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Effect of Probiotic Treated Rice Straw on Nutrient Digestibility, Milk Yield and Composition in Dairy Cows

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Abstract: Two experiments were conducted to investigate the effect of probiotic on nutrient improvement, digestibility and milk yield and composition in Crossbred dairy cows. In both the experiments (1 and 2), rice straw was treated using commercial probiotic protexin. In experiment 1, a complete randomized design (CRD) was used with eight Holstein Friesian Crossbred heifersaverage weighing 180.25±21 kg. The animals were allocated into two groups (4 in each group) based on their weight and fed untreated (T1) or probiotic-treated (T2) rice straw for 15 days. The dry matter (DM), organic matter (OM), crude protein (CP), detergent fibre (ADF) and neutral detergent fibre (NDF) intake did not vary significantly ($P<0.05$) among treatments. However, there was a significant ($P < 0.05$) difference of CP digestibility (63.60 vs 77.58%) in probiotic-treated rice straw compared to control. In experiment 2, ten Holstein crossbred dairy cows, weighing 235±17 kg, from the livestock and poultry farm of BSMRAU, Gazipur-1706, Bangladesh, were individually fed in free-stalls and milked once daily. Experiment 2 had the same treatments as of experiment 1, using a CRD and it was conducted during the first 30 days in early lactation. The fat, solid not fat (SNF), protein, lactose, ash and total solid (TS) % were in an expected range as a milk constituent but did not vary significantly ($P<0.05$) after feeding probiotic treated rice straw. The present study demonstrated that probiotic treated rice straw led to nutrient improvement, especially CP content, enhance digestibility and maintain normal milk composition in dairy cows.

Key words: Probiotic · Rice Straw · Nutrient · Digestibility · Milk Yield

INTRODUCTION

Rice straw is not a suitable feed for ruminant animals because rice straw has limited nutritive value (low crude protein and digestibility & high level of lignification and silicification. Rice straw contains about 3% crude protein (air dry basis), 35% crude fiber and 1900 kcal DE/kg of straw [1]. Common methods for the improvement of nutritive value of fibrous feeds are physical, chemical and biological treatments. Among these treatments, physical and chemical treatments are expensive, unsafe to farmers and animals, or unfavorable to the environment [2]. Many researchers [3, 4] conducted research using rice straw with urea or urea-molasses in Bangladesh. Use of NaOH to treat crop residues for ruminant feeding have been done by Berger *et al.* [5] Arieili [6]. The main advantages of the different NaOH treatment methods are increased degradability and palatability of treated straw [7]. Among numerous treatments, the biological treatment of rice straw through probiotic is the best alternative treatment because it is cost-effective and eco-friendly and can improve its digestibility. Researchers in India [8] have found significant improvement in nutrient degradability of DM, OM and NDF due to probiotic mix

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supplementation. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy micro-biota, preventing enteric pathogens from colonizing the intestine [9] and no adverse effects on animal health [10]. Probiotics compete with harmful gut flora, stimulate the immune system of the animal and increase its resistance to infectious agents in order to promote growth [11]. Ganai [12] reported higher digestibility of DM, OM, NDF and total gas production values at supplementation of yeast to bajra straw based complete feed using goat rumen liquor on *in vitro* study. Yasu d a *et al*. [13], reported 3% to 16% increase in milk production in Holstein Friesian cows by supplementing the diet with probiotics. Total daily milk productions of the cows fed with probiotics were 12.7% and 11.5% higher than those of animals in the control group [14].

Our research addresses the hypothesis that probiotic-treated rice straw would enhance nutrient content, digestibility and may have an impact on milk production and its composition. Thus, two independent experiments were performed to investigate the response of heifers in digestibility and lactation performance and milk composition of dairy cows to the probiotic treated rice straw.

MATERIALS AND METHODS

The experimental procedures were in agreement with the ethical principles in animal experimentation of the Committee of Ethics in Animal Experimentation of the BSMRAU, Bangladesh.

