per ml of SRL in T<sub>0</sub> (Control), T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups respectively. From above table it was found that the peaked value of protozoal count observed at 4h of post-feeding for all the groups and decreased to lowest value at 4h of pre-feeding for T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups and 0h of post feeding for T<sub>0</sub> (Control) group.

## **Chapter-5: Discussion**

The current study investigated the effect of feeding urea and rice gruel supplemented with rice straw on the production performance of sheep. As far as known few researcher in Bangladesh and abroad worked on the effect of UMB (Urea Molasses Block), UMS (Urea Molasses Straw) or UMS and URS (Urea Rice Gruel Straw) Supplementation in Cattle, Buffalo and others animal for its growth performance, rumen microbial, hematological and sero-biochemical profile but none of them had worked on sheep. So current study was conducted with the hypothesis that feeding urea and rice gruel supplemented straw may influence in live body weight, weekly body weight gain, nutrient digestibility, rumen physiology and microbial profile as well as the hematological and serum biochemical profile of sheep will be furnished. However, the results found in the study will be discussed in this chapter here after.

### **5.1. Effect of UMS and URS supplementation on growth performance of sheep**

#### **5.1.1. Effect on growth performance of sheep**

The final body weight and live weight gain result showed significant effect among the treatment groups where  $T_1$  ranked the higher position where UMS was offered along with other feeds followed by  $T_2$  (URS) and  $T_0$  (Control). The findings of the current study was strongly collaborated with the findings of Chowdhury and Huque (1998) who reported that final body weight was increased in  $T_1$ (UMS),  $T_2$  (URS) compared to  $T_0$  (Control) group. Furthermore, it appears from the result that virtually there was so much change in live weight of sheep of three treatment groups continuously and to change their live weight almost linearly indicating that the effect of UMS and URS in the ration can improve body weight gain compared to control group. Higher growth rate of animals on straw with urea molasses block lick is in agreement with that of the result of Kumar et al. (1983). The result of this experiment also agreed with the result of El-Fouly and Leng (1987) and Sudana and Leng (1986) where they observed a positive response on the daily live weight gain and lower feed conversion ratio in sheep fed with urea molasses block lick to ruminants. In another experiment conducted by Chowdhury and Haque (1998) who reported supplementation of rice gruel yielded medium levels of both energy and protein yielding nutrients with the consequents medium level of growth rate compared to that of the UMS. These kinds

of relationship is obvious as the live weight gain depends mainly on the supply of amino acids and energy yielding substrates delivered to the tissues, up to the genetic limit for protein synthesis (Poppi and McLennan, 1995) the same scenario happened in the current study.

### **5.1.2. Effect on digestibility of different nutrients of sheep**

In digestibility trial, there found highly significant difference in value of DM, CP, Ash and NFE. On the other hands digestibility of EE and CF was differed significant among treatment groups. Highest DM and EE digestibility found in T<sub>1</sub> (UMS) supplemented group. Better CP, CF, Ash and NFE digestibility observed in T<sub>0</sub> (Control) group. This study made close agreement with Misra et al. (2000) who found digestibility of DM, OM and fibre fractions of MS (Mustard straw) improved by the urea treatment and digestibility of DM increased by 16% units in TMS (Treated mustard straw) compared to UTMS (Untreated mustard straw). Similar agreement was also observed with the observations of Trung (1986) and Dajajanegara and Doyle (1989) where sheep and goats were fed urea treated rice straw. Similar improvement in DM digestibility of wheat (Harrera-Saldana et al., 1982), barley (Horton, 1981; Mira et al., 1983) and oat (Horton, 1981) straws by urea treatment has also been observed in cattle. Chowdhury and Haque (1998) was reported that despite higher intake of straw by the UMS and URS fed animals, digestibility of DM and OM in these animals were similar to that of the control group. This study agreement with Can et al. (2004) who reported DM and OM digestibly of wheat straw control diet were found lower than urea and molasses supplemented treatment diets. This may be explained the increment of the microbial populations caused by the urea-molasses supplementation increased OM and DM digestibility. Misra et al. (2000) also reported that urea treatment of MS (Mustard straw) significantly ( $p < 0.01$ ) improved CP intake as well as its digestibility by the ewes. Similar improvements in CP digestibility of urea treated cereals straws have been reported by Prasad et al. (1993). On the other hand, the results of the current study was disagreed with the findings of Can et al. (2004) who reported control diet consuming animals had a lower CP digestibility than urea and molasses supplemented animals.

