Research Article

Effect of TMR and Fermented TMR on Ruminal *In vitro* Digestion and Gas Production

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Abstract | Ruminants efficiently digest fibrous components resulting in high-quality milk and meat. The emerging ruminant population to meet the protein demand is one of the driving factors of increasing global greenhouse gases. The current study aimed to assess the effects of total mixed ration (TMR) and fermented TMR (FTMR) feed on digestibility, total gas production and pH of ruminants. Novel feeding techniques were implemented that combined fermentation to improve digestibility with locally accessible feed supplies using a completely randomized design. TMR feed was produced with 70% roughage of silage & rice straw and 30% concentrate mixture. FTMR feed was produced by mixing TMR feed with molasses containing *Saccharomyces cerevisiae.* Prepared TMR and FTMR feed mixed with rumen fluid buffer for *in vitro* digestion at 6h, 12h, 24h, & 48h of the incubation period. There were five replications at each of TMR and FTMR dietary treatments. Mean comparison of both TMR and FTMR group evaluated by t-test assuming equal variances. We observed decreasing tendency of pH with the increasing incubation period and no significant difference of pH between TMR & FTMR feeds at 24h, 48h incubation period. The highest mean digestibility of both TMR and FTMR feed was obtained at 34.78% (34.78±1.28) and 45.91% (45.91±1.09) respectively, at 48h incubation period. Among five replicates of both TMR and FTMR, the highest mean gas production was 74ml (73.8±1.11) and 59ml (59±0.89) respectively, at a 48h incubation period. The digestibility of FTMR was significantly higher than the TMR ($p<0.01$) feed at all incubation periods. Conversely, total gas production was significantly lower in FTMR than in the TMR ($p<0.01$) feed. It can be concluded that FTMR is better than TMR in terms of digestibility and gas production.

Keywords | Digestibility, FTMR, *In vitro* digestion, TMR, Total gas production

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INTRODUCTION

The global existent 7.3 billion population is expected to increase by 8.5 billion by 2030 and 9.7 billion worldwide by 2050 (DESA, 2015). Food production will need to increase by 70% to meet the demand for adequate nutrition in this growing population (Lagrange et al., 2015). Global milk production in 2018 was estimated to total 838 metric tons (mt), with the largest producers were India, the European Union (EU), New Zealand, and the United States (OECD/FAO, 2019). However, the demand for milk and meat production from ruminants are one of the

driving factors behind increasing global greenhouse gases (GHG) through enteric methane (CH_4) production (Niu et al., 2018). Total non-CO_2 GHG emissions from livestock in 2000 were estimated at 2.45 billion mt CO_2 equivalent, where ruminants were the largest source of GHG emissions (1.6 billion mt CO_2 equivalent) due to enteric CH_4 fermentation (Herrero et al., 2013). 14.5% of global anthropogenic GHG emissions come from livestock, with approximately 44% of livestock emissions in the form of CH_4, whereas enteric fermentation is the second largest source of emissions, contributing about 40% to total emissions (Matthews et al., 2019; Gerber et al., 2013).

Ruminants effectively convert cellulose and other fibrous components in order to produce high-quality milk and meat. Additionally, they are producing GHG (CO_2, CH_4, N_2O) through microbial fermentation of feed within the rumen (Henry & Eckard, 2009). Another important problem with gas production is the loosing of productive energy and high biological value proteins nearly 12% of total energy intake (Johnson & Johnson, 1995). This may cause confined productive performance (Kholif et al., 2014) and the release of pollutants to the environment (Calsamiglia et al., 2007). Methane emissions from cattle are influenced by a variety of factors, such as the amount of feed consumed, the kind of carbohydrates consumed, the processing of the feed, the presence of lipids or ionophores to the ration, and changes in the ruminal microflora (Johnson & Johnson, 1995). Additionally, feed production costs are rising daily in the livestock sector due to dietary dependence on raw materials. In order to reduce on-farm GHG emissions, novel feeding techniques that include various roughages and concentrate with minimal impact on animal productivity and social acceptability should ideally be implemented.