Experiment 1

Animal, Design, Diets and Straw Treatments: Eight crossbred heifers with initial average body weight (BW) of 180.25±21 kg were used in this experiment. The experiment lasted for 15 days of the feeding trial, of which five days was the adjustment period and ten days for the collection periods of digestibility trials. A Complete Randomized Design (CRD) was used in the study. The heifers were grouped based on initial BW. Each dietary treatment was randomly assigned to each heifer in a block resulting in four replications per treatment. The treatments included the feeding of untreated rice straw (Control T1) and Treated rice straw T2. The chemical composition of the diet ingredients used in the treatments is reported in Table 1.

Locally collected rice straw was chopped into 4 to 6 cm in size using an electric chopping machine. Chopped rice straw was mixed with commercial probiotics products (Protexin-Table 2) at a rate of 0.5% (1.5×10^8 cfu/g). The total viable count of Protexin was done using Plate Count Agar (PCA) incubating 24 h at 37°C. Tenfold serial dilution was performed for the viable count. Distilled water was mixed with probiotic and sprayed in the straw layer by layer. Then the straw was covered by polythene sheet tightly and incubate for two days. After two days, the polythene sheet was removed and the fermented rice straw was stored in a dry place. The diets were fed to the experimental heifers twice daily in the morning and afternoon. The experimental animals had *ad libitum* access to water.

Sample Collection and Analysis: Daily feed offer and refusals were recorded for each heifer to calculate daily feed intake. Feed and refusals were sampled per animal and pooled for each treatment. Representative samples of both diets and refusals were kept and used for further analysis. Daily fecal excretions per animal were collected and weighed every morning. Twenty percent of the total collected feces were sampled daily and kept in airtight plastic containers for each animal until the end of the collection period, during which time they were thoroughly mixed for each animal and subsampled for chemical analysis.

Samples of feed offered, refusals and feces were ground to pass through a 1-mm sieve screen and analyzed for contents of DM, CP, Ash [15], neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soes t *et al.* [16].

The apparent DM and nutrient digestibility coefficients were determined as:

Apparent digestibility (%) = (Nutrient intake – Fecal Nutrient output)/ Nutrient intake

Experiment 2

Cows, Design, Diets and Straw Treatments: Experiment 2 was performed at livestock and poultry farm of BSMRAU. Ten Holstein crossbred dairy cows, with initial average body weight (BW) of 235±17 kg, were individually fed in a free-stall and milked once a day. The trial lasted for 30 days of feeding. A Completely Randomized Design (CRD) was used in the study. The cows were divided into two groups, each consisting of 5 cows belong to each group received un-treated straw (Control group T1) and Treated rice straw T2, respectively. The composition of diet ingredients used in the treatments has been shown in Table 3.

Table 1: Chemical composition of feed ingredients used in the diets (Exp. 1.)

	DM	OM	CP	ADF	NDF	EE	Ash
Ingredients	g/100g			% DM			
Untreated rice straw	92.17	85.69	3.95	65.85	78.56	0.90	12.42
Treated rice straw	91.42	82.33	5.77	62.33	76.78	0.80	17.69
Green grass	18.4	87.38	12.15	53.22	66.08	0.82	10.73
Wheat bran	90.87	94.06	14.62	14.24	45.89	2.88	4.70
Rice bran	90.04	78.05	9.10	22.45	56.86	6.69	21.84
Broken rice	91.08	95.45	11.07	8.78	57.88	1.30	3.66
Mustard oil cake	9210	92.75	39.43	27.44	44.05	8.10	7.28

Treatment of straw: 0.5% probiotic fermented for 2 days. DM= Dry Matter, CP=Crude Protein, OM= Organic Matter, ADF= Acid Detergent Fiber, NDF=Neutral Detergent Fiber, EE= Ether Extract.

Table 2: Probiotic (Protexin) composition and its viable cells count (cfu /ml) (Exp. 1.)

