CHAPTER I

Introduction

Ruminants are an essential part of livestock sector, cause ruminant is an expert in converting cellulose and other fibrous materials into high quality milk & meat. Besides they also have great role in green-house gas (carbon dioxide, methane, nitrous oxide) production (Henry *et al.*, 2009). Another important problems facing ruminant production is the losing of energy and high biological value proteins as a result of ruminal fermentation. This may cause a limited productive performance (Kholif *et al.,* 2014; Ahmed *et al*., 2016) release of pollutants to the environment (Calsamiglia *et al.*, 2007) Many factors influence methane emissions from cattle and include the following: level of feed intake, type of carbohydrate in the diet, feed processing, addition of lipids or ionophores to the diet, and alterations in the ruminal microflora. (Johnson and Johnson, 1995). Again in the livestock industry cost of feed production increasing day by day due to dietary dependency on raw material. So, with new feeding strategies of different roughages & concentrate mixture we can decrease methane gas production and cost of feed. The total mixed ration (TMR) has been the subject of great interest from farmers because of its expected benefits in the nutrition, management and production of ruminant animals (Owen., *et al* 1984; Howard *et al*., 1986; Sirohi *et al*., 2001). Farmers raising homebred fattening cattle are showing increased interest in fibroid material assorted feed, such as the TMR allowance, over concentrates (Kim *et al*., 2003), because homebred fattening cattle (rapid growing) require more feed intake for rapid body weight gain. It has already been experimentally confirmed that fibroid materials assorted feed is advantageous in maintaining the homeostasis of ruminant stomach pH, reducing the incidence of metabolic disease, and improving milk production (Nock *et al*., 1986; Harrison *et al*., 1989; Kellems *et al*., 1991). In recent years, the expediency of feeding cattle a TMR has become widely accepted. The benefits of a TMR include increased feed intake, enhanced use of low-cost alternative feed ingredients, ability to control the forage concentrate ratio, lower incidence of metabolic and digestive disorders, and reduced labor input for feeding (Owen, 1984). TMR is a proper type of feed especially when agricultural by-products

with high moisture are to be included (Li *et al*., 2003). Silage, forage, and hay are the conventional roughages contained in TMR (Chumpawadee and Pimpa, 2009). Moreover, Silage of green grasses like Napier, Para, German is feed ingredient used to prepare TMR, but the use of domestic straw and whole barley, also available in Bangladesh. Fermented feed of TMR may change its digestibility as well as feed efficiency. However Yeast, as a natural feed additive, has the ability to stabilize rumen fermentation and prevents rumen flora disorders and disturbances (Pinloche *et al*., 2013) with increasing the numbers of viable bacterial cells. In case of fermented mixed feed, supplementation of probiotic yeast maintained a healthy fermentation in the rumen of cattle with higher rumen pH .Yeast products formulated with *Saccharomyces cerevisiae* have good effects on the dynamics of gas production, *in vitro* digestibility and there was no interaction with forage quality.(Elmasry *et al*., 2016) The principal objective of this study was to evaluate the effects of TMR and fermented TMR feed on total gas production, pH, digestibility of ruminant by ruminal in vitro digestion method.

CHAPTER II

MATERIALS AND METHODS

The study was conducted with *in vitro* experiment at Department of Animal Science & Nutrition, Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh. The chemicals and most of the instruments were provided by Animal Science & Nutrition department laboratory.

Materials:

2.1: For Bottling & Methane Gas Preservation: 40 glass serum bottles, 40 syringe, 40three way canola, 40 gas keeping tubes & 40 ruminal digested filter water tube, 40 rubber cap.

2.2: For Rumen fluid buffer: K_2HPO_4, KH_2PO_4 , $(NH_4)_2SO_4$, $CaCl_2$.$2H_2O$, $MgSO_4{\cdot}7H_2O$, trypticase peptone, yeast extract, and Cysteine HCl, rumen fluid, Sodium phosphate dibasic, Sodium phosphate monobasic, NaOH, HCL, Distilled water(DW). All the chemicals used were purchased from Merck Chemical Company.