The total mixed ration (TMR) is a widely accepted proper type of balanced mix feed with incorporation of high moisture and nutrient-containing agricultural by-products (Li et al., 2003; Wang et al., 2016). The mixed feed has been the subject of great inclination among farmers because of its anticipated benefits in the nutrition, management, and production of ruminant animals (Owen, 1984; Sirohi et al., 2001). Farmers involved with the beef fattening are demonstrating a strong preference for fibrous material diversified feed, such as the TMR allowance, over concentrates (Kim et al., 2003). It has already been established that fibrous materials in the mixed feed are convenient for maintaining the homeostasis of ruminant stomach pH, reducing the incidence of metabolic disease, and improving milk production (Harrison et al., 1989; Kellems et al., 1991).

The advantages of TMR include higher feed consumption, improved utilization of inexpensive substitute feed ingredients, the capacity to manage forage concentrate ratios, a decreased incidence of metabolic and digestive diseases, and a reduction in feeding labor (Owen, 1984). Silage, forage, and hay are the traditional and accepted roughages to prepare TMR (Chumpawadee & Pimpa, 2009). The fermented feed of TMR (FTMR) may increase its digestibility and feed efficiency. Nevertheless, Yeast as a natural feed additive has the ability to stagnate rumen fermentation and prevent rumen flora disorders and disturbances with increasing the quantity of durable bacterial cells (Pinloche et al., 2013).

Probiotic use of *Saccharomyces cerevisiae* as nutritional additive increase feed efficiency and activity of the cellulolytic flora, reduce methane production and favor keeping pH stable (Suarez & Guevara, 2018; Kim et al., 2012). In the case of fermented mixed feed, addition of probiotic yeast ensured that cattle with higher rumen pH continued to experience a healthy fermentation. The dynamics of gas production, *in vitro* digestibility, and interactions with fodder quality are all positively impacted by yeast products made using *Saccharomyces cerevisiae* (Elmasry et al., 2016).

Likewise, in developed countries, TMR feed is becoming more popular in the commercial dairy farms of Bangladesh. However, there is currently insufficient information on digestibility and gas production for TMR and FTMR prepared from silage of green grasses like Napier (*Pennisetum purpureum*), Para (*Brachiaria mutica*), and German (*Echinochloa polystachya*) grass with domestic rice straw in Bangladesh. The purpose of the study was to assess the effects of TMR and FTMR feed on ruminant digestibility, total gas production, and pH using the ruminal *in vitro* digestion method with locally available silage, rice straw, and concentrates.

MATERIALS AND METHODS

An *in vitro* ruminal fermentation technique was obtained using TMR and FTMR feed with buffered rumen fluid in serum bottles through *in vitro* tests. Total mixed concentrate feed prepared on a 90% dry matter (DM) basis with 75-77% total digestible nutrient (TDN) (Table 2). Total mixed feed homogeneously mixed with yeast culture to obtain FTMR. The prepared TMR and FTMR feed were added into separate serum bottles. Buffer was essential to have proper functioning of the rumen fluid in the *in vitro* test by maintaining the pH level of the rumen environment. So, a buffer medium (pH 6.9) to inoculate in rumen fluid was prepared.

Buffered rumen fluid was prepared by mixing the rumen fluid with a buffer medium in a 1:3 rumen fluid: buffer ra-

tio and was taken in previous serum bottles for the final *in vitro* test according to Asanuma et al. (1999). Serum bottles were kept in a shaking incubator (Model: LBSI-100A, Labnics® Equipment, USA) for 6h, 12h, 24h, and 48h incubation.

A completely randomized design was applied during the dietary treatment setup at each incubation time (6h, 12h, 24h, and 48h). At each of the 4 incubation times, the total gas production, digestibility, and pH were measured from 5 replications of both the TMR and FTMR diets.