		Viable cell (cfu/g) count	
Probiotics	Composition of Bacteria	Manufacturers claim	Our Findings
Protexin	*Lactobacillus plantarum* *Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Bifidobacterium bifidum* *Streptococcus thermophilus* *Enterococcus faecium*	5.0×10^8/g	1.5×10^8/g

Table 3: Chemical composition of diet ingredients used in the diets (Exp. 2.)

	DM	CP	Ash	NDF	ADF	EE
Feed ingredients	gm/100gm	% DM				
Green Grass	19.39	7.9	13.8	71.5	42.7	0.92
Treated Straw	92.4	6.46	15.81	84.88	48.84	0.88
Untreated Straw	92.49	5.96	15.88	84.64	52.64	0.79
Wheat Bran	90.41	16.25	4.56	39.31	12.43	3.21
Rice Polish	90.14	6.15	15.81	43.53	35.13	4.18
Mustard Oil Cake	96.62	29.56	6.25	23.38	10.65	3.44
Broken Rice	91.31	7.3	1.39	22.12	8.68	2.23

Treatment of straw: 0.5% probiotic fermented for 2 days. DM=Dry matter, CP=Crude protein, NDF=Neutral Detergent fiber, ADF=Acid Detergent Fiber, EE=Ether Extract afternoon

Table 4: Probiotic (protexin) composition and its viable cells count (cfu/g) (Exp. 2)

		Viable cell (cfu) count	
Probiotics name	Composition of Bacteria	Manufacturers claim	Our Findings
Protexin	*Lactobacillus plantarum* *Lactobacillus bulgaricus* *Lactobacillus acidophilus* *Lactobacillus rhamnosus* *Bifidobacterium bifidum* *Streptococcus thermophilus* *Enterococcus faecium*	5.0×10^8/g	1.9×10^8g

Rice straw was chopped similar to experiment 1. Chopped rice straw was mixed with commercial probiotics (Table 4) at a rate of 0.5% (3.9×10^9/g). Water was mixed with probiotic and sprayed in straw layer by layer. Then the straw was covered by polythene sheet tightly and remains for incubation for 2 days. After two days, the polythene sheet was removed and the fermented rice straw was stored in a dry place. The diets were fed to the experimental cows twice daily in the morning and The experimental animals had *ad libitum* access to water and diet supplied to each cow and the daily milk yield recorded for a period of 30 days. Collected milk samples were analyzed for fat, lactose, ash, protein, total solids (TS) and solids non-fat (SNF) every seven days interval.

Samples of feed offer were ground to pass through a 1 mm sieve screen and analyzed for contents of DM, CP, Ash, NDF and ADF were determined according to experiment 1.

Statistical Analysis: The results of experiments 1 and 2 were analyzed statistically with one-factor analysis of variance and Duncan`s tests using Statistical Analysis Systems [17]. The P-value <0.05 was considered statistically significant.

RESULTS

Effect of Probiotic-Treated Straw on Nutrient Improvement, Intake and Digestibility: Probiotic treated rice straw increased CP content from 3.95 to 5.77 % (Table 1) likely because a reduction of acid detergent fiber (ADF) 65.85 vs. 62.3 % and neutral detergent fiber (NDF) 78.56 vs. 76.78% after probiotic treatment in experiment 1. It seems that commercial probiotic products can improve CP content because fibre content is reduced. In expt. 2 (Table 3) the CP content was also increased from 5.96 to 6.47%, which is around 7% improvement (Table 3). Probiotic treatment decreased acid detergent fiber (ADF) 52.64 vs. 48.84 %; however, neutral detergent fiber (NDF) was not changed after probiotic treatment in experiment 2. Similar DM was observed in treated and untreated rice straw (92.17 vs. 91.42). Organic matter was little bit higher in untreated rice straw (85.69 vs. 82.33%). Mineral content that means total ash was higher in treated straw (Expt. 1) 12 vs. 17% whereas ash content in expt. 2 was similar (15%). The fermented rice straws produced by probiotic treatments in both the experiments had a yellowish brown colour, seemed fragile and easily broken into much smaller particle size. Moreover, the tests could not notice any bad odor after treating with probiotic.