2.3: For TMR & FTMR: rice straw, concentrate feed, green grass of Napier, Para, German for silage, *Saccharomyces Cerevisiae,* molasses.

2.4: Instrument & Apparatus: Nitrogen gas for anaerobic condition creation in buffered rumen fluid, Incubator, weighing machine, Shaking incubator, Blender for grinding dried straw, silage, concentrate mixture & homogenous mixing of all feed materials; Hot bath, Water bath, Stirrer/Glass rod, pH meter, Cheesecloth, Volumetric pipette, 3L measuring flux, 3 different 500ml cylinder, 5 100ml beakers, Feed pasting mortar, Flask, Ice box.

Respective methodology of whole action:

As for the methodology, the experiment is divided into two major parts. Firstly, buffers including rumen fluid buffer have to be prepared. Secondly, inoculation of the buffers with rumen fluid and rumen fluid buffer taken in serum bottles for final in vitro test.

To elaborate, two different kinds of buffers are to be made since the efficacy and proper functioning of the rumen fluid in the in vitro tests requires certain pH level. Thereby, to obtain the required pH level in in vitro test similar to the cattle rumen environment, it is essential that buffers are prepared. Thereby firstly buffer was prepared for rumen fluid. Then collection of fresh rumen fluid from freshly slaughtered cow from slaughtered house. The rumen fluid buffer was mixed along with constant Nitrogen gas (N2) flow for anaerobic condition creation. Afterwards, this rumen fluid buffer mixture was poured in 40 different bottles along with feed material for final in vitro test experiment. Later, bottles were put in shaking incubator for the ultimate in vitro test to occur. Then, upon incubation trails, 6hour, 12hour, 24 hour and 48 hour, bottles were removed from shaking incubator in order to avail total gas produced inside with syringes. Detail methodology for each and every step is given below:

2.5: TMR: For dairy Cows 70% good quality roughage and 30% concentrate to have the maximum production of human health beneficial conjugated linoleic acid without compromising on milk yield. (Netsanet., *et al* 2015)

Concentrate mixture:

Feed name	Percentage
1.Maize	20%
2.Wheat bran	40%
3.Khashari	10%
4.Soybean meal	12%
5.Rice polish	15%
6.Oyster shell	2%
7.Salt	1%

Roughage mixture: Roughage feed made with 60% Silage of Napier hybrid, Para, German grass and 40% Rice straw.

For preparation of 50 gm Total mixed feed sample quantity of different feed sample on Dry matter basis given below.

Feed type	DM basis weight (gm)	Fresh weight (gm)
1.Concentrate mixture	15	17.025
2.Silage	21	22.61
3.Roughage	14	14.47

2.6: FTMR: Total 100 gm feed sample maded where 50gm for TMR & another 50gm for FTMR. For FTMR production 50gm total mix feed mixed with 5ml molasses containing *Saccharomyces Cerevisiae* . The optimal values of parameters temperature, pH, substrate concentration, enzyme concentration and fermentation period are 35°C, 4.0, 300 gm/L, 2 gm/L and 72 h respectively for growth of *Saccharomyces Cerevisiae* (Periyasamy *et al.,* 2009).

2.7: Proximate Composition of Feed: The feed material of the cattle was collected from Chittagong Veterinary and Animal Science University (CVASU,) Bangladesh. The proximate composition of the commercial cattle feed used in the experiment had certain quantity. The labeling of the feed suggested that it was constituted of 75-77 % total digestible nutrients (TDN) , 14-15 % crude protein (CP) , 1.1 % calcium (minimum) , 0.8 % phosphorous (minimum) , and 90 % dry matter (DM). Feed powder of less than 1mm (<1mm) was prepared using mortar.