Ethical approval

The research protocol was discussed and approved at a meeting of the Chattogram Veterinary and Animal Sciences University Ethics Committee, dated March 9, 2020. The approval number for this research project was CVASU/Dir(R&E)EC/2020/165(9).

TMR and FTMR Feed

Maximum production in dairy cows has been reported to achieve from a mixture of 70% good quality roughage and 30% concentrate (Beyero et al., 2015). Based on that, the roughage feed was made of silage (60%) of Napier hybrid, Para, and German grass and rice straw (40%). The concentrate feed was made with wheat bran (40%), maize (20%), rice polish (15%), soybean meal (12%), khesari (10%), oyster shell (2%), and salt (1%) (Table 1). Nutrient composition of the concentrate mixture were measured before sample preparation (Table 2). A total of 100 g mixed feed sample of the prepared roughage and concentrate on dry matter basis was equipped for the study, where 50g was allocated as a TMR sample and the rest of the 50 g was isolated to prepare an FTMR feed sample. To obtain an FTMR feed sample, isolated 50 g mixed feed fermented with a cultured medium of *Saccharomyces cerevisiae*.

Table 1: Ration Formulation of the experimental TMR and FTMR feeds

Ingredient	%	Feed composition	%
Concentrate mixture	30	Maize	20
		Wheat bran	40
		Khashari	10
		Soybean meal	12
		Rice polish	15
		Oyster shell	2
		Salt	1
Roughage	70	Silage	60
		Rice straw	40

%=Percentage

Table 2: Nutrient composition of the concentrate mixture used in experimental TMR and FTMR feeds

Parameter	Composition (%)
DM	90
TDN	76
CP	14.5
Calcium	1.1
Phosphorus	0.8

DM=Dry matter; TDN=Total digestable nutrient; CP=Crude protein, %=Percentage

Yeast was cultured as 1g/L of molasses a soluble starch as medium (Asanuma et al., 1999). The optimal temperature, pH, and fermentation period were 35°C, 4.0, and 72 h respectively, for the growth of *Saccharomyces cerevisiae* (Periyasamy et al., 2009).

Rumen Fluid Collection

A sample of rumen fluid was collected from a freshly slaughtered cow in the Government slaughterhouse, Chattogram, Bangladesh which was fed rice straw and commercial feed compositions twice a day. The required buffers were made the day before of rumen fluid collection for the time constraints. Grasses present in the collected rumen fluid were squeezed immediately to obtain the rumen fluid after slaughtering the cow. Rumen fluid (1L) was filtered through four layers of cheesecloth, put in an airtight flask, and brought to the laboratory of the animal science department, CVASU. The rumen fluid was then preserved at 39°C which was essential for conducting the *in vitro* test. In order to maintain the anaerobic environment required for rumen fermentation, rumen fluid was promptly dispensed with steady N_2 gas flow.

Purification of Buffer for Rumen Fluid

The buffer media was prepared following the instructions mentioned by Asanuma et al. (1999). The buffer used for rumen fluid contained a mixture of several chemicals and solids with a weighed amount of distilled water. Then, it was kept in an anaerobic condition. The chemicals required for the buffer were 0.45 g K_2HPO_4, 0.45 g KH_2PO_4, 0.9 g $(NH_4)_2SO_4$, 0.12 g $CaCl_2.2H_2O$, 0.19 g $MgSO_4{\cdot}7H_2O$, 1.0 g trypticase peptone, 1.0 g yeast extract, and 0.6 g cysteine HCl. The chemicals were poured into 1L distilled water. Yeast extract and trypticase peptone was dissolved immediately by hand to avoid clumping in contact with air. This mixture needed to be maintained at a pH of 6.9 with the addition of NaOH. Then, the buffer for rumen fluid was kept on a hotplate to prohibit chemical chunk floating in the buffer for homologous distribution. In order to establish anaerobically conditions, the buffer was dispensed with a 100% steady N_2 gas flow. At last, the buffer was autoclaved (Autoclave Digital, Model: LAT-105, Labnics® Equipment, USA) at 121°C for 15 minutes and preserved

till the rumen fluid collection of the next day.

