International Journal of Agricultural Sciences ISSN 2167-0447 Vol. 7 (6), pp. 1323-1331, June, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of the digestibility of probiotic-treated rice straw using the *in vitro* gas production technique

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Accepted 20 June, 2017

The objectives of this study were to use the *in vitro* gas production (IVGP) technique to evaluate the pattern and parameters of anaerobic fermentation of probiotic-treated rice straw collected from local market of Gazipur district, Bangladesh. Probiotic-treated rice straw was assessed for their chemical composition, total digestible nutrients (TDN), the fractional rate of degradation (kd) characteristics, and *in vitro* gas production measurements. In general, probiotic treatment improved the crude protein (CP) content and maximum increase was 15*%.* The response to urea treatment on CP content almost doubled the CP content of untreated straw. *Trichoderma* spp. and *Aspergillus* spp. also increased the CP content. However, the ADF content in the probiotic-treated rice straw was similar to control but significantly higher in urea, *Trichordema*, and *Aspergillus* spp.The trend of total *in vitro* gas production increased up to 48 h for all treatments. Most of the probiotic-treated rice straw showed similar TDN and kd, but there were some differences. Mineral concentration did not vary among treated groups. Non-fiber carbohydrates (NFC) concentration was almost half in the probiotic-treated rice straw, suggesting a greater release of NFC for microbial utilization. Moreover, the relative feed value was higher in treated straws compared to control. In conclusion, the probiotic-treated rice straw improved nutritional quality and provided better fermentation pattern regarding the kd and gas production.

Keywords: Probiotics, urea, trichoderma, aspergillus, degradation, gas production.

INTRODUCTION

In addition to concentrate feeds, the small and medium dairy farmers of Bangladesh usually use rice straw as a primary source of feed for cattle with limited grazing on roadside and community land. Rice straw alone contributes to 87% of the roughage feed of animals in Bangladesh (Akter et al., 2013). However, the high level of lignification and silicification, which slows and limits the ruminal degradation of the carbohydrates, and the low content ofNare the main deficiencies of rice straw, hinderingits nutritive value as feed for ruminants (Sarnklong et al., (2010).Extensive research has been carried out for several decades on improving the nutritive value of cereal straws using physical, chemical, and biological treatments with varying degree of success in Bangladesh as well as other parts of the

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world (Hossain et al., 2010; Akter et al. 2013; and Rangnekar, 2005). However, only a few of the treatment methods can be applied at farmer's level. Among these treatment methods, rice straw with urea or urea-molasses have been considered as a simple and practical method for a consistent increment of Ncontent and *in vitro* organic matter digestibility (OMD). Some have, however, reported no consistent effect of urea treatment on the chemical components (Yuliastini et al., 2000). Moreover, Yuliastini et al., (2003) indicated that DMI of urea treated rice straw was lower than that of untreated rice straw.

Probiotics can increase the crude protein (CP) level of rice straw (Aguset al., 2000; Thalib et al., 2007), improve fiber digestion in the rumen, reduce the number of pathogenic microbes in the digestive tract, and help in balancing the microbial consortium by optimizing the fermentation process (Amlius, 2008). Several researchers have reported advantages in treating rice straw with probiotics regarding digestibility and palatability (Haryanto etal., 2003; 2004), and improved *in vitro* dry matter digestibility (DMD) and OMD (Haryanto et al., 2003; 2004). Despite the potential of probiotics-treated rice straw as cattle feed, there is limited information available in some parts of the world, specifically in the tropics, on its application in cattle production. Preston (1990) suggested that tropical regions can take full advantage of their natural resources (i.e., solar energy, soil, water, and biological diversity) to be competitive with livestock production. For quite some time, the development and application of technology and scientific knowledge have been used to improve feeding and nutrition value of sugar-cane in the tropics (Leng and Preston, 1976). It is time to obtain the same advancements for rice straw. Based on the facts presented above, the objective of this study was to evaluate the nutritive value of probiotic treated rice straw regarding chemical composition, total digestive nutrients (TDN), fractional rate of degradation, and total *in vitro* gas production obtained using the *in vitro* gas production (IVGP) technique.