However, digestibility of different nutrients in sheep was found similar with so many researchers over the globe except CF digestibility which was significantly higher in

the T<sub>0</sub> (Control) group than T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups. One possible reason could be that higher DM intake by the UMS and URS fed animals resulted in increased fractional outflow rate of solid digesta, with the consequent reduction in the fiber digestibility (Hemsley and Moir, 1963).

## **5.2. Effect on hematological changes**

Hematological constituents reflect the physiological responsiveness of animal to its internal and external environment, which includes feed and feeding (Esonu et al., 2001). In this study, Hemoglobin and PCV values of hematological parameter were significantly differed among the treatment groups and there was no significant difference of the values of TEC and TLC among the treatment groups. Hb range in the current study was observed lower the range of 9–15 g/dl reported by (Kaneko, 1997; Patra et al., 2003), but almost similar the values of 5 to 6 g/dl obtained by Belewu and Ogunsola (2010) for goats fed treated *Jatropha curcas* kernel cake rations. Generally, increase in the Hb concentration is associated with greater ability to resist disease infection and low level is an indication of disease infection and poor nutrition (Tambuwal et al., 2002). The Highest PCV value was found in T<sub>1</sub> (UMS) group than T<sub>0</sub> (control) group and T<sub>2</sub> (URS) group respectively. PCV values obtained in this study were within the physiological range of 27.0 – 45.0 % given by Jain (1993), and slightly higher than the range of 25–30% reported by (Opara et al., 2010). In contrast to this, Taiwo and Ogunsanmi (2003) reported higher values (36.9% and 35.5%) for clinically healthy West African dwarf sheep. This result was in agreement with Kioumarsis et al. (2011) who found highest value of PCV (%) in goats fed with the ration contain UMB+MUMB (Medicated urea molasses block) have highest percentages while the goats with MUMB, UMB and the control group ranked second, third and forth respectively.

The result also revealed not significant differences of the percentage of neutrophil, eosinophil, basophil and monocyte among all the treatment groups except lymphocyte that have significant differences among the treatment groups. This result has agreement with earlier report made by Kioumarsis et al. (2011) that fed with urea molasses block in goats had no significant effect among these factors. The neutrophil, eosinophil, basophil, lymphocyte and monocyte levels showed slight and uneven changes in their parameters. The white blood cell differentials (lymphocytes and

neutrophils) levels are comparable among the breed, age and sex groups of animals. The high lymphocyte counts in the animals in this study are favored by the findings of (Milson et al., 1960) and (Wilkins and Hodges, 1962).

### **5.3. Effect on serum biochemical changes**

From the current study it was revealed that the serum glucose and cholesterol had highly significant variation among the treatment groups. The serum glucose concentration was relatively higher in T<sub>0</sub> (Control) group than T<sub>2</sub> (URS) and T<sub>1</sub> (UMS) groups respectively. On the other hand highest serum cholesterol was found in T<sub>2</sub> (URS) supplemented group than T<sub>0</sub> (Control) and T<sub>1</sub> (UMS) groups respectively which was not agreed with the study of Kioumarsis et al. (2011) who found that serum glucose and cholesterol appeared insignificant that fed with urea molasses block in goats.

