Research Article



Effect of Feeding Probiotic Fermented Rice Straw-Based Total Mixed Ration on Production, Blood Parameters and Faecal Microbiota of Fattening Cattle

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Abstract | The current study was planned to determine the effect of feeding probiotic fermented rice straw-based total mixed ration (TMR) on production performance, haemato-biochemical indices, and faecal bacteria of fattening cattle. A total of eighteen crossbred male cattle of 14-16 months were divided into three equal groups at random. Cattle in the control group (C) were offered ration containing 40% untreated rice straw, 20% green fodder, and 40% concentrate mixture (dry matter basis) fed separately as a conventional feeding system. Cattle in the other two treatment groups were fed TMR, where one fed TMR (TMR₁) with control ration and the other fed TMR (TMR₂) with the same feed ingredients in the same quantity except probiotic fermented rice straw instead of untreated rice straw of TMR₁. The trial lasted ninety days. Feeding of TMR₂ improved the growth rate (P<0.05) of fattening cattle compared to others. Furthermore, TMR₂ fed cattle had higher (P<0.05) values for hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and lactobacillus count. Serum glucose, triglyceride, and blood urea nitrogen (BUN) concentrations and coliform count were significantly (P<0.05) lower in the TMR₂ fed group relative to the TMR₁ and control ration fed groups. It may be concluded that feeding of rice straw as such or after its probiotic fermentation along with concentrate mixture in the form of TMR produced a better response in fattening cattle than the conventional system of feeding concentrate and straw separately.

Keywords: Total mixed ration, Blood profile, Fattening cattle, Performance, Faecal bacteria, Probiotic

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INTRODUCTION

In Bangladesh, the potential genetic superiority of livestock has not been fully exploited due to a lack of feed resources and underutilization of appropriate technologies to fulfil the nutrient deficiencies in their ration. It is crucial to increase the feeding value of available feed resources, especially crop residues, in order to achieve economic productivity in livestock husbandry (Kannan et al., 2011). To solve the problem, new feeding habits and strategies are clearly required. The total mixed ration (TMR) has fascinated farmers' interest due to the benefits it has provided in terms of nutrition, management, and production of ruminant animals (Hayasaka et al., 1991; Sirohi et al., 2001). TMR or complete feed is a method of feeding concentrates and roughages in a blended form without allowing the animal to select a specific ingredient (Khan et al., 2010), and every bite can give the required quantity of

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nutrients (Wadhwa and Bakshi, 2006). TMR not only increases the nutritional value of feedstuffs but also simplifies feeding and reduces wastage. TMR helps in maintaining a more stable and ideal rumen environment for microbial activities, which in turn can boost and economize ruminant productivity (Hundal et al., 2004; Rao et al., 2014). Feeding complete diets improved animal performance when compared to conventional feeding systems (Mane et al., 2006; Dhuria et al., 2007). TMR feeding to cattle has recently gained widespread acceptance.

Rice straw is a common agricultural by-product in many tropical countries (Nguyen et al., 2019). Smallholder farmers typically store and use it as ruminant feed all of the year, especially during the dry winter season. Rice straw is a low-density feed made up primarily of lignified cellulose and hemicellulose that has long been used as a staple feed ingredient for ruminants in Bangladesh. Because of its high lignification (which makes crop residue less palatable and digestible) and low nutritional content, it is classified as a sub-maintenance feed for ruminants. The nutritive quality and voluntary consumption of straw can be maximized by using different treatments, supplementing with some concentrate, or combining both techniques (Chaudhry, 2000). Microbial degradation is becoming a preferred alternative for pre-treatment of crop straw since it breaks down cellulosic polymers into cellulose, which may subsequently be digested by various cellulases and hemicellulases (Wu et al., 2004; Hughes et al., 2008). Improvement in the nutritional value of straw and their voluntary intake following probiotic fermentation makes it a method of choice. The use of probiotics in rice straw fermentation benefits both pre-treatment and normal digestion in the rumen. However, data on the use of probiotic fermented rice straw in complete rations/TMR and its effects on cattle are limited. Therefore, the study was carried out to determine the effect of feeding either rice straw-based or probiotic fermented rice straw-based total mixed rations (TMRs) versus the conventional feeding system on feed intake, growth performance, haemato-biochemical parameters, and faecal microflora in fattening cattle.