MATERIALS AND METHODS

Collection of rice straw and commercial probiotics

Rice straw and seven commercial probiotics (P1, P2, P3, P4, P5, P6 and P7; Table 1) were collected from a local market near Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. *Trichoderma* spp. and *Aspergillus* spp. were donated by the Department of Biology and Aquatic Environment, Faculty of Fisheries, BSMRAU and Department of Pathology, Faculty of Agriculture, BSMRAU, Bangladesh, respectively.

Treatment of rice straw using probiotics and urea

A total 500 g of rice straw was added in a 1000 ml Erlenmeyer flask and water was added to maintain the moisture at 65%. The flask with rice straw was autoclaved for 15 min at 121^{o}C. After that probiotic at a level of 1% was added to the rice straw followed by incubation at 0, 2, and 4 days at 37°C. The treatment methodology of rice straw with *Trichoderma* spp. and *Aspergillus* spp. were essentially thesame as above, with 65% moisturization level. In this case, incubation was done at 32°C. Then, the sample was kept in the refrigerator in polythene bags for further use. Urea treatment was also done in similar way adding 3% urea into the straw. The sample ground to pass through a 2-mm screen using a Wiley mill (Model 3375-E25, Thomas Scientific, Swedesboro, NJ 08085). The ground sample was used for fermentations using the fermentation chamber (Tedeschi et al., 2008).

In vitro gas production measurements

As described by Tedeschi et al., (2008), the fermentation chamber included an incubator (chamber) with a multi-plate stirrer, pressure sensors attached to incubation flasks (125-ml Wheaton flasks), an analog-to-digital converter card, and an IBM-PC provided with appropriate software (Pico Technology, Eaton Socon, Cambridgeshire, UK). The software was set to collect pressure signal every 5 minutes for 48 h. The strict anaerobic technique was employed in all transfers (Bryant, 1972; Hungate, 1950) by venting all containers with CO_2 for at least 5 minutes using low to the medium flow rate of CO_2.

Preparation of the rumen fluid

The ruminal fluid inoculum was obtained from rumen-cannulated crossbred cattle. The collected ruminal content was transported in a pre-warmed with hot water, closed plastic container (Thermos) full of ruminal content to the Ruminant Nutrition Laboratory. Immediately upon arrival, the rumen content was filtered through four layers of cheesecloth and then through glass wool into an Erlenmeyer flask withan O_2-free headspace. The ruminal fluid was mixed continuously with $CO_2$to minimize changes in microbial populations and to avoid CO_2contamination and was maintained at 39^{o}C at all times.

Preparation of the medium

The *in vitro* medium used was the phosphate-bicarbonate medium which reduce the solution of Goering and Van Soest (1970) (trypticase was not added). The medium flask was ventilated with CO_2 all the time; no CO_2 was added in the medium. The medium was heated separately to just below boiling temperature and then cooled to room temperature. At this point, cysteine hydrochloride was added. The medium pH and CO_2 saturation were controlled by color change of resazurin indicator from purple to pink/colorless;

Table 1. Probiotics and their microbial composition.

Probiotics name with code given in the study	Expiry date	Composition of Bacteria	Viable cell (cfu) count	
			Manufacturers claim	Our Findings
1. P1	April 2017	*Lactic acid bacillus* *Bacillus subtilis* *Bacillus licheniformis* *Bacillus polymyxa* *Bacillus megaterium* *Bacillus mesentricus*	5×10^{9}/g	$6x10^{10}$/g
2. P2	October 2016	*Pediociccus sp.* *Lactobacillus sp.* *Bifidobacterium sp.* *Enterococcus sp.*	5.0×10^{12}/g	$7.5x10^{10}$/g
3. P3	September 2017	*Lactobacillus acidophilus* *Lactobacillus plantarum* *Pediociccus pentosaceus* *Saccharomyces cerevisiae* *Bacillus subtilis* *Bacillus licheniformis*	1.0×10^{9}/ml	$1.8x10^{9}$/ml
4. P4	April 2017	*Lactobacillus plantarum* *Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Bifidobacterium bifidum* *Streptococcus thermophilus* *Enterococcus faecium*	2.0×10^{9}/g	$1.8x10^{8}$/g
5. P5	February 2017	*Lactobacillus plantarum* *Lactobacillus bulgaricus* *Lactobacillus casei* *Lactobacillus acidophilus* *Bifidobacterium bifidum* *Streptococcus thermophilus* *Streptococcus faecium* *Aspergillusoryzae* *Torulopsis bovina*	1.0×10^{10}/g	$5x10^{7}$/g
6. P6	October 2016	*Lactobacillus acidophilus* *Lactobacillus bulgaricus* *Lactobacillus plantarum* *Streptococcus faecium* *Bifidobacterium bifidus*	2.0×10^{9}/g	$4x10^{7}$/g
7. P7	April 2017	*Bacillus* spp. *Bacillus subtilis* *Bacillus licheniformis* *Saccharomyces boulardii* *Saccharomyces boulardii* *Aspergillus niger* *Aspergillus oryzae*	4×10^{9}/g	$1.7x10^{8}$/g

