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Effects of probiotic-treated rice straw on blood parameters and gut microbes of heifers

Abu Sadeque Md. Selim[1*], Mahbub Sobhan[1], Mueena Jahan[2], Md. Morshedur Rahman[3], Md. Kaosar Niaz Bin Sufian[4] and Shilpi Islam[1]

[1]Department of Animal Science and Nutrition, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur - 1704, Bangladesh.
[2]Department of Microbiology and Public Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh.
[3]Department of Dairy and Poultry Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh.
[4]Department of Genetics and Animal Breeding, Patuakhali Science and Technology University, Out Campus, Barishal, Bangladesh.

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The study investigated the effects of probiotic treatment on nutrient content of rice straw and its impact on blood parameters and gut microbes of heifers. Eight Holstein crossbred heifers with initial average body weight (BW) of 180.25±21 kg were used in this study. The experimental period lasted for 33 days of feeding, of which 5 days were adjustment period. The treatments included feeding of untreated rice straw (Control) and probiotic treated rice straw. In each treatment, four animals were randomly assigned. Feed were offered twice daily and ensured *ad libitum* water supply. Probiotic treatment improved crude protein (CP) level from 4.35 to 6.37%. Crude fiber content (acid detergent fiber, ADF) was decreased from 64.83 to 61.30% after treatment of straw using probiotic. Blood glucose, albumin and high density lipoprotein (HDL) level were significantly (P<0.05) increased from 65.62 to 125.08 mg/dl; 3.70 to 4.18 g/dl, and 106.28 to 165.28 mg/dl, respectively in probiotic treated straw fed animals. Low density lipoprotein (LDL) level was significantly (P<0.05) decreased from 8.77 to 0.79 mg/dl and 247.6 to 162.2 mg/dl in probiotic treated rice straw fed animals which are worthy for animal health. Probiotic treated group had a normal range of microbial community in the intestine. In conclusion, probiotic treated straw improves nutrient content of rice straw and maintains normal blood parameters and microbial gut flora of heifers.

Key words: Probiotic, rice straw, crude fiber, crude protein, acid detergent fiber, blood parameters, fecal microbiota.

INTRODUCTION

Rice straw is one of the agricultural by-products that are abundantly available in many tropical countries (Nguyen et al., 2017). Small holder farmers usually store it and use rice straw as ruminant feed throughout the year

*Corresponding author. E-mail: asmselim@bsmrau.edu.bd

especially in the dry winter season. Although, ruminants are able to convert low quality agriculture by-products into high quality protein but the use of rice straw as ruminant feed is hampered by its low nutritional value such as high neutral detergent fiber (NDF) and lignin content, low protein content and their low digestibility (Sarnklong et al., 2010). Various methods have been used to improve the nutritive value of fibrous feeds including physical, chemical, and biological treatments for ruminants. However, physical and chemical treatments can be expensive, harmful to users or unfriendly to the environment (Tuyen et al., 2012). Among them, biological treatment of rice straw through probiotic is the best alternative treatment because it is cost effective and ecofriendly and can improve its digestibility. Researchers in India (Gowher et al., 2017) have found significant improvement in nutrient degradability of DM, OM and NDF due to probiotics mix supplementation with maximum values at 3 g kg to 1 DM.

Probiotics are live microorganisms, beneficial to animal health when consumed in adequate quantities. Several *Lactobacillus* strains have been found to reduce total cholesterol (TC) and triglyceride (TG) concentrations. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens (Coliforms) from colonizing the intestine, increasing digestive capacity, lowering the pH, and improving mucosal immunity. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota, preventing enteric pathogens from colonizing the intestine (Yutaka et al., 2015). 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 (Kritas and Morrison, 2005). Intestinal bacteria play a vital role in digestion of feed and maintaining good health of animal. However, no information is available on the effect of probiotic on cattle diets on health and population of intestinal bacteria. The mode of action of the probiotic on nutrient improvement of rice straw is also poorly understood. To assess the beneficial effects of commercial probiotic treated straw, a thorough investigation is needed. Therefore, the objectives of this study were to evaluate the effects of probiotic on straw nutritive value, blood parameters and microbial community of heifers.