MATERIALS AND METHODS

The growth trial was conducted at Sher-e-Bangla Agricultural University's Animal Farm in Dhaka-1207, Bangladesh, and Microbial study was performed in the Laboratory of the Department of Animal Production and Management of the same University.

ANIMALS, DIETS, AND MANAGEMENT

Eighteen crossbred male cattle of 14-16 months of age having an initial average body weight of 135±15.87 kg were randomly divided into three equal groups of six cattle

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in each. In control group (C), cattle were offered ration containing untreated rice straw, green fodder (Pennisetum purpureum), and concentrate mixture fed separately as conventional feeding system. In treatment group 1, cattle were fed the control (C) ration as TMR (TMR₁) but group 2 was offered TMR (TMR₂) with the same feed ingredients of the same quantity except probiotic fermented rice straw instead of untreated rice straw of TMR₁. Ration was formulated as per National Research Council (2001) standards, and straw, green fodder and concentrate mixture were added in the ratio of 40:20:40 on dry matter basis (Table 1). The proximate composition of feeds and fodder was estimated as per AOAC (2007). The Van Soest et al. (1991) procedure was used to determine the acid detergent fibre (ADF) and neutral detergent fibre (NDF). The chemical composition of the feedstuffs has been presented in Table 2. All the cattle were stall-fed. The experiment lasted 105 days, with 15 days adjustment period followed by data and sample collection period for 90 days. Ad libitum feeding was practiced twice daily during the period of adjustment for determining voluntary feed intake (VFI). The amount of supplied diet was adjusted every 15 days based on the live weight of each animal. Cattle had free access to fresh, clean, and safe drinking water. Cattle were dewormed and vaccinated against common contagious diseases before beginning the experiment. They were housed in well-ventilated concrete-floored sheds with individual feeding and watering facilities, as well as identical care and management.

Table 1: Ingredients	used	in	the	experimental	rations	(%
DM basis)						

Ingredients (%)	Dietary Treatments			
	Control	TMR ₁	TMR ₂	
Untreated rice straw	40	40	-	
Probiotic fermented rice straw	-	-	40	
Green fodder (<i>Pennisetum purpureum</i>)	20	20	20	
Wheat bran	5	5	5	
Kheshari bran	4	4	4	
Rice polish	3.8	3.8	3.8	
Soybean meal	15	15	15	
Molasses	10	10	10	
Mineral mixture	0.8	0.8	0.8	
Salt	0.4	0.4	0.4	
Total	100	100	100	

Note: Mineral mixture consisted of Vitamin A- 6,000,000 I.U, Vitamin D₃- 3,000,000 I.U, Vitamin E- 300mg, Vitamin B₂- 600mg, Vitamin B₆- 60mg, Vitamin B₁₂- 4mg, Vitamin K₃- 40mg, Nicotinic Acid- 440mg, Folic Acid- 100mg, Cu-12000mg, Mg- 24000mg, Zn-24000mg, Se- 180mg, Essential Amino Acid- 108000mg.

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Table 2: Chemical composition of feedstuffs (% DM basis)

Parameters	Untreated rice straw	Probiotic fermented straw	Green fodder	Concentrate mixture
Dry matter (DM)	91.05	28.88	24.00	89.50
Crude protein (CP)	4.46	7.72	8.83	21.56
Acid Detergent Fibre (ADF)	63.93	60.79	46.43	17.06
Neutral Detergent Fibre (NDF)	78.86	77.87	72.05	53.02
Ether Extract (EE)	1.21	1.28	1.69	3.96
Ash	18.68	17.49	8.54	11.47