the optimum pH utilized was between 6.8 and 6.9.

Preparation of feed samples and incubator

Feed samples (200 mg) were transferred to 125-ml Wheaton flasks, which contained a small Teflon-covered stir inside. Inside the flasks, feed samples were wetted with 2.0 ml of boiled, double-distilled water that had been previously cooled to room temperature; the water was addedto avoid particle dispersion and discounted by the

media. Each flask was filled with 14 ml of media as described above, closed with previously unused, lightly greased with petroleum grease base (Lubriseal; stopcock grease, Thomas Scientific, Swedesboro, NJ (8085) butyl rubber stoppers (Geo-Microbial Technologies, Ochelata, OK 74051), and crimp sealed with Aluminum caps. All flasks were placed in the fermentation chamber, and the respective sensor for each bottle was inserted using needles. When the fermentation chamber reached 39°C, 4 ml of the filtered mixed ruminal bacteria inoculum was injected into the Wheaton flasks. The fermentation chamber was closed, and when the internal temperature reached 39°C, the pressure inside each bottle was zeroed by puncturing the stopper with a needle for 5 seconds. The fermentation chamber was closed and when the temperature reached 39°C, the recording of the pressure was initiated. The atmospheric pressure was recorded at the beginning and the end of all fermentation rounds.

Preparation of fermentation residue

After 48 h of fermentation, 2880 data points per sample were collected. Each flask was depressurized, the pH and oxidation/reduction (redox) potential were measured, and 40 ml of neutral detergent solution (Van Soest et al., 1991) was added to each Wheaton flask to determine neutral detergent residue (NDR; Mertens, 2002); sodium sulfide and amylase were not used. Wheaton flasks were crimp sealed and cooked in an autoclave for 60 min at 105°C to determine the undegraded fiber, filtered by a gravimetric method using a Whatman 54 filter paper, and dried in the oven using the micro-method for determination of residual fiber (Pell and Schofield, 1993). The NDR was determined gravimetrically.

Total viable count of probiotic bacteria

The total viable count was done using Plate Count Agar (PCA) incubating 24 h at 37°C. Ten-fold serial dilution was performed for the viable count.

Chemical analyses

Chemical analysis was performed by Cumberland Valley Analytical Services (Hagerstown, MD 21742; http://www.foragelab.com), USA, as follows: DM was performed in two steps; the first step was according to Goering and Van Soest (1970), and during the second step oven temperature increased to 105ºC, according to National Forage Testing Association (2002); ash was determined according to AOAC (2002, method 942.05); CP and non-sequential ADF analyses were performed according to AOAC (2002; methods 2001.11 and 973.18), respectively; NDF analysis was determined according to Van Soest et al. (1991); ether extract (EE) was determined by AOAC (2002; method 920.39); and lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid, with modifications. Total silica content was measured directly starting from the ADF procedure of the sequential analysis and termedacid-detergent insoluble silica (ADISi) after Van Soest et al. (1991).

Statistical Analyses

Statistical analyses were performed with R 2.7.2 (R Development Core Team, 2008) and with SAS (SAS Inst. Inc, Cary, NC) packages.