2.8: Rumen Fluid Collection: Rumen fluid was collected from a freshly slaughtered cow from slaughter house. The rumen fluid was collected early in the morning, whereas the required buffers were made the day before for time constraint. On an important note, it is essential to preserve the rumen fluid temperature for the in vitro test. Thereby, immediate collection of rumen fluid is vital after slaughtering of the cow. The rumen contained rumen fluid in the digested grass. The grasses were squeezed to obtain the rumen fluid. Thereby, 1L of rumen fluid was filtered with four folded cheesecloth and poured in an airtight flask. The usual temperature for rumen

fluid is 39°C. It was maintained since immediately after filtering the rumen fluid in flask, the flask was sealed and kept in ice box. Afterwards, it was immediate transfer of the ice box was done to laboratory of department of Animal science & Nutrition for a balanced temperature management. The rumen fluid was immediately dispensed with Nitrogen gas for maintaining an anaerobic condition that is vital for rumen fermentation. The rumen fluid was collected from a cow which was fed rice straw and commercial feed compositions twice in a day. The cow feed, times of feed and the cow breed were recognized after consultation with the workers and owner of the slaughterhouse.

2.9: Purification of Buffer for Rumen Fluid: The buffer medium was prepared according to the method described by Asanuma *et al.* (1999). The buffer used for rumen fluid contained mixture of several chemicals solids with measured amount of distilled water. Then, it was kept in an aerobic 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 gm yeast ex- tract, and 0.6 g cysteine HCl. The chemicals were poured in distilled water of one liter. Firstly, all the chemicals were poured and a very small amount of distilled water was put for the solution to mix evenly. Yeast extract and trypticase peptone were dissolved by hands since they clump immediately when these come in contact with air. They soak the moisture in air. Thereby, immediate mixture of these chemicals was needed. In this process, a certain pH is required for the efficient function of the in vitro test the required and desired pH is 6.9. However, after the solution was made, pH was low Consequently, Sodim Hydroxide (NaOH) was poured drop by drop until the pH risen to 6.9 while pouring, if the pH rises above 6.9, the pH was balanced by adding one to two drops of Hydrochloric Acid (HCL). Then, the buffer for rumen fluid was seated on a hotplate in order to prohibit chemical chunk floating in the buffer for homologous distribution of the buffer. Afterwards, the buffer was dispensed with 100 % Nitrogen (N_2) gas for creating anaerobic condition. Lastly, the buffer was autoclaved at 121°c for 15 minutes. Finally, the buffer was collected after almost one hour when the buffer was cooled after autoclaving and preserved till the next day for mixing with freshly slaughtered rumen fluid.

2.10: Preparation of Buffered Rumen Fluid: The rumen fluid was mixed with the buffer the next day after collection of freshly slaughtered cow and rumen fluid. It followed a ratio where rumen fluid was 625 millilitres and buffer was 1875 millilitre. Thereby, the total amount of buffered rumen fluid was 2500 millilitre or 2.5 litres. Although, 2000 ml of total liquid was required, but excessive 500 ml was prepared in order to prohibit shortage of liquid in case liquid is lost while pouring in serum bottles. Lastly, the bottle containing buffered rumen fluid was dispensed with 100 percent Nitrogen gas (N_2) atmosphere in order to make it oxygen free as per suggested by Asanuma *et al*. (1999) The reason is fermentation method is inhibited in aerobic conditions as per claimed and suggested by Goering & Van Soest (1970) . Finally, the rumen fluid buffer was prepared to be poured in 40 different serum bottles for the ultimate *in-vitro* experiment.

2.11: Transfer of Buffered Rumen Fluid to Serum Bottles by Anaerobic Condition: The rumen fluid solution mixed with buffer was then taken in to 40 experimental serum bottles. The flow of buffered rumen fluid in serum bottles were done by volumetric pipette in order to pour accurate amount required. After each time dispensing 50 ml of buffered rumen fluid in each bottle, Nitrogen gas (N_2) gas was flowed extensively by carefully avoiding powder form fire extinguisher cylinder. Afterwards, immediately the rubber caps were capped in order to allow any kind of air gas especially oxygen to flow inside as part of maintaining anaerobic condition.