Table 3: Dry matter intake, growth performance and feeding system

Parameters	Control	TMR ₁	TMR ₂	Level of significance
Initial body weight (kg)	136.52±13.76	134.07±14.35	136.61±12.49	NS
Final body weight (kg)	166.85 ^b ±17.15	168.09 ^{ab} ±15.67	181.07ª±18.32	*
Total weight gain (kg)	30.33 ^b ±4.25	$34.02^{ab} \pm 4.15$	44.46 ^a ±6.95	*
Average daily gain (g/d)	337 ^b ±39	$378^{ab} \pm 46$	494ª±41	*
DM intake (kg/d)	3.70 ± 0.02	3.76±0.04	4.07±0.06	NS
DMI (% BW)	2.43±0.02	2.48±0.05	2.56±0.04	NS

Control-Concentrate mixture, green fodder and rice straw fed separately; TMR_1 -Control ration offered as TMR; TMR_2 -Concentrate mixture, green fodder and probiotic treated rice straw; Data were presented as mean ± standard deviation; Means with different superscripts at the same row differ significantly; *, P<0.05; NS, non-significant.

PROBIOTIC FERMENTATION OF RICE STRAW

Rice straw and probiotics (Super Madangbal, KVGMP, Korea) were collected from the local market. The probiotic product was a freeze-dried preparation of *Lactobacillus acidophilus* (15×10^9 CFU/kg), *Bacillus subtilis* (15×10^9 CFU/ kg), *Saccharomyces cerevisiae* (25×10^8 CFU/kg), and *Aspergillus oryza* (1×10^4 CFU/kg). Rice straw was chaffed to 2-3 cm in size using an electric chopping machine. Water was mixed with commercial probiotic @ 0.5% of straw as per Selim et al. (2019) and sprayed into the straw layer by layer. Then, the straw was tightly wrapped in a polythene sheet and incubated for two days. The polythene sheet was removed after two days, and then fermented rice straw was stored in a dry place.

FEED INTAKE AND WEIGHT GAIN CALCULATION

Every day, feed offered and refused was recorded in order to calculate dry matter intake. The body weight of the cattle was recorded using an electronic weighing balance at the beginning and end of the experimental feeding, as well as at fortnightly intervals until the study was completed.

BLOOD COLLECTION AND ANALYSIS

Blood samples were drawn aseptically by venipuncture of the jugular vein in two test tubes, one containing ethylene diamine tetra acetate (EDTA) and the other for serum separation on the 90th day of the feeding trial. Blood in EDTA was used for hematological analysis. Serum was used for biochemical evaluation.

Hematological parameters i.e., red blood cell count (RBC),

hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), white blood cell count (WBC), and platelet count (PLT) were analyzed using an automated hematology analyzer (Sysmex XN-450, Japan). Biochemical parameters viz., total protein, albumin, glucose, cholesterol, triglycerides, creatinine, and blood urea nitrogen (BUN) were assayed by an automatic serum biochemistry analyzer (Erba Chem 7, Germany).

FECAL COLLECTION AND ENUMERATION OF BACTERIA

Fecal sample was collected at the end of the feeding trial (on the 90th day) using the sterilized spatula and immediately transferred to the laboratory to enumerate the number of fecal lactobacilli, coliforms, and total viable bacteria. The total viable bacteria, *Lactobacillus* spp., and coliforms were determined using Nutrient Agar (NA); de Man, Rogosa, and Sharpe (MRS) agar; and Eosin Methylene Blue (EMB) agar media, respectively. After incubation of agar plates at 37°C for 48 h, plates were assessed for growth, and colonies were counted using a digital colony counter. All the bacteriological cultures were maintained according to the methods described by Merchant and Packer (1967). Before statistical analysis, the number of bacteria was converted to Log_{10} values and expressed as arithmetical means \pm SE (Log_{10} CFU/gm of fecal material).

STATISTICAL ANALYSIS

The SPSS software for Windows (IBM SPSS Statistics 22) was used for the analysis of data. Means were tested when statistically varied significantly using Duncan's Multiple Range Test (DMRT).