RESULTS

Improvement of nutritional values based on chemical composition

The results of chemical composition treating with commercial probiotics, urea, *Trichoderma*, and *Aspergillus* are shown in Table 1. In general, probiotic treated rice straw increased CP content compared to control (5.8 vs 6.1, 5.9, 6.4, 6.8, 6.5, 5.9, 6.7, 6.6 and 6.2%) except for P1 (d4), P3 (d2) and P5 (d0). Urea treatment increased CP contents more than two-fold compare to control (5.8 vs. 13.1, 10.5, 12.1). *Aspergillus*-treated rice straw also increased CP content. However, *Trichoderma*-treated straw had a lower CP at day 2 (5.8% vs. 5.5%). Probiotic (P1 to P7)-treated rice strawhad almost thesimilar range of ADF in all time points ranging from 36.6 to 45.5% compare to control 45.1, but surprisingly urea-, *Trichoderma*-and *Aspergillus*-treated straw had increased ADF content (56.7 vs. 57.1, 57.4,59.8, 57 and 58.8%). The NDF content was reduced by P2 at d2 and d4, P3 at d0, d2, and d4. It was also reduced by urea, *Trichoderma* and *Aspergillus* treatments at all-time points (Table 2). In the current study, lignin content was reduced only after treating straw with P5 at d4 (7.1% vs. 6%), P7 at day 4 (7.1 vs. 6.9%), urea days 0 and 4 and *Trichoderma* spp. at day 0 (7.1 vs. 2.6%). However, higher percentages of non-fiber carbohydrate (NFC) were released from all treatments compare to untreated rice straw (Table). Ca concentration increased only in P6 and P7.A mineral of P, Mg, K and Na concentration were similar in untreated and treated straws.

Total Digestible Nutrients

The effect of untreated, probiotic, urea, *Trichoderma*, or *Aspergillus* on predicted total digestible nutrients (TDN), assuming kp of 4 and 6%/ h, are shown in Figures 1 and 2, respectively. There was a significant difference of predicted TDN among Aspergillus-control, Asp-urea, and control-P1. Besides these substantial differences of TDN between Asp-Tric at 0d, Asp-Urea at 0 andd4, Asp-control at d2, control-Tric d0 andd2, control d2-P1d2, P5d4, and P6d4 were also observed in kp 4%/h. There was also a significant difference of TDN in P4

