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NUTRITIVE VALUE AND PRODUCTION PERFORMANCE OF LIMPO GRASS (*Hemarthria altissima*) IN DAIRY CATTLE

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ABSTRACT

In developing countries rice straw and other different low quality forages are used as basal feed of ruminants. Animal longevity and production are adversely affected when ruminants are reared with poor quality forages. High quality forages can offer complementary properties for their use to formulate forage based balanced diets to optimize the degradability and utilization of low quality forages. Limpo grass is potential as a forage crop which can tolerate wet soils and intermittent flooding during warm conditions may be adaptable with the sudden flash flood condition of this region. In the present study, chemical analyses of rice straw and limpo grass were carried out. Rice straw was lower in crude protein, ether extract but higher in fibre contents than Limpo grass. *In vitro* and *in vivo* digestibility trials were conducted to test the potential use of Limpo grass as in ruminant diets. Limpo grass was added at 0, 250, 500, 750, 1000g/kg level with rice straw in different incubation times (0h, 6h, 24h and 48h). In vitro DM and OM degradability of rice straw increased in the presence of limpo grass. In vivo study showed that milk production did not changed by replacing Limpo grass, however fat% and total solid% increased in presence of Limpo grass. It could be concluded that the digestibility and utilization of low quality forage could be improved by supplementation of limpo grass.

Keywords: Limpo grass, *In vitro* degradability, milk fat percentage

1. INTRODUCTION

Bangladesh is a developing country and the economy of this country mainly based on agriculture. The term agriculture is combination of crop, livestock, fisheries and forestry. Livestock plays a vital role in the agricultural economy. Rearing large or small animals

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especially ruminants are one of the most appropriate income generating activities for rural women especially for landless and marginal farmers. It plays an important role in the rural economy.

Shortage of feed was identified as one of the primary causes of low productivity of animals (Roy *et al.*, 2012). In Bangladesh, there is a requirement of 70 mmt of green grass for cattle feed in a year but produced only 24 mint. Thus, there is a deficit of animal feeds for about 60 percent, which are hampering the livestock development to a great extent (Roy *et al.*, 2012). The scarcity of fodder is one of the limiting factors for increasing milk production on uprising small-scale dairy farms.

The main constraint to forage production is the scarcity of land. Usually farmers do not spare cultivable land for fodder production. In Bangladesh poor farmer does not provides the high quality forages especially green grass for feeding their ruminants and due to increasing the population number the cultivated and agricultural land is diminishing day by day. As a result the pasture land is not available and hence the farmers depend on the low quality forages especially agricultural by products such as cereal straws, sugar-cane top, bagasse, tree leaves, road side grasses and stover etc. For example in Bangladesh the total area for fodder is about 6,312 hectare, producing only about 47,000 metric tons of fodder crops whereas very little grain is available for feeding the animals in the country. It is estimated that about 0.19 million tons of grain are available for livestock feeding, contributing only about 15.7 % of the total amount of concentrate feed required for farm livestock in Bangladesh (Khan, and Chaudhry, 2012). Rice straw is low in protein, fat, minerals, vitamins and other nutrients. The productions are adversely affected when ruminants are reared with these poor quality forages like rice straw (Khan and Chaudhry, 2012). Feeding ruminants with rice straw only will cause live weight and health loss (Sarnklong *et al.*, 2010). Khan and Chaudhry (2011) suggested that the selected forages can offer complementary properties for their use to formulate forage based balanced diets to optimize the degradability and utilization of LQF in ruminants.

However, different studies showed that in milk pocket areas, the dairy farmers started cultivating fodder instead of cereal crops. The farmers of Munshigonj kept cultivable land for local grass known as Baksa (Limpo grass) and they fed the animals throughout the year by preserving them in a stack or heap. Now-a-days some of the farmers in the study areas started cultivation of high yielding Napier (*Penisetum purpureum*) varieties on some plots of their blocked land for high yielding variety of rice. Irrespective of areas, farmers started fodder marketing, which was absent in the past. This development occurred with the increase of demand and knowledge on fodder cultivation.