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DRY MATTER INTAKE AND BODY WEIGHT CHANGE

The intake of dry matter did not vary significantly (P>0.05) across the various experimental groups (Table 3), which is consistent with the findings of Hundal et al. (2004) and Khan et al. (2010) who also provided the same feeds in the form of TMR. Titi et al. (2008) and Jiang et al. (2020) reported that probiotics had no significant effect on feed consumption in small ruminants. However, a slightly higher intake of DM in cattle received TMR containing fermented straw may be attributed to increased nutrient density as well as changes in the physical characteristics of probiotic treated rice straw such as wilting and small particle sizes of total mixed rations.

The total weight gain and daily weight gain were improved (P<0.05) on feeding TMRs over the conventional feeding system, but the difference between TMR₁ and the control group was not significant (Table 3). In contrast to the findings (insignificant variation between TMR_1 and control group regarding weight gain), a higher (P<0.05) growth rate was reported by Mane et al. (2006) and Dhuria et al. (2007) following the feeding of pelleted TMR to goats and lambs, respectively. However, in our study, pelleting of TMR was not performed, which might be the reason for the insignificant effect on growth rate. The significantly (P<0.05) improved weight gain for TMR, group cattle may be due to the nutritional superiority of the probiotic fermented straw based TMR over the untreated straw based TMR. Besides, the possible positive effect of TMR having probiotic fermented rice straw on body weight gain could be attributed to increased microbial cellulolytic activity, leading to improved fibre fermentation and better synthesis of microbial protein, resulting in increased supply of post-ruminal amino acid (Chaucheyras-Durand et al., 2008; Erasmus et al., 1992). Additionally, better growth performance in TMR, feeding animals may also be associated with increased consumption, fibre degradation (El-Waziry and Ibrahim, 2007), and efficient feed utilization (Antunović et al., 2005; Musa et al., 2009). Probiotics have been shown in studies to have a significant effect on body weight gain (Krehbiel et al., 2003; Tricarico et al., 2007).

HEMATOLOGICAL PROFILE

The concentration for hematological indices (Table 4) was well within the normal range reported for cattle, indicating that TMRs had no adverse effect on the physiological status of the cattle, which is also a sign of good health. Hemoglobin concentration and RBC count were statistically (P<0.05) greater in the TMR₂ group than in the other groups, but did not differ significantly between the TMR₁ and control groups. Similar results were reported by

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Milewski and Sobiech (2009) and Ghazanfar et al. (2015) in ewes and heifers fed probiotics supplemented feed, respectively. The observed elevated effects would be explained as the dietary supplementation of probiotics triggered blood-cell formation processes as a result of better iron salt absorption from the small intestine and vitamins B production (Kander, 2004). Counts for leukocytes were also higher (P<0.05) in the cattle fed TMR, than in the control and TMR, groups. Our findings were consistent with those of Al-Saiady et al. (2010) and Ghazanfar et al. (2015), who found that probiotic-supplemented cattle had a significant improvement in the number of white blood cells. Increased blood WBC count may be involved in the production of more immune cells (Gaggia et al., 2010), which play an important role in defending the body against various disease-producing agents. The increased total leukocyte count in probiotic supplemented groups might be indicative of gut microbiota stimulating intestinal immune system responses. However, platelet count, PCV, and MCV levels were similar (P>0.05) across all the treatments, which was consistent with the previous findings of Malik et al. (2020), who fed goats wheat straw based TMR.

BIOCHEMICAL PARAMETERS

Total blood protein and albumin concentrations did not vary significantly among the conventional and TMRs feeding groups, whereas numerically higher values in TMRs feeding groups indicate efficient nitrogen utilization (Table 5). Our findings were in accordance with those of Hundal et al. (2004), who reported that TMRs and conventional feeding systems had no effect on blood protein and albumin levels. Abas et al. (2007) and Soren et al. (2013) also observed that blood total protein and albumin concentrations were not influenced by the dietary supplementation of probiotics in lambs.