Table 2. Chemical analysis of untreated and probiotic treated rice straw

	Feed treated in days																					
	Control	P1			P2			P3			P4			P5			P6			P7		
Item		0	2	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
DM % as fed	94.6	27.1	38.5	34.3	48.8	34.7	29.3	30.6	22.0	30.2	33.4	36.8	27.5	33.5	38.5	31.9	36.6	35.6	34.5	33.5	40.1	26.5
CP, % of DM	6.9	6.1	5.9	5.8	6.4	6.8	6.5	5.9	5.5	5.9	6.7	6.7	6.5	5.7	6.6	6.2	5.9	6.6	5.8	6.2	6.7	6.4
Adjusted Protein, %5 of DM	5.8	3.6	3.7	4.4	4.5	5.0	4.7	4.1	2.8	4.2	4.5	4.8	4.9	3.9	4.2	4.9	4.1	4.5	4.3	4.0	4.7	5.1
Soluble Protein, % of CP	-	1.4	1.3	5.4	2.6	1.4	1.8	1.8	1.1	1.6	1.9	1.3	1.7	1.6	1.4	1.4	1.5	1.7	1.5	1.2	1.5	1.9
ADF Protein, % of DM	1.8	3.1	2.8	1.9	2.5	2.5	2.5	2.3	3.2	2.3	2.9	2.6	-	-	-	1.9	2.4	2.7	2.1	2.9	2.7	1.9
Rumen Degr. Protein, % of DM	-	3.8	3.6	5.6	4.5	4.1	4.1	3.8	3.3	3.7	4.3	4.0	4.1	3.6	4.0	3.8	3.7	4.2	3.7	3.7	4.1	4.2
ADF, % DM	45.7	43.4	42.0	40.6	40.9	40.5	38.7	41.6	38.5	38.6	45.5	44.9	40.8	42.9	42.7	36.1	41.8	42.0	40.2	40.3	44.5	38.1
aNDF, of DM	72.3	71.4	73.0	73.4	71.0	68.5	70.9	69.8	65.9	68.2	75.4	75.8	71.9	71.4	75.0	66.5	71.2	71.4	72.1	69.8	76.7	68.8
aNDFom, % of DM	68.3	67.0	62.8	62.1	62.9	59.7	58.8	62.0	58.5	58.2	69.7	69.3	59.2	63.7	67.4	55.2	63.3	63.6	60.7	63.0	68.7	57.1
Lignin, % DM	7.1	8.8	8.2	6.8	7.1	7.8	7.3	7.2	8.4	6.7	8.8	9.3	7.5	7.4	9.8	6.0	7.8	8.0	6.8	8.7	7.1	6.9
TDN (%DM)	48.3	44.5	40.0	41.2	44.4	44.9	41.3	44.5	45.5	43.1	41.9	43.1	40.3	44.5	42.9	46.5	43.7	43.6	40.4	41.7	43.3	41.7
RFV %	73.0	76.3	8.3	86.0	84.0	89.0	93.3	85.0	94.0	94.0	71.0	72.0	90.0	81.0	77.0	102.0	83.0	82.0	88.0	85.0	74.0	96.0
NFC, % of DM	24.8	12.3	10.4	10.0	13.3	16.0	12.8	14.6	2.0	14.8	7.1	9.4	11.6	13.0	10.5	18.4	13.5	12.4	10.3	11.4	7.5	13.5
Ash, % of DM	-	14.6	20.9	22.0	17.4	17.4	21.9	17.5	16.3	21.1	16.4	14.6	22.6	17.0	15.5	20.1	17.3	17.3	23.1	19.3	17.5	23.0
Minerals																						
Calcium, % of DM	0.39	0.5	0,5	0,5	0,4	0,5	0,5	0,4	0,5	0,4	0,5	0,6	0,5	0,5	0,5	0,4	0,6	0,5	0,6	1,9	0,7	1,0
Phosphorus, % of DM	0.11	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2
Magnesium, % of DM	0.19	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
Potassium, % of DM	2.13	1.5	2.1	2.0	2.0	1.7	1.8	2.1	1.8	1.9	2.1	2.1	1.8	2.1	1.9	2.0	2.0	2.0	2.0	1.8	2.0	1.8
Sodium, % of DM	0.03	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,1	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0
Iron, ppm	326	375	293	263	304	399	271	313	326	235	397	391	268	415	393	337	310	348	359	539	348	325
Manganese, ppm	869	983	853	822	1012	796	751	991	841	712	925	1016	815	1009	911	693	1039	837	796	1081	969	758
Zinc, ppm	47.0	87.0	38.0	22.0	39.0	54.0	26.0	43.0	65.0	36.0	90.0	92.0	22.0	44.0	83.0	26.0	48.0	65.0	28.0	73.0	111.0	32.0
Copper, ppm	5.0	6.0	5.0	5.0	5.0	6.0	5.0	5.0	5.0	5.0	7.0	6.0	5.0	6.0	7.0	5.0	5.0	6.0	5.0	6.0	7.0	6.0

Pro= probiotic

RFV= relative food value

P1-P7=probiotic

and other treatments but no interactions between treatments and time were observed. However, P1 has the lower degradation rate (29 %) among the treatments.

Fractional rate of degradation

The fractional rate of degradation of various treatments is presented in Figure 3. The higher fractional rate of degradationwas observed in P4 (5.8%) compared to all other treatments. There was a significant difference of rate of degradation in P3, P4, P5, P6, urea, *Trichoderma* and *Aspergillus* compared to control. However, there was no difference of rate of degradation among treatments in terms of time.

Total Gas Production

Total gas production of untreated and treated rice straw is shown in Figure 4. Gas production of straw during *in vitro* incubation varied significantly among various treatments. Significantly higher (P>0.05) gas yield was in P4 (70 ml/100gDM) at d0 and lowest in control d0. In general, probiotic, urea, *Trichoderma*, and *Aspergillus* treatments also showed a good pattern of total gas production.