The serum total protein concentration had significant variation in T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups compared to T<sub>0</sub> (Control) group. Result for total protein which strengthens the study of Muralidharan et al. (2011) who found that urea molasses mineral block supplementation increased total protein concentration in sheep. Muralidharan et al. (2011) was found that animals fed with more proteins in the diet through concentrate feed and UMMB (Urea molasses mineral block) had higher total protein levels. This finding of the current study was in close agreement with Dhore et al. (2005); and Abdel-Hameed et al. (2013). The total protein in the blood can be affected by various different factors. The serum albumin concentration was significantly higher in T<sub>2</sub> (URS) group followed by T<sub>1</sub> (UMS) and T<sub>0</sub> (Control) groups. Serum albumin in this study ranged from 2.04 -2.65(g/dl) which was agreed with the statement of Coles (1986). This result was in agreement with Muralidharan et al., (2011) who found that Serum albumin levels was significantly differed and higher values in (Grazing + concentrate feed) group than free grazing group and (garzing + UMMB) group in a experiment of sheep.

Similarly, serum calcium concentration was significantly differed among the different treatment group and highest value found in T<sub>2</sub> (URS) group than T<sub>0</sub> (Control) and T<sub>1</sub> (UMS) groups respectively which was moderately agreed with Muralidharan et al., (2011) and disagreed with the findings of Kioumarsis et al., (2011). Serum Phosphorus

concentration was significant and higher values found in T<sub>1</sub> (UMS) group which was in close agreement with Muralidharan et al., (2011) and disagreed with the findings of Kioumarsis et al., (2011). The serum Sodium and Potassium levels in this study were within the range of 107.75-139.15mmol/l for Sodium and 4.3-6.1mmol/l for potassium, which compares with the report of Borjesson (2000). This study result of all electrolytes (Na, K, Mg, Cl, Ca and Mg) were significant among the treatment groups which come to the agreement with Muralidharan et al. (2014); Oboh and Olumese, (2008) and contradict with Davies et al. (2007) and Hatfield et al. (1998).

#### **5.4. Effect on rumen ecology**

In the present investigation, T<sub>2</sub> (UMS) group was highest irrespective of post-feeding intervals as compared to T<sub>0</sub> (Control) and T<sub>1</sub> (URS). It might be due to higher secretion of alkalizing agents through saliva, (Hasanuzzaman et al., 2014). The color, odor, consistency, motility shown in table 4.6 were within the physiological limit as supported by Radostits et al. (2000) and Hasanuzzaman et al. (2014). The microbial population (cell x 10<sup>10</sup>) in case of rumen bacteria ranged from 4.95±0.05 to 6.10±0.20, 4.45±0.25 to 6.35±0.15 and 4.95±0.05 to 5.7±0.20 in T<sub>0</sub> (Control), T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) group respectively which recommended by Kurihara et al. (1967). The bacterial population attained peak level at 8h of post-feeding and lowest values found at 4h of pre-feeding (Hasanuzzaman et al., 2011). These results were supported by Thakur (2006); Kurihara et al. (1967) and Hasanuzzaman et al. (2014). The rumen mixed protozoal population (cell x 10<sup>6</sup>) ranged from 2.08±0.085 to 2.84±0.045, 3.12±0.075 to 4.28±0.02 and 2.31±0.03 to 3.42±0.04 per ml of SRL in T<sub>0</sub> (Control), T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) groups respectively. From table 13 it was found that the peaked value of protozoal count observed at 4h of post-feeding for all the groups and decreased to lowest value at 4h of pre-feeding for T<sub>1</sub> (UMS) and T<sub>2</sub> (URS) and 0h of post-feeding for T<sub>0</sub> (Control) group. The concentration of protozoal population in the SRL (Stained rumen liquor) was supported by the (Kurihara et al. 1967).

## Chapter-6: Conclusions

In Bangladesh, most of the village people rear small ruminants-sheep and goats especially around homes by feeding them kitchen wastes or most of the times leaving them to graze on surrounding herbs and shrubs. It can't full fill the demand of nutrition of sheep or goats. There are many unconventional feed resources present in our country which is not properly identified. So the current study was designed to identify the effect of feeding urea and rice gruel supplemented straw on the production performance of sheep.