MATERALS AND METHODS

Collection and treatment of rice straw

Rice straw and probiotic (protexin) were collected from local market. The probiotic was a mix culture compost of *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophiles* and *Enterococcus faecium.* The total CFU of the probiotic 1.8×10^8/g was determined against manufacturer claim 2.0×10^9 g^{-1}. Rice straw cut into 2 to 3 cm, water added to give moisture up to 65% and then mixed 0.5% probiotic with straw sealed in polythene bag and incubated for 2 days.

Feeding of experimental animals

Eight Holstein crossbred heifers with initial average body weight (BW) of 180.25±21 were used in this study which lasted for 33 days of which 5 days adjustment period. The Holstein crossbred 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 feeding of untreated rice straw (Control T1) and treated rice straw T2. Composition of diet ingredients and chemical composition of rice straw and treated rice straw used in the experiment has been shown in Table 1.

The diets (Table 1) were fed to the experimental heifers twice daily in the morning and afternoon. The experimental animals had *ad libitum* access to water. Daily feed offers and refusals were recorded for each heifer to calculate daily feed intake. Samples of feed offers were taken on batches. 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. The experiment was conducted at the Livestock and Poultry farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gzipur-1706, Bangladesh.

Collection of blood sample

Blood samples were collected from experimental animals at days 0, 7, 14, 21 and 28 of the research period. Five milliliters of blood was collected from the jugular vein of animals with the help of 5cc sterile syringe. In every case, a new sterile syringe was used to collect blood and after collecting blood, all syringes were disposed carefully. After collecting, the blood was kept in a heparinized tube to prevent clotting and transported to the lab for further use.

Determination of blood parameters

Albumin, glucose, high density lipoprotein (HDL) and high density lipoprotein (LDL) were determined using selective kits purchased from Elabscience® China and with the support of Double Beam Spectrophotometer, Aarson Scientific, India.

Collection of fecal sample and monitoring fecal microbiota

Fecal sample was collected at days 0, 7, 14, 21 and 28 using sterilized spatula and immediately transferred to the laboratory for further analysis. De Man, Rogosa and Sharpe agar (MRS), Eosin Methylene Blue (EMB) and Plate Count Agar (PCA) were used for the determination of lactobacillus, coliform and total bacteria in heifers at different periods incubating Petri dish at 37°C for 48 h.

The animals were maintained and monitored by university ethical committee.

Chemical analysis

DM was determined in two steps, the first step was according to Goering and Van Soest (1970) and during the second step, oven temperature was increased to 105°C. The CP and EE levels were determined according to AOAC (2005). The NDF and ADF contents were analyzed according to Van Soest et al. (1991).

Table 1. Composition of ingredients and chemical composition of rice straw and treated rice straw used in the experiment.

Treatment	Control (T1) (%)	Treated Straw (T2) (%)
Diet ingredients		
Green grass	52.26	52.36
Treated rice straw	-	23.63
Untreated rice straw	23.78	-
Wheat bran	11.72	11.74
Mustard oil cake	5.40	5.41
Rice polish	4.13	4.13
Broken rice	2.11	2.13
Salt	0.60	0.60
Parameter	**Untreated rice straw (%)**	**Treated straw (%)**
Dry matter (DM)	93.18	92.42
Crude protein (CP)	4.35	6.37
Acid Detergent Fiber (ADF)	64.83	61.30
Neutral Detergent Fiber (NDF)	76.46	77.68
Ether Extract (EE)	0.07	0.90
Ash	13.42	18.68

T1: Control; T2: Probiotic treated straw

Statistical analysis

Data were analyzed by analysis of variance using the General Linear Models procedure of SAS (Kuehl, 1994; SAS, 2000). Differences among treatments, when significant, were also ordered using Tukey's test (Kuehl, 1994). Statements of statistical significance were based on P<0.05 or P<0.01.