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Climate change acts as another barrier of sustainable livestock production in Bangladesh (Chawdhury *et al.*, 2016). The country is one of the highest vulnerable countries in the world for climate change (UNDP, 2009). The Sylhet Division (North East Part of Bangladesh) consists of some hilly areas and hundreds of haors and beels varing in size from a few hectares to several thousand hectares. In monsoon these haor basins remain full of flood water but in winter remains dry and remain bare, so ruminants cannot get quality forage. As a result, overall livestock production is very poor. In 2016 and 2017 early flood washed away green grasses available at road side. To get optimum production it is essential to provide green fodders to the ruminants. In Sylhet district, there are some rural areas where farmers are very poor and animals are suffering from mal nutrition during lean period (rainy and winter season). So they need alternative techniques to improve their socio-economical status.

Limpo grass is a warm season perennial grass used in dairy cattle. In Bangladesh Limpo grass is known as Baksha grass. Limpo grass is potential as a forage crop which can tolerate wet soils and intermittent flooding during warm conditions which may be adaptable with the sudden flash flood condition of Sylhet region. It is also preferable to ruminants than any other summer grass with a high palatability and in vitro digestibility of 70% (Newman et al., 2009) in compared with the other grasses.

To our knowledge, the chemical composition and feeding value of these forages have never been compared in a single study where growing conditions and fertilization practices were standardized across all forage types. The current study was designed to test our hypothesis, which stated that significant differences exist in the feeding value, relative of these commonly used tropical forages. The present study was conducted to evaluate nutritive value of Limpo grass in different places of Sylhet region in Bangladesh and to examine the effect of Limpo grass to improve degradability and utilization of rice straw in dairy cattle. The results of this research will assist cattle producers within the subtropics to better utilize various pasture forage options. In addition, these data will assist in the development of supplementation programs designed to fortify the nutritional needs of Limpo grass.

2. MATERIALS AND METHODS

2.1 Collection of forages

Limpo grass (*Hemarthria altissima*) and rice straw (*Oryza sativa*) (RS) were selected on the basis of their availability, low cost and potential for use as feed materials. Representative samples of RS was collected from Friends Dairy Farm, Khadimnogor, Sylhet. Limpo grass was collected from nearest hilly areas of Friends Dairy farm, Khadimnogor, Sylhet. Limpo Grass was also collected from Plain land and haor (locally named water body in Bangladesh) areas.

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2.2 Grinding the forages

The representative samples of forages (rice straw and grass) were dried in the sun. The feed samples also dried again in an electric oven (WTB binder) at 90^{o}C for 24 hours. After drying rice straw and grass were grounds separately to approximate 2 mm size by using an electric grinder (blender machine) and measured by analytical balance (Setra, M- EL- 410s, USA). A part of these samples were taken for chemical analysis and rest were used for experiment.

2.3 Chemical composition of rice straw and Limpo Grass

For chemical analysis, respective samples of feed were analyzed for determination of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), and ash/ total mineral following the methods of AOAC (2004). Organic matter (OM) and nitrogen free extract (NFE) were also estimated.

2.4 In vitro trial

2.4.1 Collection of Rumen Fluid and Preparation of Rumen-Buffer solution

The rumen fluid was collected from immediately after slaughtered mature cattle. The rumen was opened with the help of scalpel and the rumen fluid was collected as soon as possible. The fluid was then transferred into a flask after filtering with a filtering cloth. For preparing the flask hot water was put in it to maintain the rumen temperature and dropped out the hot water before pouring the rumen fluid into the flask. The phosphate-bicarbonate buffer solution was made by following (McDougall, 1948) formula for synthetic saliva as mentioned by Khan and Chaudhury[7]. The solution was prepared by dissolving the chemicals in water and the pH of the solution was recorded 6.9. Then the whole prepared solution was screw capped and kept at 38-39^{o}C in a water bath (WB 10, Germany). After collecting the rumen fluid it was filtrated again with two layers of cotton cloth to remove the large feed particles. Then the rumen fluid and buffer solution were mixed at the rate of 3:1 (buffer: rumen fluid). For preparing 3000 ml solution 2250 ml buffer were added with 750 ml rumen fluid. Then the prepared rumen buffer solution was transferred into bottles which were covered with black polythene sheet.