The experiment was conducted during the period of January -June, 2016. A total of 12 indigenous sheep of about fourteen months of age with an average body weight of  $15.14 \pm 0.07$  kg were distributed into three treatment groups and supplemented with urea, molasses and rice gruel with rice straw arranged in a Completely Randomized Design.

The study identifies that body weight gain was significantly varied among the treatments groups. Highest body weight gain was observed in T<sub>1</sub> (UMS) group followed by T<sub>2</sub> (URS) and T<sub>0</sub> (Control) groups. In case of growth performance, rice gruel with urea was found efficient as like as molasses with urea when supplemented 3% level of urea and 15% level of molasses or rice gruel with rice straw. In nutrient digestibility, highest value of DM and EE were found in T<sub>1</sub> (UMS) group. On the other hand highest value of CP, CF, Ash and NFE were found in T<sub>0</sub> (Control) group.

In hematological study, Hemoglobin and PCV result showed significantly differed among the treatment groups where highest value was found in T<sub>1</sub> (UMS) group. Different blood cell observed no significant difference among the treatment groups except lymphocyte. Among the serum biochemical parameters the glucose, total protein, albumin and cholesterol were significantly varied among the treatment groups. The highest value of glucose and total protein were found in T<sub>0</sub> (Control) and T<sub>1</sub> (UMS) groups respectively. While the highest value of albumin and cholesterol were found in T<sub>2</sub> (URS) group. Different minerals level in blood was significantly differed among the treatment groups. The highest level of P and K were found in T<sub>1</sub> (UMS) group. Ca, Mg and Cl were significantly higher in T<sub>2</sub> (URS) group, while highest level of Na was found in T<sub>0</sub> (Control) group.

In the rumen ecological study, pH value in different feeding time had no significant difference among the treatment groups. The pH of the rumen liquor peaked at 8h post-feeding and lowest pH value was attained at 4h post-feeding in all the treatment groups. Bacterial and protozoal count in rumen liquor showed significant difference among the treatment groups.

The present study provide evidence that rice gruel together with urea can improve productivity of sheep on an absolute rice straw based diet. However, as supplement, molasses was much more effective than rice gruel apparently due to higher fermentable carbohydrate and mineral content in the former. However, in situation where molasses is not available or costly, rice gruel does appear to have a place as readily fermentable energy source on urea supplemented straw diet.



## **Chapter-7: Recommendation**

This study on the investigation of effect of feeding urea and rice gruel supplemented straw on the production performance of sheep suggests the following recommendations:

- URS (Urea rice gruel straw) supplemented with straw showed increased body weight gain, improved rumen ecological, hematological and biochemical parameters in blood. So, farmers can add URS in sheep ration for better digestibility and growth of sheep.

### **Future perspective**

The current study only indicates 3% urea +15% rice gruel and 3% urea + 15% molasses with straw and duration of experimental trial was 10 weeks. The supplementation level could be increased in higher level of rice gruel and duration could also be increased for further study. Some extra parameters of hematological and biochemical study could be taken into consideration. Effect of male and female reproduction of sheep and meat composition was not studied in this study so further study could include reproduction and meat composition effects by supplementing URS in sheep.

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## Annexure 1: Serum Biochemical Study

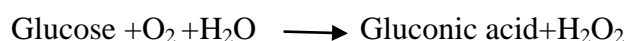
### Biochemical tests

Different biochemical test were performed using the commercial kits of RANDOX company (<http://www.randox.com/reagent>). The biochemical tests were performed according to manufacturer's direction. A brief description of procedure is given below:

### Estimation of serum glucose:

**Principle:** Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (GOD).The hydrogen peroxide formed reacts, under catalysis of preoxidase (POD), with phenol and 4-aminophenazone to form a red-violet quineimine dye as indicator.