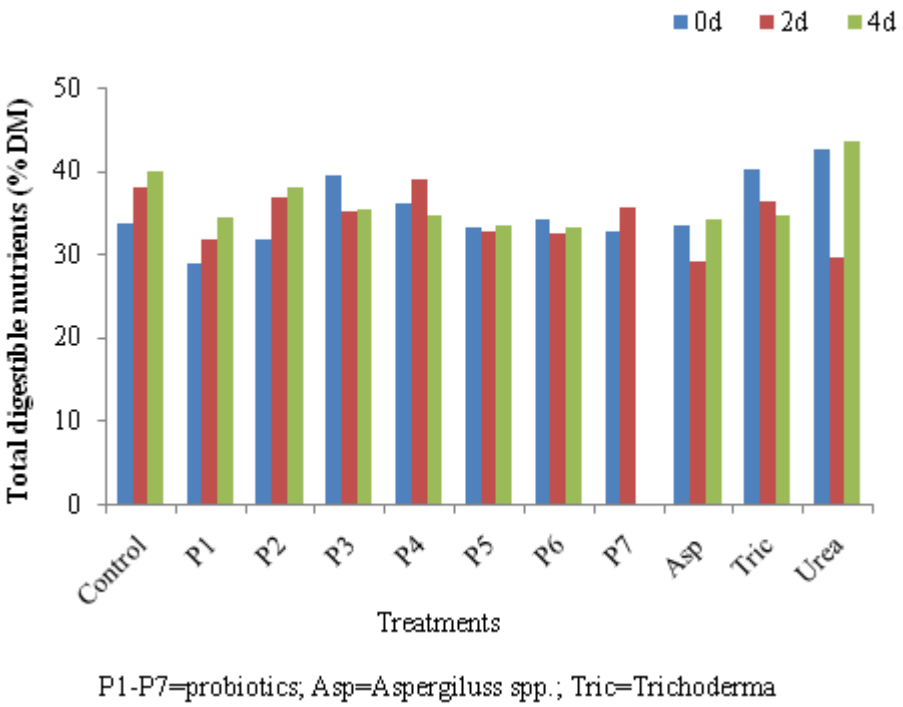

Figure 1. Effect of probiotics, urea, *Trichoderma* and *Aspergillus* on predicted total digestible nutrients (TDN, dry matter basis), assuming a kp of 4%/h.

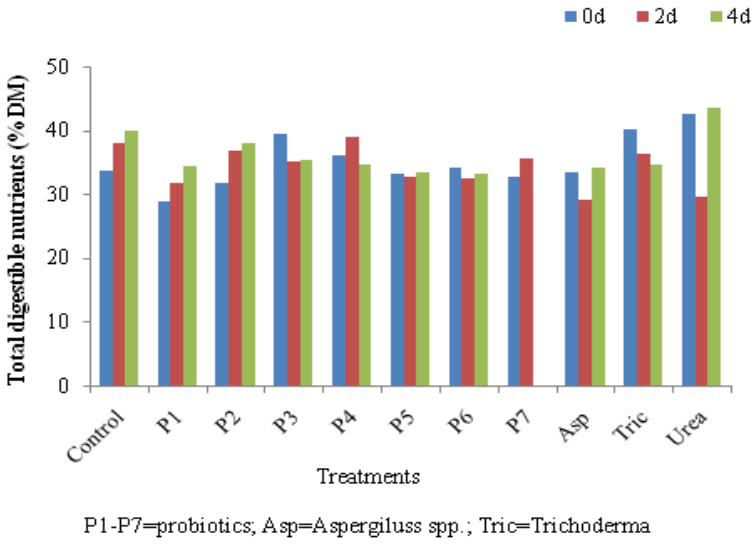

Figure 2. Effect of probiotics, urea, Trichoderma and Aspergillus on predicted total digestible nutrients (TDN,dry matter basis), assuming akpof6%/h.

DISCUSSION

Improvement of nutritional quality of probiotic treated rice straw

Chemical composition, alone, as measured by the proximate and elemental analysis system, is accepted as an inadequate indicator of nutritive values of feedstuffs. These measurements take no account for the form of nutrient availability and, at best, may provide information on potential nutrient contents. The values of DM content of untreated rice straw was observed around 90%, which was similar to that of DM content of different varieties of rice straw varied from 88 to 92%