2.4.2 Experimental design

A duplicate was used to assess the degradability of two forages (rice straw and limpo grass) each at five levels of 0, 250, 500 750 and 1000 mg/g DM for each of the four different times (0, 6, 24 and 48 h) for the experiment.

2.4.3 *In vitro* incubation

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The incubations of forages were conducted in 50 ml polypropylene tubes each containing about 0.4 g of ground forage to which 0.4, 0.3, 0.2, 0.1 and 0 g rice straw and 0, 0.1, 0.2, 0.3 and 0.4 g limpo grass respectively according to the experimental design. Then 40 ml of rumen buffered fluid were added to each tube. The tubes were sealed with rubber stoppers fitted with pressure release valves. Incubation was conducted at 39^{o}C in a temperature controlled water bath. After 0, 6, 24 and 48 hour the tubes were collected from water bath and waterlogged in an ice box to stop further fermentation. Then the pH of the buffered rumen fluid was measured immediately with a pH meter (Jenway Ltd, model 3340 Ion Meter). The liquid and residue were separated by filtering with a filter paper and filter cloth. The supernatant of the buffered rumen fluid was collected to determine ammonia concentration in rumen fluid. For ammonia concentration 20 ml of supernatant were acidified with 10 ml of 1 (N) HCl and kept in a tube. Residues were washed with distilled water and used to determine DM and OM to estimate their respective degradabilities.

2.4.4 Determination of DM and OM degradability

The *in vitro* DM (IVD) and OM degradability (IVOMD) were determined according to Khan and Chaudhury (2010).

2.4.5 Determination of Ammonia-Nitrogen in Rumen Fluid

The acidified rumen fluid samples which were kept in a sample tube analyzed for determining the ammonia nitrogen (NH_3-N) concentration in rumen fluid by using kjeldahl method. For determining the NH_3-N, at first 20 ml 2% boric acid solution were taken into 250 ml conical flask and mixed 1 drop mixed indicator (Methylene blue and Methylene red) then placed it into distillation set. Secondly 40 ml NaOH (40%) solution were taken into kjeldahl flask where 30 ml acidified rumen fluid samples were taken. Then some glass rod & zinc pieces added into the flask and placed into the upper portion of distillation set and allowed to distillation the sample. After completed the distillation the sample was then allowed to titration with 0.1N HCl and when the pale greenish color removed the titration value was calculated. Finally the NH_3-N concentration of rumen fluid was determined by following formula:

NH_3-N level = 0.1 × 0.014 × 1000 × 30/20 × Titration value.

2.5 Assessment of milk production and milk quality after using limpo grass

2.5.1 Animals, Housing and Feeding routine

A total of six crossbred (Holstein Friesian x local) dairy cows were used to assess the milk production and milk quality after using limpo grass. They were housed on concrete floor tin

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shaded roof at Friends Dairy Farm, FIVDB (Friends in Village Development Bangladesh), Khadimnogor, Sylhet. Experiment was conducted for a period of 3 weeks. Three of the cows were in control group and three were in experimental group. In 1st week, both groups of cows i.e. six cows were given roadside grass with rice straw. In 2nd week, control group cows were given roadside grass with rice straw and experimental group were given 50% Limpo grass and 50 % roadside grass with rice straw. In 3rd week same procedure was maintained like 2nd week. Animals were kept at a fixed level of feeding (750 g/day/animal) with 2:1 chopped forage: concentrate diet to fulfill their maintenance requirement[1].

2.5.2 Collection of milk sample

Milk sample was collected every Saturday and Tuesday from the six cows in every week. The animals of both control and experiment group were observed in close supervision during the periods of experiment.

2.5.3 Chemical analysis of milk

After collection, the qualities of collected milk samples were evaluated by conducting several tests in the Laboratory. The tests were as follows: Fat (%), Total solids (TS) content (%), Solids not fat (SNF) content (%), Protein content (%).