### Reaction



### Procedure

The sterile eppendorf tubes were taken. Then 1000 $\mu$ l glucose reagent was taken in an eppendorf tube and 20  $\mu$ l of sample serums were taken in each eppendorf tube. The eppendorf tube was then kept in room temperature for 20 minutes. Then all eppendorf tubes containing sample serum reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

The test was then run with water blank and glucose standard provided by manufacturer. Absorbance of sample and standard was performed against reagent blank with the wavelength 500 nm and expressed as mg/dl after calculation as follows-

$$\text{Glucose conc.} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{standard conc. (mg/dl)}$$

## Serum Calcium

### Principle:

Calcium ions form a violet complex with O- Cresolphthalein complexone in an alkaline medium.

### Reagents

All reagents were pre-prepared and ready for use. The buffer and chromogen were mixed together and kept at +2 to +8°C.

### Procedure:

After measuring the sample absorbance ( $A_{\text{sample}}$ ) according to the assay procedure, one drop of EDTA was added to the sample to make it colorless. After 10 second the absorbance of sample was taken again.

Therefore,  $A_{\text{sample}}(\text{Corrected}) = A_{\text{sample}} - A_{\text{sample/EDTA}}$

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Pipette into test tubes

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	Reagent Blank	Standard	Sample
Sample	-	-	25 $\mu$ l
Distilled Water	25 $\mu$ l	-	-
Standard	-	25 $\mu$ l	-
Working Reagent	1.0 ml	1.0 ml	1.0ml

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The absorbance of the sample ( $A_{\text{sample}}$ ) and standard ( $A_{\text{standard}}$ ) against the reagent blank were measured after 5 to 50 minutes.

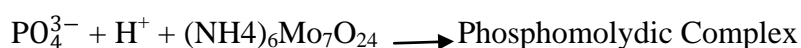
### Calculation:

$$\text{Concentration (mmol/L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 2.50$$

$$\text{Concentration (mg/L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 10$$

### **Serum Phosphorus**

Inorganic phosphate reacts with aluminium molybdate in the presence of sulfuric acid to form phosphomolybdic complex which is measured at 340 nm.



Absorbance of sample and standard was measured against reagent blank at 340 nm

### **Estimation of Total Protein:**

Principle: Cupric ions form chelates with the peptide bonds of proteins in an alkaline medium. Sodium potassium tartrate keeps the cupric ions in solution. The intensity of the violet colour that is formed is proportional to the number of peptide bonds which, in turn, depends upon the amount of proteins in the specimen.

### **Reagents**

- (i) Biuret Reagent – 3 gm of copper sulphate is dissolved in 500 ml of water. 9 gm of sodium potassium tartrate and 5 gm of potassium iodide are added and dissolved. 24 gm of sodium hydroxide, dissolved separately in 100 ml of water is added. The volume is made up to 1 litre with water. The reagent is stored in a well-stoppered polythene bottle.
- (ii) Biuret blank – this is prepared in the same way as the biuret reagent with the difference that copper sulphate is not added.
- (iii) Standard protein solution – the best way is to determine the total protein concentration in pooled human serum by Kjeldahl method, dilute it to bring the protein concentration to the desired level, say 6 gm/100 ml and use it as standard. Alternatively, a 6 gm/100 ml solution of bovine albumin in water may be prepared and used as standard.

Procedure: label 3 test tubes 'Unknown', 'Standard' and 'Blank', Measure 5 ml of biuret reagent into each. Wash 0.1 ml of serum into 'Unknown', 0.1 ml of standard protein solution into 'Standard' and 0.1 ml of water into 'Blank'. Mix and allow to stand for 30 minutes.

Read 'Unknown' and 'Standard' against 'Blank' at 540 nm or using a green filter.

Calculations:

$$\text{Serum total protein (gm/dl)} = 6 \times \frac{A_u}{A_s}$$

**Estimation of serum Albumin:**

**Bromocresol Green Method:**

**Principle:**

The method is based on the protein error of indicators. Binding of a protein to an indicator changes its colour. Among serum proteins, only albumin binds to BCG this binding produces a change in the colour of BCG which is measured colorimetrically. The pH is maintained during the reaction by a buffer.