Glucose and blood urea nitrogen (BUN) levels in the TMR, group were significantly lower (P<0.05) than in the other groups (Table 5). Concentrations of glucose and BUN decreased in lambs fed a probiotic supplemented diet, as previously reported by Antunovic et al. (2005) and El-Sayed and Mousa (2020). The lower serum glucose levels in fermented straw based TMR fed cattle could be explained by reduced gluconeogenesis. In ruminants, blood glucose is contributed partly by direct absorption through the GIT (gastrointestinal tract) and partly by gluconeogenesis from propionate (Kaneko et al., 2008). Probiotic fermentation of straw can result in lower blood glucose concentrations because of improved digestion of fibre, leading to increased acetic acid and decreased propionic acid formation in the rumen (Antunović et al., 2005; Bruno et al., 2009). The lower concentrations of BUN in cattle fed TMR containing fermented straw could be related to better rumen bacterial utilization of nitrogen. Probiotic supplementation boosts rumen microbial activity, which leads to higher am-



Table 4: Haematological parameters of fattening cattle and feeding system

Haematological variables (units)	Control	TMR ₁	TMR ₂	Level of significance
RBC (million/ µl)	$7.87^{b} \pm 0.20$	$8.18^{b} \pm 0.18$	8.93ª±0.04	*
WBC (per µl)	9650 ^b ±295	$10103^{b} \pm 340$	11950°±372	*
Platelets (per µl)	348360±17303	306090±8990	343500±13601	NS
Hb (g/dl)	$10.28^{b} \pm 0.15$	10.59 ^b ±0.23	11.87ª±0.21	*
PCV (%)	28.93±2.36	30.08±2.50	29.79±1.93	NS
MCV (fl)	40.93±0.84	42.04±0.67	41.25±0.75	NS

RBC: red blood cell count, WBC: white blood cell count, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume; Control-Concentrate mixture, green fodder and rice straw fed separately; TMR_1 -Control ration offered as TMR; TMR_2 -Concentrate mixture, green fodder and probiotic treated rice straw; Data were presented as mean ± standard deviation; Means with different superscripts at the same row differ significantly; *, P<0.05; NS, non-significant

Table 5: Serum biochemical profile of fattening cattle and feeding sys	Table 5: Ser	rum biochemical	profile of fattening	g cattle and feeding syste
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Biochemistry variables (units)	Control	TMR ₁	TMR ₂	Level of significance
Total protein (g/dl)	6.29±0.40	6.41±0.52	6.57±0.67	NS
Albumin (g/dl)	3.67±0.08	3.74±0.05	3.83±0.09	NS
Glucose (mg/dl)	71.3ª±3.18	$66.14^{a} \pm 2.08$	$62.8^{b} \pm 1.48$	*
Cholesterol (mg/dl)	111.29±5.48	105.6±3.48	101.71±4.48	NS
Triglyceride (mg/dl)	48.33 ^a ±6.88	43.01 ^{ab} ±5.67	$41.15^{\text{b}}\pm 5.35$	*
BUN (mg/dl)	17.11 ^a ±2.1	$14.61^{b} \pm 1.9$	11.87°±1.8	*
Creatinine (mg/dl)	1.24±0.02	1.26±0.03	1.27±0.01	NS

Control-Concentrate mixture, green fodder and rice straw fed separately; TMR_1 -Control ration offered as TMR; TMR_2 -Concentrate mixture, green fodder and probiotic treated rice straw; Data were presented as mean ± standard deviation; Means with different superscripts at the same row differ significantly; *, P<0.05; NS, non-significant.