2.5.4 Determination of fat (%) in milk sample

Fat percentage was determined by Gerber Fat test method, according to the procedure describrd by Eckles, *et al*. (1951). At first 10 ml concentrated sulfuric acid (Sp. Gr.1.82) was taken in butyrometer with the help of pipette. Then 11ml of milk was added gently into the concentrated sulfuric acid containing butyrometer and 1 ml amyl alcohol was taken in butyrometer. The open end of the butyrometer was closed by lock stopper with the help of key. The butyrometer containing sample was mixed thoroughly and then placed in the Gerber centrifuge machine for 5 minutes at 1100 rpm. The fat percentage was estimated from the upper meniscus of the fat column in the butyrometer.

2.5.5 Determination of protein content of milk by formal titration method

Protein was determined by formol titration method (Bennenberg *et al*., 1949). 10 ml of milk were taken in a small beaker or white cup to which 10 ml of distilled water, 0.4 ml aqueous potassium oxalate solution and 1 ml of 1% alcoholic phenolphthalein indicator were added and mixed them carefully with the help of stirrer. After mixing there were allowed to stand for 2 minutes and it was titrated against 0.1N NaOH solution. The required amount of NaOH was recorded. After that 2 ml of 40% formaldehyde (H-CHO) solution was added with the mixed sample and the

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pink color was disappeared to white. Again the milk sample was titrated against the same NaOH solution. The amount of NaOH (0.1N) solution required in the second titration was found by subtracting the first reading from the second reading. A blank was run by titrating 2 ml of 40% formaldehyde solution plus 10 ml distilled water with 0.1N NaOH. The ml of 0.1N NaOH solution used was multiplied by 1.74 factors to obtain percent protein in milk sample.

% Protein= $(V_a - V_b) \times 1.74$

$= \{(R_3 - R_2) - (R_4 - R_3) \times 1.74$

Here,

R_2 = First titration value
R_3 = Second titration value
R_4 = Blank titration value
1.74= formal factor for cow's milk.

2.5.6 Determination of Solids-not-fat and Total Solids content of milk sample

Total solids of the milk samples were determined by oven drying method as described by AOAC[2]. The SNF content and total solids contents were estimated using the Gerber method-

$$\text{SNF \%} = \frac{\text{CLR}}{4} + (0.2 \times \text{Fat \%})$$

Total solids %=Fat% + SNF%

Where,

CLR= Corrected lactometer reading and
F = Fat percentage of the milk by Gerber method.

2.6 Statistical Analyses

The data were analyzed by completely randomized design (CRD) using General Linear Model of Minitab. The effects of different level of forages were considered for IVD, IVOMD and ammonia level. Again, CRD using General Linear Model of Minitab were used to analyze milk production and milk quality after using limpo grass and road side grass with rice straw. Significant differences between means for each main effect were compared by using the Tukeys test at $P<0.05$.

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3. RESULTS

3.1 Chemical composition of roughages

The proximate composition of rice straw and limpo grass (baksha grass) from 3 different areas of Sylhet region used during the experimental period were shown in the Table 1. The DM and CF were higher in rice straw than limpo grass however CP was higher in limpo grass than rice straw and no difference for ash. The limpo grass from water land contained more DM and CP than plain land and hilly areas. But CF and Ash is less than those two areas.

Table 1: Chemical composition (g/100g DM) of roughages

Feed items	DM	CP	CF	EE	Ash
Rice straw	91.41	3.56	31.37	1.2	7.41
limpo grass (plain land)	22.89	6.13	27.25	2.0	8.08
limpo grass (hilly area)	18.69	6.59	25.42	1.6	7.97
limpo grass (Water land)	24.41	7.12	24.51	1.4	7.16

3.2 *In vitro* dry matter degradability at different incubation time

Table 2 shows IVD of rice straw and limpo grass at different incubation times. The IVD of forages were highest at 48 h. Higher portion of limpo grass also increased the IVD of forages. The degradability of limpo grass alone was higher than degradability of rice straw alone and mixer of rice straw and limpo grass. Mixer of limpo grass had higher degradability than rice straw alone.