Preparation of Buffered Rumen Fluid

625 ml of rumen fluid was mixed with 1875 ml buffer the next day after collection from freshly slaughtered cow. The bottle containing buffered rumen fluid was dispensed with 100% N_2 gas to make it O_2-free anaerobic condition (Asanuma et al., 1999). Fermentation method was inhibited in aerobic conditions as asserted and suggested by Goering & Van Soest (1970). At last, the rumen fluid buffer was prepared to be poured into 40 different serum bottles for the ultimate *in vitro* experiment. Buffered rumen fluid solution was then taken into 40 experimental serum bottles. 50ml of buffered rumen fluid dispensed in serum bottles with a volumetric pipette to pour accurate amounts into each bottle. After each time dispensing, N_2 gas was flowed extensively in each bottle for anaerobic condition creation. Afterward, immediately rubber caps were capped so that any kind of air gas especially O_2 cannot flow inside as part of maintaining anaerobic conditions.

Serum Bottle Setup

The final bottle setup was made keeping five replicates of both TMR and FTMR (T1, T2, T3,T4, T5) for each incubation time. Thereby, incubation times were 6h, 12h, 24h, and 48h. As for bottles, two types of serum bottles were made, where 20 serum bottles with TMR and another 20 bottles with FTMR. Each incubation period at the TMR and FTMR groups had 5 fixed bottles. Firstly, 0.5g prepared TMR feed material was added to each 20 serum bottles of TMR group and 0.5g prepared FTMR feed also added in another 20 serum bottles of FTMR group. Secondly, 50 ml of buffered rumen fluid was added to all 40 serum bottles. Gradually, all the bottle openings were sealed with the rubber cap and locked with a tin lid to prohibit gas leakage after *in vitro* gas production. Finally, all the bottles of both TMR and FTMR groups were put into a shaking incubator (Model: LBSI-100A, Labnics® Equipment, USA) at 37°C temperature for *in vitro* gas production as described by Hattori and Matsui (2008). For each incubation time, five replicates of both TMR and FTMR (T1, T2, T3, T4, T5) per experimental treatment were used.

Total Gas Collection

The gas generated during the *in vitro* test was collected using a calibrated gas syringe composed of plastic and glass. The syringe was attached with a three-way canola to regulate the gas flow in and out of the bottle and syringe. Firstly, a syringe was locked before entering in each bottle to prohibit atmospheric gas input in each syringe. Secondly, three way canola was regulated in a way to open the entrance of the syringe. Thirdly, syringe was put into the serum bottles. To clarify, any kind of extra pushing on the syringe tail was not made so that the natural flow of total gas conquered the inside vacuum of each syringe. Thereby, after each push of total gas accumulated from serum bottles, the plunger of the syringe went backward due to the total gas pressure. After the push ended, three way canola were regulated to close the entrance of the syringe. Thereby, it stopped further entrance of atmospheric gas inside the syringe. Thus, finally, syringes were prepared to measure the total gas. Total gas measured in mL and noted for further research.

Digestibility and pH Measurement

Firstly, TMR and FTMR feed sample weight was taken before digestion. After digestion of each incubation period weight of undigested dried feed of each serum bottle was taken. The difference of feed weight before digestion and digested feed was measured. Then digestibility was calculated as the percentage. The pH meter (Hanna HI 2211 bench pH meter) was used to determine the pH value.