RESULTS

Improvement of nutritional value of rice straw after probiotic treatment

Table 1 shows that probiotic treatment improved crude protein (CP) content from 4.35 to 6.37%. This result is almost similar with Sariubang et al. (2002), Sembiring et al. (2002) and Antonius (2009) who observed CP content in the range of 5.63 to 11.25% fed starbio, starbio and pobion, respectively.

Acid detergent fiber (ADF) was 64.83% and after treatment it decreased to 61.30%. The reduction of ADF (4.89%) is similar with the results of Syamsu (2001) when rice straw was treated with chicken manure.

Effect of probiotic treated rice straw on blood parameters in heifers

Table 2 shows the effect of probiotic treated straw on blood albumin, glucose and LDL. These are the indications of animal health status. Table 2 shows that there was no significant difference (P<0.05) of albumin content in days 14 to 28 among treated and untreated group. However, a significant difference (P<0.05) of albumin content at days 0 and 7 was observed, may be due to individual variation of animals, not might be probiotic impact. The range of albumin content was observed between 3.70 and 5.69 g/dl in the current study. Glucose is a measurement of the blood sugar level. It was found that the range of blood glucose varied (48.41 vs. 125.8 mg/dl) (Table 2). The highest value (125.8 mg/dl) was found in treated straw at day 21 and lowest value was found in untreated straw at day 0. The certain fall of glucose (52.44 mg/dl) in untreated straw at day 21 was due to illness of two animals. Glucose level was increased after probiotic supplementation (65.62 vs. 115.43 mg/dl). This result is consistent with the results of Dlamini et al. (2017) who found 127.98 mg/dl. In other study, Ahmad et al. (2004) reported higher values in cyclic cross breed cow (50.72±1.12 g/dL).

Low-density lipoproteins (LDL), or "bad" cholesterol, may make arterial narrowing worse. The normal range of LDL in blood is 3 to 800 mg/dl. From the Table 2, it was found that there was a significant reduction (P<0.05) of LDL concentration in blood of experiment animal at day 28 fed probiotic treated straw compared to untreated straw. However, a significant (P<0.05) increased LDL concentration was observed in blood of animal at days 14 and 21 fed probiotic treated straw. Highest value was found at day 0 (247.6 mg/dl) and lowest value was found at day 28 (162.2 mg/dl) in treated straw compared to untreated straw group. However, in probiotic treatment, there was no difference in time points. LDL level was decreased after probiotic supplementation which is

Table 2. Effect of probiotic treated rice straw on albumin (g/dl), glucose (mg/dl), HDL (mg/dl) and LDL (mg/dl) concentration in heifers.

Parameter	Treatment	Days				
		0	7	14	21	28
Albumin (g/dl)	T1	5.69[a]	4.16[a]	4.05[a]	4.37[a]	4.24[a]
	T2	3.70[b]	3.80[b]	4.08[a]	4.15[a]	4.18[a]
	SEM	0.31	0.35	0.35	0.43	0.18
	P-value	0.0002	0.0052	0.9461	0.3817	0.7747
Glucose (mg/dl)	T1	48.41[b]	93.20[b]	100.6[a]	52.44[a]	123.01[a]
	T2	65.62[a]	95.07[b]	109.45[a]	125.08[b]	115.43[b]
	SEM	1.48	2.20	1.68	2.08	3.03
	P-value	0.0002	0.0637	0.0360	0.0001	0.0001
LDL (mg/dl)	T1	245.2[a]	241.7[a]	184.9[b]	195.8[b]	251.5[a]
	T2	247.6[a]	243.1[a]	233.5[a]	232.3[a]	162.2[b]
	SEM	7.05	9.30	6.18	4.89	5.30
	P-value	0.0637	0.0360	0.000	0.0001	0.0001
HDL (mg/dl)	T1	120.42[a]	30.29.7[a]	119.92[a]	135.75[a]	136.19[a]
	T2	106.63[a]	139.891[b]	148.46[c]	165.28[d]	148.09[c]
	SEM	6.50	2.30	3.18	3.89	2.30
	P-value	0.003	0.060	0.0043	0.0021	0.0031

[a-b]Mean values within a row with different superscripts are significantly different ($P<0.05$). T1=Control group, T2=treatment group, SEM=standard error mean, LDL=low density lipoprotein.