Table 6: Population of faecal bacteria in fattening cattle (\log_{10} cfu/g ± SD) and feeding system

Parameters	Control	TMR ₁	TMR ₂	Level of significance
Total bacterial count	7.90±0.13	7.88±0.19	7.91±0.21	NS
Coliforms	3.48 ^a ±0.32	3.43 ^{ab} ±0.19	$3.24^{b} \pm 0.19$	*
Lactobacilli	5.67 ^b ±0.11	$5.76^{ab} \pm 0.17$	6.14 ^a ±0.25	*

Control-Concentrate mixture, green fodder and rice straw fed separately; TMR_1 -Control ration offered as TMR; TMR_2 -Concentrate mixture, green fodder and probiotic treated rice straw; Data were presented as mean ± standard deviation; Means with different superscripts at the same row differ significantly; *, P<0.05; NS, non-significant.

monia capture for the production of microbial protein (Erasmus et al., 1992). BUN reflects the efficiency of dietary protein utilization in the rumen (Fadel-Elseed et al., 2014).

In our study, there was a significant variation (P<0.05) in triglyceride levels among the experimental groups, with the TMR₂ group having the lowest triglyceride level. This could be due to an improved metabolism as well as a positive energy balance with probiotic additions. Such a reduction was reported by Abas et al. (2007) and Ayad et al. (2013) in lambs and cows supplemented with probiotics, respectively. The serum cholesterol concentration was lower for cattle fed probiotic fermented straw based TMR compared to others, but the difference was not statistically (P>0.05) significant (Table 5). Similar results were reported by Malik et al. (2020) after feeding fattening goats with

TMR containing wheat straw. Probiotic supplementation had no effect on blood cholesterol concentrations, according to Soren et al. (2013) and Yasmin et al. (2021). Contrary to these results, Ooi and Liong (2010) stated that probiotic administration is beneficial in terms of improving lipid profiles as well as lowering blood cholesterol. However, there were no significant changes in creatinine levels in treated groups compared to the control group, which corresponded with the earlier findings of El-Sayed and Mousa (2020).

FECAL BACTERIAL COUNTS

The effects of feeding TMR containing probiotic treated straw on the number of coliforms and lactobacillus were significant (P<0.05), but the effect on total viable bacteria was not significant (P>0.05) (Table 6). In present studies, the results showed that feeding of fermented rice straw

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based TMR had positive effect on increasing (P<0.05) the numbers of fecal Lactobacillus population. The present results were agreed with Kawakami et al. (2010) and Bayatkouhsar et al. (2013) who found that probiotic administration increased the fecal population of lactobacilli. The significantly lower (P<0.05) number of coliforms was found in the TMR, group than others. A decrease of coliform in feces is a good sign for animal health. According to Agarwal et al. (2002) and Mallo et al. (2010), feeding probiotics significantly decreased fecal coliform. The number of coliforms is lower than that of lactobacilli in healthy animals (Signorini et al., 2012) which is also consistent with the present findings. One possible explanation for the lower coliform count is that probiotic bacteria inhibit the growth of coliform bacteria through the release of carboxylic acids such as acetic acid and lactic acid, which lower gut pH.

CONCLUSION

Feeding TMR with rice straw co-fermented with probiotics improved growth performance. In addition, the use of this fermented straw had also an improving effect on hematological and serum biochemical variables and altered the faecal bacterial community toward the increased relative abundance of lactobacillus. The study may lead to the conclusion that feeding rice straw in the form of total mixed ration is a practical method for improving the growth performance and health status, but TMR containing probiotic fermented rice straw could even be better than that of untreated straw based TMR.

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NOVELTY STATEMENT

The main objective of this research work is to find out the impact of feeding probiotic fermented rice straw-based total mixed ration (TMR) on dry matter intake, growth performance, haemato-biochemical parameters, and faecal bacteria of crossbred fattening cattle. Even though many researchers have worked on the probiotic fermented rice straw and its effects on animal performances, but very few researchers have reported on the feeding performance of probiotic treated rice straw in the form of TMR with crossbred male cattle under tropical environmental conditions like Bangladesh.

CONFLICT OF INTEREST

No conflict of interest.

AUTHORS CONTRIBUTION

Conceptualization, MEK and MJA; experiment execution, MEK and MMH; data analysis and interpretation of the data, MEK, MJA and MMH; writing—original draft, MEK and MMH; writing —review and editing, MEK, MJA, MMH and ZF; All authors have read and agreed to the published version of the manuscript.

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