Statistical Analysis

Data of digestibility%, Total gas production, and pH at 6h, 12h, 24h, and 48h incubation period for 40 serum bottles in both TMR and FTMR groups compiled in MS Excel (Microsoft Office Excel 2013). Mean comparison of both TMR and FTMR group evaluated by t-test assuming equal variances in STATA 13 (StataCorp LP 4905 Lakeway Drive College Station, TX 77845, USA). The p-value of ≤ 0.05 was considered as significant.

RESULTS

Data of total gas (ml) production, digestibility% and pH were recorded during *in vitro* digestion trial at 6h, 12h, 24h, and 48h of the incubation period. At each incubation period digestibility% was significantly higher in FTMR than TMR ($p<0.01$). Average digestibility of five replicates in both the TMR and FTMR group was 33.54% and 43.14% respectively, for the 24h incubation period ($p<0.001$) and 34.78% and 45.91% respectively, for the 48h incubation period ($p<0.001$) (Table 3). Total gas production increased gradually with a higher incubation period in all TMR and FTMR digestion trials.

In contrast, gas production differed evidently at all incubation periods ranging from 6h to 48h between the TMR and FTMR groups ($p<0.01$). FTMR had good digestibility and less gas production than TMR at the 48h incubation period for all 5 replicates between TMR and FTMR groups (Figure 1 and Figure 2). The decreasing tendency of pH value with increasing incubation period without significant difference was noticed (Figure 3).

Table 3: Mean comparison of Digestibility (%) in each incubation period

Incubation period	TMR	FTMR	P- value	95% CI
	Mean ± SEM*	Mean ± SEM*		
6h	25.01±0.55 [a]	32.49±1.79 [b]	0.0041	[25.288, 32.207]
12h	30.53±0.95 [a]	40.01±0.67 [b]	0.0000	[31.488, 39.055]
24h	33.54±0.99 [a]	43.14±0.86 [b]	0.0001	[34.457, 42.218]
48h	34.78±1.28 [a]	45.91±1.09 [b]	0.0002	[35.785, 44.910]

*SEM= Standard Error Mean; TMR=Total mixed ration; FTMR=Fermented total mixed ration; [a, b]Means in the same row with different superscript are significantly different ($p<0.05$)

Table 4: Mean comparison of total gas (ml) production in each incubation period

Incubation period	TMR	FTMR	P- value	95% CI
	Mean ± SEM*	Mean ± SEM*		
6h	27.8±1.16 [a]	17.4±0.68 [b]	0.0001	[18.426, 26.773]
12h	35.8±0.73 [a]	28.8±0.58 [b]	0.0001	[29.477, 35.122]
24h	54.8±2.22 [a]	45.2±0.66 [b]	0.0033	[45.616, 54.383]
48h	73.8±1.11 [a]	59±0.89 [b]	0.0000	[60.615, 72.184]

*SEM= Standard Error Mean; TMR=Total mixed ration; FTMR=Fermented total mixed ration; [a, b]Means in the same row with different superscript are significantly different ($p<0.05$)

Table 5: Mean comparison of pH in each incubation period

Incubation period	TMR	FTMR	P-value	95% CI
	Mean ± SEM*	Mean ± SEM*		
6h	6.34±0.05 [a]	6.60±0.01 [b]	0.0017	[6.355, 6.586]
12h	6.26±0.01 [a]	6.45±0.01 [b]	0.0000	[6.280, 6.431]
24h	5.91±0.04	5.84±0.05	0.2741	[5.801, 5.950]
48h	5.61±0.03	5.64±0.02	0.3642	[5.583, 5.668]

*SEM= Standard Error Mean; TMR=Total mixed ration; FTMR=Fermented total mixed ration [a, b]Means in the same row with different superscript are significantly different ($p<0.05$)