Table 5: Nutrient intake and apparent digestibility in heifers fed treated and untreated rice Straw

Parameters	T1	T2	SEM	LS
Dry matter (DM) intake (kg/day)	7.68	7.64	0.94	NS
Crude protein (CP) intake (kg/day)	0.95	0.98	0.32	NS
Organic matter (OM) intake (kg/day)	6.60	6.76	0.87	NS
NDF intake (kg/day)	4.65	4.84	0.56	NS
ADF intake (Kg/day)	2.62	2.65	0.32	NS
Apparent Nutrient Digestibility (%)				
DM	63.30	64.0	01.5	NS
OM	61.35	62.70	1.46	NS
CP	63.60	77.58	1.78	**
NDF	59.20	62.90	1.98	NS
ADF	59.70	58.10	1.20	NS

DM=Dry matter; OM=Organic matter; CP=Crude protein; NDF=Neutral Detergent Fiber; ADF=Acid
Detergent fiber, SEM= Standard Error of Mean. LS=Level of Significance, T1=Control. T2=Treatment group.

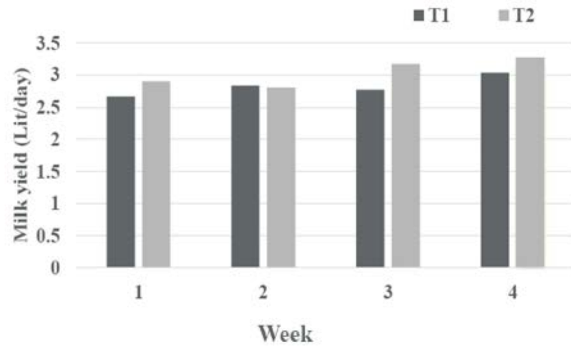

Fig. 1: Effect of probiotic treated rice straw on average milk yield (Liter/day) of Holstein

Nutrient intake and digestibility of feeding probiotic (Expt. 1) have been shown in Table 5. The average Value of DM (7.68 vs. 7.64), OM (6.60 vs. 6.76), CP (0.95 vs. 0.98), ADF (2.62 vs. 2.65) and NDF (4.65 vs. 4.84 kg/d) intake did not vary significantly (P<0.05) among treatments. However, there was a significant (P<0.05) increased CP digestibility (63.60 vs. 77.58%) was found in probiotic treated rice straw compared to control. We observed OM digestibility of 61.35 and 62.70% in the control and treated group, respectively. Thus, indicating similar trend as that of DM digestibility. In the present study, there was not similar improvement in the digestibility of organic matter of probiotic treated straw when compared with the control group. The NDF digestibility improved slightly (59.20 vs 62.90), but ADF remained almost the same in the current study.

Effect of Probiotic Treated Rice Straw on Average Milk Yield and its Composition: The effect of probiotic treated rice straw on average milk yield in different weeks (1-4 weeks) has been reported in Fig. 1. Milk yield did not vary significantly (P<0.05) among treatments during the experimental periods. However, an increasing tendency of milk yield in week 3 (2.77 vs 3.17) and week 4 (3.03 vs 3.27 kg/week) in probiotic treated rice straw was observed compared to control.

Table 6 shows the difference in the milk composition of the treated group and the control group. There were no significant (P<0.05) differences of fat, solid not fat (SNF), protein, lactose, ash and total solids (TS) percentage after feeding probiotic treated rice straw. However, we observed an increasing tendency of fat % (3.83 to 4.50) and Total solid % (12.53 to 14.57) in treated straw.