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Table 2: In vitro dry matter (DM) degradability of rice straw with Baksha grass at different incubation time

Treatment	**DM**			
	0 hr	6 hr	24 hr	48 hr
T_0 (rice straw + baksha) 4.0+0	231.7 ab	240.8 b	277.5 b	315.8 b
T_1 (rice straw+ baksha) 0.3+0.1	220.0 b	237.5 b	280.0 b	310.0 b
T_2 (rice straw + baksha) 0.2+0.2	239.2 a	239.2 b	290.0 ab	333.3 a
T_3 (rice straw + baksha) 0.1+0.3	241.7 a	246.7 a	295.0 a	332.5 a
T_4 (rice straw + baksha) 0+0.4	237.5 a	249.2 a	297.5 a	339.2 a
Level of significance	*	**	*	**

The values are expressed as the Mean ± SE. ** = Significant at ($p<0.01$). NS= Non significant. Figures with similar superscripts mean did not differ significantly among respective groups. Figures with dissimilar superscripts mean differed significantly among respective groups as per DMRT.

3.3 In vitro organic matter degradability at different incubation time

Table 3 shows IVOMD of rice straw and limpo grass at different incubation times. IVOMD was highest for T2 where rice straw and baksha are treated with equal proportion (0.2+0.2). In vitro OM degradability of forages were highest at longer incubation time.

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Table 3: In vitro organic matter (OM) degradability of rice straw with Baksha grass at different incubation time

Treatment	**OM**			
	0 hr	6 hr	24 hr	48 hr
T_0 (Rice straw + Baksha) 0.4+0	312.6 b	350.5 b	397.5[a]	396.8 b
T_1 (Rice straw + Baksha) 0.3+0.1	308.0 b	371.3 ab	393.9[a]	436.3 a
T_2 (Rice straw + Baksha) 0.2+0.2	373.4 a	430.9 a	356.8 b	433.6 a
T_3 (Rice straw + Baksha) 0.1+0.3	319.8 ab	346.6 b	365.9 b	420.0 b
T_4 (Rice straw + Baksha) 0+0.4	295.4 b	346.0 b	394.5a	416.4 b
Level of significance	NS	NS	*	**

The values are expressed as the Mean ± SE. ** = Significant at ($p<0.01$). NS= Non significant. Figures with similar superscripts mean did not differ significantly among respective groups. Figures with dissimilar superscripts mean differed significantly among respective groups as per DMRT.

3.4 Ammonia nitrogen concentration at different incubation time

Ammonia nitrogen concentration was higher at higher portion of baksha grass (Table 4). Ammonia nitrogen concentration also increased at higher incubation time.

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Table 4: In vitro NH3-N concentration

Treatment	**NH_3N**			
	0 hr	6 hr	24 hr	48 hr
T_0 (Rice straw + baksha) 0.4+0	52.00 b	62.83 c	61.17 c	63.50 c
T_1 (Rice straw+ baksha) 0.3+0.1	52.83 ab	62.17 d	72.00 b	82.17 c
T_2 (Rice straw + baksha) 0.2+0.2	53.33 ab	73.33 b	72.33 b	83.83 c
T_3 (rice straw + baksha) 0.1+0.3	52.00 b	76.20 a	83.83 a	93.17 b
T_4 (Rice straw + baksha) 0+0.4	54.50 a	82.83 c	91.50 b	135.7 a
Level of significance	*	**	**	**

The values are expressed as the Mean ± SE. ** = Significant at ($p<0.01$). NS= Non significant. Figures with similar superscripts mean did not differ significantly among respective groups. Figures with dissimilar superscripts mean differed significantly among respective groups as per DMRT.

3.5 Effect of Limpo grass supplementation in milk production and milk quality

Total milk production in control group and experimental group had been shown in Table 5. There was no significant difference for milk production of limpo grass. The chemical compositions of milk were shown in Table 6. The fat percentage was significantly higher ($P<0.001$) in treatment group i.e. in the presence of limpo grass than control (Fig 1). The protein and solid not fat (SNF) percentage had no difference between control and treatment (Table 6). The total solid percentage was also significantly higher ($P<0.001$) in treatment group than control (Fig 2).