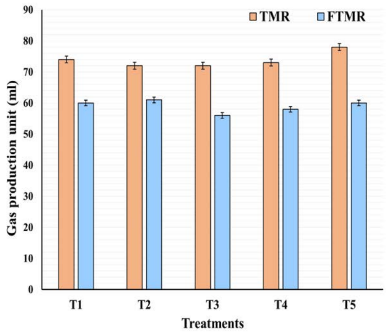

Figure 1: Total Gas production of FTMR and TMR feed at 48h incubation period for each replication
(In this figure 1: Total gas production at 48h incubation period is high in TMR than FTMR (Fermented TMR) feed in all replications)

Ruminal pH was not affected by TMR & FTMR group and the average pH value at 6h and 12h incubation period was nearly 6 ($p<.01$) (Table 5). At 24h and 48h incubation period pH value remained the same between TMR and FTMR groups. On the other hand, the lowest pH was insignificantly found at 48h incubation period in both the TMR and FTMR groups respectively, 5.61 and 5.64 ($p>0.05$) (Table 5 and Figure 3).

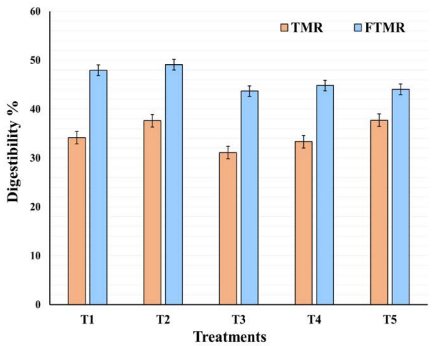

Figure 2: Digestibility (%) of FTMR and TMR feed at 48h incubation period for each replication
(In this figure 2: Digestibility at 48h incubation period is high in FTMR (Fermented TMR) than TMR feed in all replications)

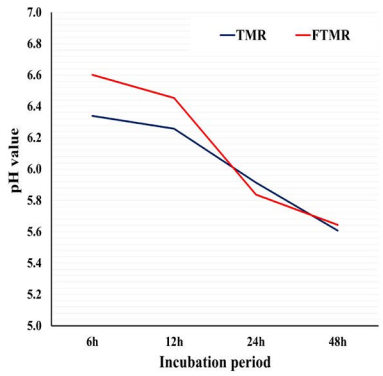

Figure 3: The trends of pH at each 6h, 12h, 24h, and 48h for both FTMR and TMR feed
(Decreasing tendency of pH was observed with increasing incubation period in both FTMR and TMR feed)

DISCUSSION

Irrespective of the variable incubation period digestibility was better in fermented total mixed feed in each replicates. 32.5% and 25% digestibility for FTMR and TMR feed found at 6h incubation period (Table 3). On the contrary, digestibility significantly increased at supreme level with 46% for FTMR and 35% for TMR feed in 48h incubation period ($p<.001$) (Table 3 and Figure 2). This finding is supported by many earlier studies. Cao et al. (2012) reported increased digestibility of FTMR compared with fresh TMR at three the incubation period of 2h, 4h, and 6h. Although they used different feed content in mixed ration preparation and lactic acid bacteria for feed fermentation. Effect of FTMR on diet digestibility and efficient feed utilization for optimum milk production have good improvement (Du et al., 2020). In this study, *Saccharomyces cerevisiae* was used as yeast supplementation which has positive effect to improve rumen fermentation (Desnoyers et al., 2009). Desnoyers et al. (2009) found organic matter digestibility significantly increased by yeast supplementation. Yeast supplementation also increases dry matter intake, milk yield, and milk fat content, but no satisfactory effect on milk protein content.

An increased proportion of concentrate in the dietary feed decreases the positive effect of yeast supplementation on digestibility, whereas dietary neutral detergent fiber increases the positive effect. The positive effects of yeast supplementation on digestibility described by Desnoyers et al. (2009) also consistent with this current study. Yeast culture of *Saccharomyces cerevisiae* as a feed additive can enhance digestibility, growth performance and economic income without any side effects on the physiological status (Elenin et al., 2016). Poppy et al. (2012) revealed strong evidence of commercially available yeast culture of *Saccharomyces cerevisiae* with dietary feed which significantly improve dry matter intake, digestibility, and milk production in lactating dairy cows (Poppy et al., 2012).