similar with the results of Petkova et al. (2008) who found 1.04 mmol/l. Similar findings were also observed by Kumar and Sharma (1993) in dairy cows and Jayachandranl et al. (2007) in buffaloes who found 2.55 and 3.45 mmol/l. After probiotic supplementation LDL (bad cholesterol) was greatly decreased which is good for animal health condition. HDL or “good” cholesterol protects the body against narrowing blood vessels. HDL interprets its levels in the opposite manner of LDL. Table 1 shows that there was no significant difference ($P<0.05$) of HDL concentration in the blood of treated and untreated straw fed animals. The highest value (165.28 mg/dl) was found at day 28 in treated straw and the lowest value (119.92 mg/dl) was found at day 14 in untreated straw.

Monitoring microbial status

Figure 1 shows the effect of probiotic treated rice straw on fecal microbiota in heifers. The number of lactobacilli was in a range of 4.8×10^5 to 6.0×10^5 cfu/ml after probiotic treatment. This result is consistent with the results of Mallo et al. (2010), who found similar concentration while *Enterococcus faecium* added to diet. This result was in agreement with the findings of Timmerman et al. (2005) who reported that fecal counts of lactobacilli was not different compared to control, while six *Lactobacillus* species was administered with one-week-old veal calves for eight weeks. The use of probiotic bacteria to inhibit *Escherichia coli* O157:H7 in cattle is a promising method to control this food-borne pathogen. Previous reports have shown the potential of beneficial *E. coli* strains and lactic acid bacteria to reduce O157:H7 fecal shedding or prevalence in cattle. Coliforms are pathogenic micro-organism and are also responsible for many diseases.

Figure 1 shows that the number of coliforms decreased (6.8×10^5-4.0×10^5 cfu/g) after probiotic treatment. This result is similar with the results of Zhao et al. (1998) who observed 3.2×10^4 cfu/ml after probiotic supplementation isolated from cattle feces. This result is consistent with the results of Mallo et al. (2010) who found 2.76×10^5 cfu/ml. But this result disagreed with the findings of Timmerman et al. (2005) who found 5.69×10^5 cfu/ml in dairy goats. Decreasing amount of coliform in feces is a positive sign for animal health. Figure 1 indicates that in general total number of total bacteria did not vary (8.4×10^{11} to 4.8×10^{11} cfu/ml) after probiotic treatment. This result is close to Srinivas et al. (2013) who found 1.8×10^{10} cfu/ml of total bacteria supplementing of yeast culture (Levucell SC 20) 0.5 g/animal/day in the diet of graded Murrah buffalo bulls.

DISCUSSION

Rice straw, a by-product of the rice production is mainly used as a source of feed for ruminant livestock. Rice

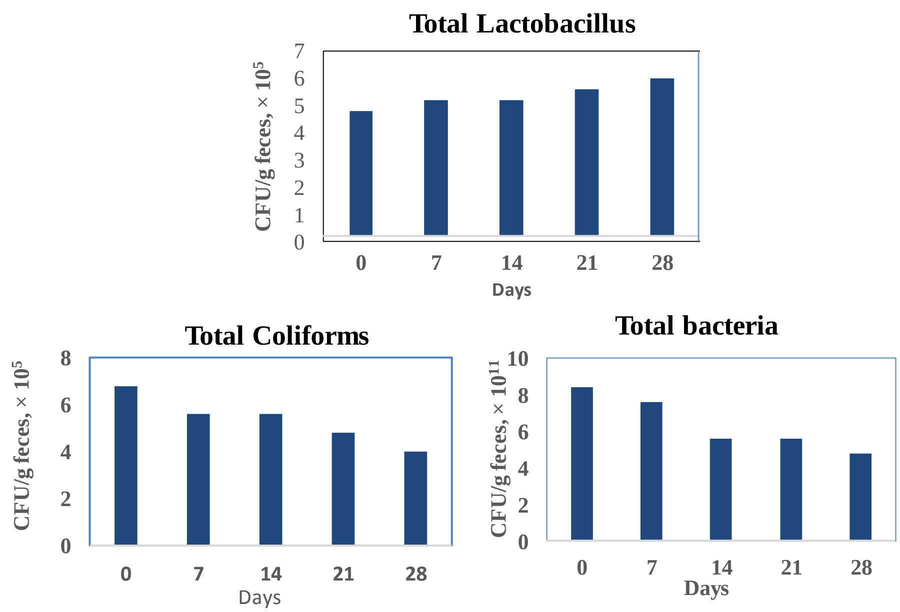

Figure 1. Effect of probiotic treated rice straw on faecal microbiota.