The amount of lactose ranged between 4.61 to 4.98% in this study. Maximum percentage of lactose was observed at day 21 in untreated group. The lowest total

Table 6: Effect of feeding probiotic treated straw on milk composition (Exp. 2)

		Days				
Parameters (%)	Treatments	0	7	14	21	28
	T1	4.17	4.10	4.00	4.17	4.07
	T2	3.83	3.90	4.17	4.43	4.50
Fat	SEM	0.34	0.28	0.38	0.27	0.29
	P	NS	NS	NS	NS	NS
	T1	8.67	8.07	8.33	8.17	8.93
	T2	8.55	8.05	8.00	8.17	8.40
SNF	SEM	1.32	1.40	1.36	1.29	145
	P	NS	NS	NS	NS	NS
	T1	3.22	3.24	3.38	3.22	3.25
	T2	3.35	3.35	3.32	3.36	3.52
Protein	SEM	0.02	0.01	0.02	0.04	0.02
	P	NS	NS	NS	NS	NS
	T1	4.64	4.68	4.94	4.98	4.70
	T2	4.75	4.61	4.87	4.59	4.83
Lactose	SEM	0.40	0.36	0.35	0.39	0.40
	P	NS	NS	NS	NS	NS
	T1	0.80	0.73	0.67	0.80	0.67
	T2	0.77	0.73	0.63	0.73	0.80
Ash	SEM	0.04	0.06	0.05	0.06	0.04
	P	NS	NS	NS	NS	NS
	T1	14.40	14.50	14.13	12.97	13.67
	T2	12.53	12.77	14.10	14.70	14.57
Total solid	SEM	1.45	1.49	1.83	1.69	1.70
	P	NS	NS	NS	NS	NS

T1=Control group, T2=Treated group, SEM=Standard Error of Mean, P = (<0.05), SNF= Solid Not Fat.

solid (TS) was found 12.53% in treated group. It was increased up to 21 days of feeding and then decreased. The heights value (14.70%) was found in treated group at 21 days of feeding.

DISCUSSION

A New Optimized Probiotic treatment improved crude protein (CP) content in two individual experiments. Similar improvement was also reported by several researchers [18, 19] in rice straw after fermented with probiotic. Increased crude protein (CP) level may be due to the action of probiotics, which contains some bacteria that grew during fermentation and contributed to the higher crude protein level. Hadriana *et al.* [20] and Selim *et al.* [21] reported similar increased crude protein levels while rice straw was treated using rumen liquor a probiotic, respectively. The fermented rice straws produced by probiotic treatments in both the experiments seemed fragile and easily broken into much smaller particle size, that may indicate to be more digestible than the untreated straw [19]. The advantage of probiotic in rice straw fermentation is contributing both to pretreatment and improve the fiber digestion in the rumen. Acid Detergent Fiber (ADF) content was lower in probiotic treated straw.The reduction of ADF is similar to the results of Syamsu [22], when rice straw was fermented with chicken manure. Such results were obtained by AkInfemi and Ogunwole [23], when *Pleurotus ostreatus* cultured on rice straw causing a decrease in the content of hemicelluloses, OM, CF, ADF, NDF and ADL. [24] reported that the activity of enzyme cellulolytic of microbes probiotic caused degradation, reorganization, expanded and break of bonded lignin with the cell wall of rice straw. The acid detergent fiber value refers to the cell wall portions of the forage that are made up of cellulose and lignin. These values are important because they relate to the ability of an animal to digest the forage. As acid detergent fiber increases, the ability to digest or the digestibility of the forage decreases. Treating rice straw with probiotic decreased the acid detergent fiber component, thus making it more digestible and the animal will get more digestible energy (DE). The neutral detergent fiber (NDF) values in the rice straw treatment did not show any significant differences. This is similar to the results of Syamsu [24]. NDF value is the total cell wall, which comprised of the acid detergent fiber fraction plus hemicellulose. Neutral detergent fiber percent increases, the dry matter intake generally decreases[18]. The decreased crude fiber level of rice straw fermented assumed that probiotic microbes are able to penetrate the fibrolytic structure and cleave the binding of lignified carbohydrate and, to some extent, degrade cellulose and hemicellulose.