The experiment findings showed that gas generation increased as the incubation time progressed, which is consistent with the findings of Ahammed et al. (2021). However, fermented ration feed produced significantly less gas production ($p<.01$) than total mixed ration in each incubation period of 6h to 48h (Table 4). The lowest mean gas production for both TMR and FTMR digestion trial was at 6h respectively 27.8ml and 17.4ml ($p<0.001$) (Table 4) whereas maximum mean gas production for both TMR and FTMR digestion trial was at 48h incubation period respectively 73.8ml and 59ml ($p<0.001$) (Table 4 and Figure 1). Kim et al. (2012) indicated total gas production increases gradually with incubation and fermentation period up to 48h in both mixed feed and fermented mixed feed. Total gas production also differed substantially from 12h to 24h of incubation among the TMR and fermented TMR feed. This consistency ascertains the equivalency between present and previous research results. Mao et al. (2007) also ascertained that the total gas production would increase with superior rumen fermentation period. Feeding a nutritionally balanced diet reduces enteric methane gas emissions in dairy cows (Sherasia et al., 2016). According to Cao et al. (2011) fermented feed with silage produce lower gas in the rumen, although they used lactic acid for the fermentation of vegetable residue silage. A remarkable decrease in gas production with fermented TMR feed is also supported by different previous studies (Cao et al., 2012; Arangsri et al., 2019). Conversely, total gas production may be higher ($p<0.001$) in Fermented TMR than TMR due to fermentation procedure or composition of TMR that changes in the degree of gas generation (Wang et al., 2016).

The pH values of the present experiment did not differ according to the effects of TMR and FTMR, but gradually decreased with the period at all replicates (Figure 3) (Ahammed et al., 2021). At 6h incubation period mean pH was 6.34 and 6.60, whereas at 48h incubation period mean pH was 5.60 and 5.64 respectively for TMR and FTMR feed (Table 5). Slightly pH variation may be differed due to components of ration formulation. Kim et al. (2012) used tall fescue, mammoth wild rye forage, whole-crop barley, rice straw with a fermented feed of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. Kim et al. (2012) mentioned at the beginning of the experiment pH value was 6.01 and the pH values measured at different incubation periods up to 48h also have decreasing tendency with the lowest pH value up to 4.82 which aligned with the current study (Figure 3).

Although, the insignificant ruminal lowest pH value was 5.6 ($p>0.05$) between TMR and FTMR feed at 48h in-

cubation period (Table 5). Cao et al. (2012) entitled final pH 5.62 to 5.66 at a total mixed feed of whole crop rice, rice bran, dried beet pulp, and concentrate while fermented with lactic acid bacteria. According to Meenongyai et al. (2017) silage utilization or TMR fermentation has no detrimental effects on ruminal pH. However, Microorganism growth rate and protease activity might increase at ruminal pH above 6.5 (Bach et al., 2005). Allen and Ying (2012) mentioned ruminal pH (mean=6.0) was not affected by treatment responses with *Saccharomyces cerevisiae* supplementation in silage.

CONCLUSIONS

The current *in vitro* study concluded that FTMR has a potential effect to decrease total gas production and increase digestibility. So, we can assume that FTMR is better than TMR in terms of productivity and environment-friendly livestock production. It also needs to conduct further research on animal trials for the conclusion.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

NOVELTY STATEMENT

The study suggests that fermented TMR feed is better than TMR feed in terms of higher digestibility and lower gas production up to 48h incubation period.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Data curation & formal analysis, Writing—original draft: O.B. Paul; writing—review and editing: S.S. Urmi; Project administration & Resources, writing—review and editing, Supervision: M.A.A. Biswas; All authors have read and agreed to the published version of the manuscript.

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