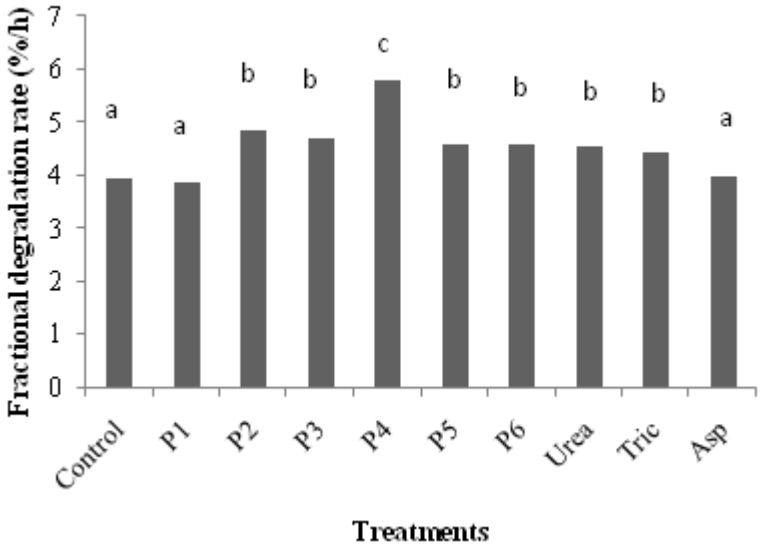

Figure 3. Effect of probiotic, urea, *Trichoderma* and*Aspergillus* on *in vitro* fractional rate of degradation of rice straw.P1 toP6= probiotic; Tric= *Trichoderma* spp.; Asp= *Aspergillus* spp.; and different letters indicated statistical difference (*P*<0.05).

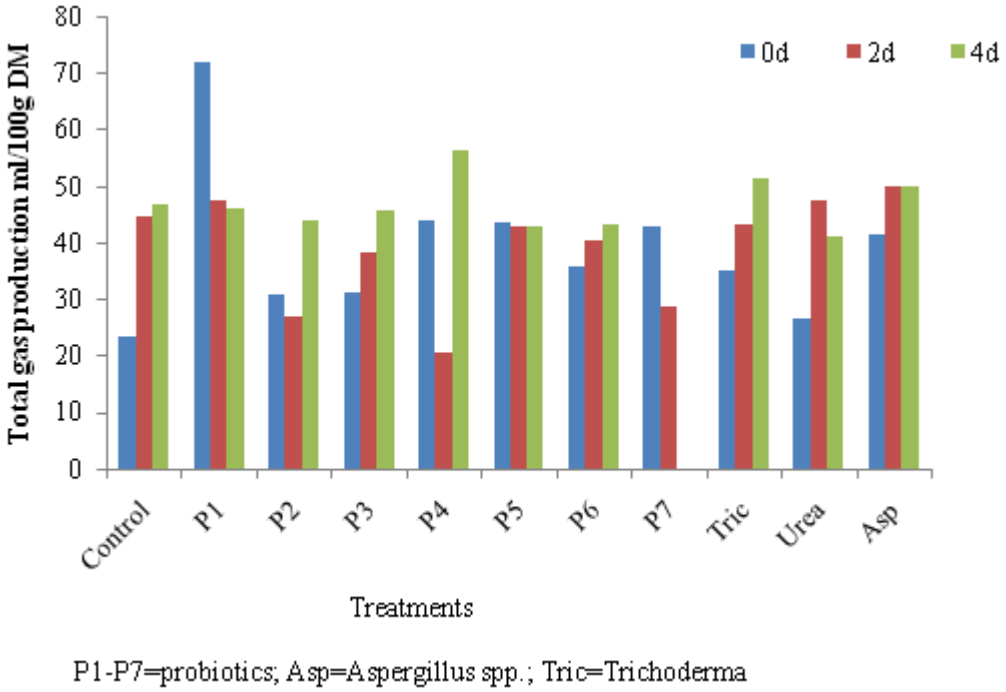

Figure 4. Effect of treatment of rice straw using probiotics, urea, Trichoderma and Aspergillus on in vitro total gas production.

reported by Modak (1985). However, DM content of probiotic-treated rice straw ranged between 22.0 to 48.8%. The CP content of untreated rice straw was 5.8% in the current studies which were alittle bit higher than that of different varieties of rice straw as reported by Rahman et. al., (2010). In general, CP content in probiotic-, *Trichoderma*, *Aspergillus*, and urea-treated rice straw increased compared to control. The level of the CP content of rice straw fermented with probiotic was in the range of the other reports (Antonius, 2009;

Sariubang et al., 2002; Sembiringetal., 2002). Increased CP was believed to be due to the addition of microbial protein and urea.