straw has very low nutritive values, especially its crude protein content and digestibility. Probiotic treatment increased CP content tremendously in the current study (Table 1). From the current findings, it was assumed that fermentation process with addition of microbes might improve crude protein content of rice straw. Sairubang et al. (2002) and Antonius (2009) strongly suggests this that animal may receive more digestible protein for their production performance. ADF concentration was also decreased in the present study (Table 1). This was a good sign of release of cellulose and hemicellulose from straw. The decreased crude fiber level of rice straw suggests that probotic microbes are able to penetrate the fibrolytic structure and cleave the binding of lignified carbohydrate and in some extent, degrade cellulose and hemicellulose. The addition of beneficial microbes in the straw seemed to have an effect in the breakdown of fiber materials in rice straw. The acid detergent fiber value refers to the cell wall portions of the forage that are made up of cellulose and lignin. Treating rice straw with effective microorganisms decreased the acid detergent fiber (ADF) component thus making it more digestible and animal will get more digestible energy (DE).

Albumin, glucose and LDL are important blood parameters of dairy cows. Blood albumin level was in a normal range (Table 2) when fed probiotic treated rice straw to the animals indicating a good health condition of those animals. A lower level of albumen was found compared to untreated straw group in the current study. The reasons of lower content of albumin in the present study may be due to individual variations of animal, breed or seasonal effect. The interesting thing is that probiotic treated straw feeding could not affect normal range of glucose, LDL and HDL concentration in the blood of animals. After probiotic supplementation, LDL (bad cholesterol) was greatly decreased (Table 2) that strongly suggest that probiotic straw feeding is not harmful for the animals. The present study stated that, there was an increasing tendency of HDL after probiotic treatment. These results are in accordance with results of Yu et al. (2004) who found 179 mg/dl of HDL concentration in blood after supplementation with direct fed microbes in cows. After probiotic treatment, HDL (good cholesterol) was significantly increased which is good for animal health status.

The beneficial effects include increased number of lactobacillus in feces on growth performance, nutrient retention, diarrhea reduction and for the balance of intestinal microflora. By this process, the health status of the animals will be increased.

The use of probiotic bacteria to inhibit *E. coli* O157:H7 in cattle is a promising method to control this food-borne pathogen. Previous reports have shown the potential of beneficial *E. coli* strains and lactic acid bacteria to reduce O157:H7 fecal shedding or prevalence in cattle. Coliforms are pathogenic micro-organism and are also responsible for many diseases. It was found that the number of coliforms decreased (6.8×10^5-4.0×10^5cfu/ml) after probiotic treatment (Figure 1). This result is similar with the results of Zhao et al. (1998) and Harman et al. (1996) who observed 3.2×10^4 cfu/ml after probiotic supplementation in buffalos. This result is consistent with the results of Mallo et al. (2010) who found 2.76×10^5 cfu/ml. But this result disagreed with the findings of Timmerman et al. (2005) who found 5.69×10^5 cfu/ml in dairy goats. Decreasing number of coliform in feces is a positive sign for animal health. Disease incidence related to coliforms may be reduced in animals after probiotic supplementation.

Conclusion

Pobiotic (protexin) treated rice straw improves crude protein content and decreases fiber content compared to untreated rice straw. An *in vivo* trial showed that blood metabolites such as glucose, albumin, LDL, HDL and fecal microbiota were almost in a normal range after probiotic treated straw feeding. Therefore, probiotic can be considered as a straw treating agent and an environment safe alternative to the hazardous chemicals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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