**Reagents**

- (i) Succinate buffer - 11.8 gm of succinic acid is dissolved in about 800 ml of water. The pH is adjusted to 4.0 with 0.1 N sodium hydroxide. The volume is made up to 1 litre with water. This solution should be stored in refrigerator.
- (ii) BCG solution - 419 mg of bromocresol green is dissolved in 10 ml of water. The solution is stored in refrigerator.
- (iii) Buffered BCG solution – 250 ml of BCG solution is mixed with 750 ml of succinate buffer. The pH is adjusted to 4.2 with 0.1 N sodium hydroxide solution. 4 ml of Brij – 35 solution (30%) is added.
- (iv) Standard albumin solution – an aqueous solution of human albumin with a concentration of 4 gm/100 ml can be prepared and used as a standard. Sodium azide should be included in this solution (50 mg in every 100 ml) as a

preservative. Pooled human serum (preserved with sodium azide) or a control serum having an albumin concentration of 4 gm/100 ml can also be used as a standard.

**Procedure:**

Level 3 test tubes 'Unknown', 'Standard' and 'Blank'. Measure 4 ml of buffered BCG solution into each. Wash 0.02 ml of serum into 'Unknown', 0.02 ml of standard albumin solution into 'Standard' and 0.02 ml of water into 'Blank'. Mix and allow the tubes to stand for 5 minutes.

Read 'Unknown' and 'Standard' against 'Blank' at 630 nm or using a red filter.

Calculations:

$$\text{Serum Albumin (gm/dl)} = 4 \times \frac{Au}{As}$$

**Estimation of serum cholesterol:**

**Test Principle:**

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase.

**Procedure:**

Both reagent and sample brought at room temperature and mixed 1.0 ml reagent with 10µl sample in test tube. Let waited for 10 minutes and placed mixture in cuvette. The cuvette was sated in spectrophotometer at 550 nm and recorded the reading. The reading was calculated by comparing with standard value and multiplied by 200mg/dl. So the result was expressed as mg/dl.

## Annexure 2: Ration formulation of sheep

Group Name	Sl. No	Body weight (kg)	3% DM (gm)	40% Concentrate of DM (gm)	Fresh weight of concentrate (gm)	60 % Roughages of DM (gm)	50% Green Grass DM (gm)	Fresh weight of green grass (gm)	50% Rice Straw ,URS and UMS of DM (gm)	Fresh weight of rice straw, UMS and URS
Group T <sub>0</sub> : Control	1	14.96	449	180	200	269	134	536	135	154
	2	15.28	458	183	203	275	137	548	138	157
	3	15.42	463	185	205	278	139	556	139	158
	4	14.98	450	180	200	270	135	540	135	153
Total					<b>808</b>			<b>2180</b>		<b>622</b>
Group T <sub>1</sub> : UMS	5	15.3	459	184	204	275	138	552	137	238
	6	15.54	466	186	207	280	140	560	140	243
	7	14.89	447	179	199	268	134	536	134	232
	8	14.92	448	179	199	269	135	540	134	232
Total					<b>809</b>			<b>2188</b>		<b>945</b>
Group T <sub>2</sub> : URS	9	15.2	456	182	202	274	137	548	137	238
	10	15.42	463	185	205	278	139	556	139	241
	11	14.94	448	179	199	269	134	536	135	235
	12	14.87	446	178	198	268	134	536	134	233
Total					<b>804</b>			<b>2176</b>		<b>947</b>

## **Brief Biography of the author**

This is **Md. Emdadul Haque**; son of **Md. Shamaul Haque** and **Parul Akter** from Parshuram Upazilla under Feni district of Bangladesh. He has passed the secondary school certificate examination in 2005 followed by higher secondary certificate examination in 2007. He obtained his Doctor of Veterinary Medicine Degree in 2013 (held in 2015) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh with CGPA 3.42 (Out of 4.00). Now, he is a candidate for the degree of MS in Animal and Poultry Nutrition under the Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, CVASU.