The decreased crude fiber (**CF**) level of rice straw fermented with commercial probiotic was 6.07% untreated straw (Bansi et al*.,* 2012). It was lower than reported by Antonius (2009) and Sairubang et al. (2002) who reported that CF content in rice straw fermented using probiotic decreased by 25.73 and 14.79%. The decreased CF level of rice straw fermented assumed that probiotic microbes can penetrate the fibrolytic structure and cleave the binding of lignified carbohydrate and to some extent, degrade cellulose and hemicellulose. In the present study, the ADF content was almost similar in all probiotics treated rice straw, and it were close to untreated rice straw.However, higher ADF content was observed in remaining treatments. Syamsu (2006) indicated that the activity of the cellulolytic enzyme of microbes probiotic caused degradation, reorganize, expanded, and break of bonded lignin with the cell wall of rice straw.

Relative feed value (RFV) has been used for years to compare the quality of legume and legume/grass hays and silages. Having one index to price hay and predict animal performance has been very useful for livestock producers and hay farmers. In our studies, we observed a higher RFV in all treatments compared to control indicating a good quality animal feed.

All treatments released more non-fiber carbohydrate (NFC) than untreated rice straw. The NFC is made up of different amounts of starch, simple sugars, beta-glucans, galactans, and pectins; and it usually makes up 35 to 40% of the dry matter (DM) in a ration designed for high production. NFC is more rapidly digested than fiber. It is a significant source of energy for the rumen microbes. Volatile Fatty Acids (VFA), primarily propionate, are producedfrom the fermentation of NFC. They are absorbed from the rumen and used as a source of energy by the cow. The microbes also use NFC to grow.

Total digestible nutrients (TDN) and fractional rate of degradation

The TDN is the sum of the percentages of CP, CF, ether extract (**EE**), andnitrogen-free extract that are digested in the gastrointestinal tract of the animal (Weisset al., 1992).Estimated rates of degradation of the different carbohydrate fractions provide additional information on the nutritive value of the feed. Based on our evaluations of the probiotic-treated rice straw, a kp of 4%/h may reflect the slower typical passage rate in dairy cows at maintenance level. The average TDN, assuming a kp of 4%/h, ranged from 40.7 to 43.4% in treated groups. The NRC (2000) suggested that TDN ranges from 53 to 57% in forages when the passage ratewas 4%/h.In the current research, the TDN value was lower than the NRC (2000) recommendation likely due to the fact of loss of nutrient during straw treatment.

Total Gas Production

The *in vitro* gas production technique has been used as a means of ruminal of feeds (Menke andSteingass, 1988; Blummel et al., 1997) and as an indicator of digestible DMI and growth rate of cattle fed cereal straws (Blummel and Orskov, 1993). This technique also has the potential to investigate associates effects between feeds. In the current study, probiotic, urea, *Trichoderma*, and *Aspergillus* treatments had an increasing trend for gas production during the 48 h of incubation. These results indicated a productive activity of microbes for fermentation. The growing tendency of total gas production shows the availability of readily fermentable material as an energy source, which stimulated the activity of the rumen microorganisms which in turn would accelerate the digestion of treatedrice straw.

CONCLUSION

Although several treatments have been used to improve the nutritional values and improved fermentation patternof rice straw, such as physical or chemical treatments,the practical use of these treatments is still restrictedregarding safety concerns, costs, and potentially negative environmental consequences. Commercial probiotic and *Aspergillus* spp. may be potential alternatives to provide a more practical and environmental-friendly approachfor enhancing the nutritive value and better fermentation pattern of rice straw.

ACKNOWLEDGEMENTS

The authors thank the Krishi Gobeshona Foundation (KGF), Bangladesh Agricultural Research Council (BARC), Dhaka, Bangladesh, for their financial support of the project (CGP/TF 21-DL/15). The authors also thankthe Departmentof Animal Science at Texas A&M University for hosting Dr. Selim and providing laboratory support, and the Borlaug Institute at Texas A&M University for their logistical support. Our sincere gratitude to BSMRAU for overall mentoring of the project. The first author was a recipient of Borlaug International Agricultural Science and Technology Fellowship Program, funded by the U.S. Department of Agriculture, Foreign Agricultural Service.

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