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Table 5: Total milk Production (avg) in 3 weeks

Cow no.	1st week(avg L)	2nd week(avg L)	3rd week (avg L)
Control group			
1	17	18	18
2	18	17	18
3	18	17	18
Experimental group			
4	18	19	19
5	17	18	19
6	18	18	18

Table 6: Chemical compositions (g/100g DM) of spices milk

	Fat %		CP%		SNF%		Total solid%	
	Cont	Treat	Cont	Treat	Cont	Treat	Cont	Treat
1st wk	3.51	3.52	2.94	3.02	8.70	8.74	12.20	12.26
2nd wk	3.59	4.00	3.26	3.29	8.30	8.66	11.89	12.66
3rd wk	3.34	5.50	3.42	3.28	8.39	7.97	11.73	13.47

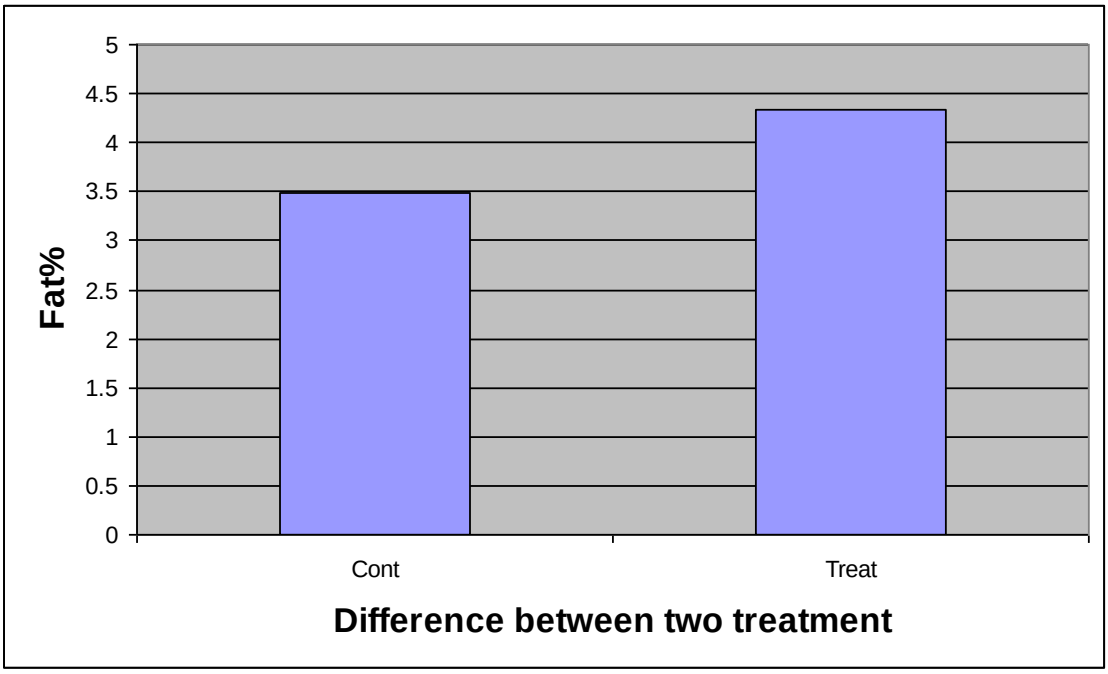

Figure 1: Fat percentage of milk of two treatment group

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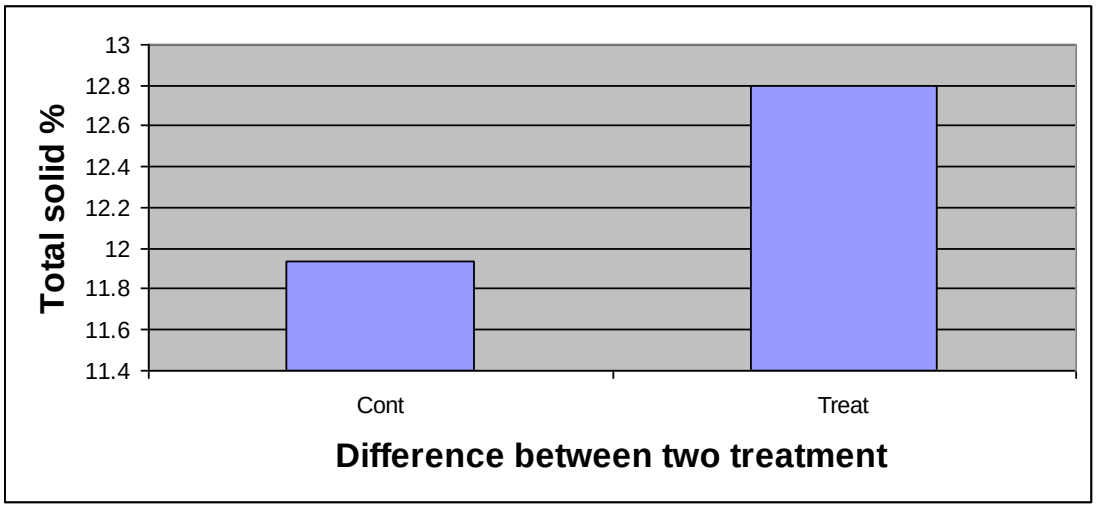