Diet intake is considered a basic case to support primary needs, growth, production and reproduction [25]. In our study, nutrient intake was not affected by probiotic treatment (Table 5), which agreed that ration had a similar influence on DM intake. Similar DM intake indicates that the treated group was being consumed in a similar amount with control treatment. In other words, treatments with probiotic had similar taste and palatability with control. Based on the observation during the study, cattle likes treated rice straw due to its color and taste. Similar DM intake was caused by similar rumen's capacity of each cattle, which related to the homogenous cattle used in this study. Chemical analysis results regarding DM intake showed no differences among values, i.e., 93.18-92.42. This could be a factor that caused the amount of feed consumed by cattle to show no difference. On the side, the energy amount (not determined), which was consumed, may prove by similar TDN intake.

There was a significant ($P<0.05$) difference of CP digestibility (63.60 vs 77.58%) in probiotic treated rice straw compared to control. This was lower than that of Wohlt *et al*., (1998), who found higher crude protein digestibility (80.9 and 79.5 %) in Holstein cows after supplementation of 10 and 20 g yeast culture, respectively, over the control (78.5 %). The improved CP digestibility might have contributed to the greater DMI by cows fed supplemental yeast. Arambel and Kent [26] noticed no significant difference in the digestibility of crude protein after supplementation of 90g yeast culture/day. This is may be due to perhaps too little yeast culture was provided to demonstrate a response in fiber digestion.

The dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility was not affected by probiotic treatment. Erasmus *et al.* [27] found greater ADF digestibility in cows supplemented with 10 g yeast culture/day. It is evident from the table 5 that average digestibility coefficient for dry matter was 63.30 and 64.0 in untreated and treated group, respectively. Arriola *et al.* [28], reported increase in DM digestibility in Holstein cows supplemented with fibro lytic enzyme. He also observed significant ($P<0.05$) in the digestibility of all nutrients in lactating buffaloes supplemented with enzymes. We observed OM digestibility of 61.35 and 62.70% in control and treated group respectively. Yoon and Stern [29] reported increased organic matter digestibility in Holstein cows fitted with ruminal and duodenal cannulas after supplementation of yeast culture. Bass Iouni *et al.* [30] found increased digestibility of organic matter in cows after the supplementation of enzymes. In the present study, there was not such a similar improvement in the digestibility of organic matter when compared with the control group.

The average weekly milk yield did not vary significantly ($P<0.05$) in untreated and treated groups. It was noted that average milk production of around 6.60 % increased in the treated group, which was lower than that of Mostafa *et al*. [31], who found a 17% increase of milk production during dietary supplementation of probiotics AVI-BAC in dairy cows. Similar improvement was also found by Hossain *et al.* [32]. In our study, it was not evidently confirmed whether this improvement is due to the probiotic effect or not, needs further investigations. There were no significant ($P<0.05$) differences of fat, solid non-fat (SNF), protein, lactose, ash and total solid % after feeding probiotic treated rice straw. Similar results were observed when Kung *et al.* [33] in milk composition conducted a trial using Holstein Friesian fed yeast and enzyme (10g/d). However, we observed an increasing tendency of fat % (3.83 to 4.50) and Total solid % (12.53 to 14.57) in treated straw. The findings of the present study are in agreement with Doležal *et al*. [34] but did not confirm whether the improvement of fat and total solid were due to probiotics.

CONCLUSION

Commercial probiotic, protexin is a potential alternative to provide a more practical and environmental-friendly approach for enhancing the nutritive value of rice straw, especially crude protein and its digestibility and maintaining expected milk composition in dairy cows. Additional studies are needed using more numbers of dairy cows for a more extended period of feeding (at least 60 days) to better understand the impact of protexin on milk yield and composition.

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