Figure 2: Total solid percentage of milk of two treatment group

4. DISCUSSION

Rice straw is an exceptional LQF containing very low levels of lignin than other forages. Normally rice straw contains high level of ash[8] than other forages and it might have been partly due to the higher amount of silica(khan and et, al, 2012)(woods and et. Al, 1993) and other minerals. But in the present experiment the ash value of rice straw was similar with limpo grass. It may be due to different in variety, soil quality and any other environmental effect. Limpo grass is more succulent and cultivated fodder. The CF was lower in limpo grass than rice straw so it was expected that Limpo grass was more digestible than rice straw.

The DM and OM degradability of rice straw without any limpo grass was very low due to various ligno-cellulose bonds. On the other hand, the DM and OM degradability of rice straw were higher in presence of limpo grass. The higher content of CP and other nutrients available of limpo grass might have increased the *in vitro* degradability of rice straw in presence of limpo grass. Supplements having higher CP and EE can be useful to complement the nutritive value of Limpo grass. In case of rice straw and Baksha combined treatment with equal proportion show better result for OM degradability. Combination effect gradually increases with time. In every group DM and OM digestibility significantly increased at longer incubation time, because microbial activity was higher at longer incubation time. Same result was found in case of ammonia concentration. Ammonia concentration in rumen fluid increased at higher proportion of Limpo grass because due to presence of higher CP content in limpo grass. Khan (2012) observed that to maintain *in vitro* ammonia level constant in longer time CP in feed should be 6%. Less than 6% CP will decrease ammonia level and more than will increase ammonia concentration in

rumen fluid. In the present study CP of rice straw was less than 6%, whereas in limpo grass CP was higher than 6%,that might have increased ammonia concentration in rumen fluid at higher incubation time.

Despite high digestibility, however, protein concentration is generally low in Limpo grass than the requirement of milk yielding cow or growing ruminants (khan and et.al,2011) , so protein supplementation is needed to meet the requirements of most livestock that are fed Limpo grass. If the nutrient supplement is well balanced, it will increase the overall production of ruminants as well as it will also be helpful for the milk quality.

In the *in vivo* experiment, control group cows were supplied roadside grass with rice straw and experimental group were given 50% Limpo grass and 50 % roadside grass with rice straw. Milk production did not changed by replacing Limpo grass however fat% and total solid% increased in presence of Limpo grass. In Bangladesh many places milk prices depends on the fat% of milk. If fat % could be increased without hampering the milk production and other composition of milk the farmers will be benefited.

Limpo grass can offer complementary properties for their use to formulate forage based balanced diets to optimize the degradability and utilization of low quality forages in ruminants. In vitro DM and OM degradability of rice straw increased in the presence of limpo grass. Ammonia concentration in rumen fluid also increased at presence of higher proportion of Limpo grass. Limpo grass supplemented animal showed better result in case of milk composition. It could be concluded that the digestibility and utilization of low quality forage could be improved by supplementation of limpo grass. However, protein concentration is generally low in Limpo grass than the requirement of milk yielding cow or growing ruminants so protein supplementation is needed to meet the requirements of most livestock that are fed Limpo grass. Finally limpo grass can provide better result as well as the production which may lead profit to the farmers. Further research could be carried out for improving the production performance of limpo grass for the benefit of our farmers to get maximum benefit with minimum cost.

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