2.12: Serum Bottle Setup: The final bottle setup was made keeping triplicates of each incubation time. Thereby, the incubation times were 6 hour. 12 hour, 24 hour, 48 hour. As for bottles, two types of bottles were made, where 20 bottles for TMR & another 20 bottles for FTMR. There were 5 bottles fixed for every 6 hour. 12 hour, 24 hour, 48 hour at both TMR & FTMR group. The control contained firstly, 50 ml of buffered rumen fluid was added in 40 serum bottles. Secondly, 0.5gm prepared TMR feed material added in each 20 serum bottles of TMR group & 0.5gm prepared FTMR feed also added in another 20 bottles of FTMR group. Gradually, all the bottle openings were sealed with rubber cap & locked with tin lid in order to prohibit gas leakage after in vitro gas production. Finally, all the bottles of both TMR & FTMR group were put into shaking incubator at 37°C temperature for in vitro gas production as described by

Hattori and Matsui (2008). For each incubation time, five replicates per experimental treatment were used.

Fig.2.1: Serum bottles with feed sample and buffered Rumen fluid in shaking incubator

2.13: Collection of Total Gas: Calibrated gas syringe made of plastic & glass was used to collect the gas produced in the in vitro test. The syringe was attached with three way canola in order to regulate the gas flow in and out of the bottle and syringe. Firstly, the syringe was locked before entering in each bottle in order 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 tail of the syringe went backwards due to the total gas pressure. After the push ended, three way canola was regulated to close the entrance of the syringe. Thereby, it stopped further entrance of atmospheric gas inside the syringe. Thus, finally the syringes were prepared to measure the total gas. Total gas measured & noted for further research.

Fig.2.2: Collection of Total gas from serum bottles

2.14: pH Measurement: The pH meter used to determine the pH value was Hanna HI 2211 bench pH meter.

Figure.2.3: After digestion pH measuring with pH meter

Digestibility 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 bottles was taken. Difference of feed weight before digestion and digested feed measured. Then Digestibility calculated as percentage.

Figure 2.4: Drying of undigested feed with incubator.

CHAPTER III

Results

Data of pH, Total gas (ml) production and Digestibility% were collected of In-vitro digestion trial at 6hr, 12hr, 24hr & 48hrs of incubation period. The mean of Total Gas production at 6hr, 12hr, 24hr, & 48hr in TMR feed 27.8ml, 35.8ml, 54.8ml & 73.8ml and in FTMR feed 17.4ml, 28.8ml, 45.2ml, 59ml respectively. Which express significantly less gas production in Fermented total mixed ration then TMR feed ($p<0.01$). Decreasing tendency of pH value with increasing incubation period where not significant different was noticed such as Average pH value of TMR & FTMR was at 6hr 6.29 & 6.60 and at 48hr 5.61 & 5.64 respectively ($p>0.05$). At each hour incubation period digestibility% is significantly higher in Fermented mixed ration then total mixed ration ($p<0.01$). Average digestibility of TMR & FTMR at 24hr were 33.54% & 43.14% and at 48hr are 34.78% & 49.11% respectively. Fermented TMR have good digestibility and less gas production which is expressed by 48hr incubation in-vitro trial Figure 1.

Table 3.1: Mean and P-value of Digestibility% in each incubation period:

Incubation period	TMR	FTMR	P- value
6hr	25.008	32.488	0.0041
12hr	30.532	40.012	0.0000
24hr	33.536	43.14	0.0001
48hr	34.784	45.912	0.0002

TMR= total mixed ration; FTMR= Fermented total mixed ration.

Table 3.2: Mean and P-value of Total gas (ml) in each incubation period:

Incubation period	TMR	FTMR	P- value
6hr	27.8	17.4	0.0001
12hr	35.8	28.8	0.0001
24hr	54.8	45.2	0.0033
48hr	73.8	59	0.0000

TMR= total mixed ration; FTMR= Fermented total mixed ration

Table 3.3: Mean and P-value of pH in each incubation period:

Incubation period	TMR	FTMR	P-value
6hr	6.34	6.602	0.0017
12hr	6.258	6.454	0.0000
24hr	5.914	5.838	0.2741
48hr	5.608	5.644	0.3642

TMR= total mixed ration; FTMR= Fermented total mixed ration

Table 3.4: At 6hr incubation period pH, Total gas (ml), Digestibility%:

Sample	pH	Total gas(ml)	Digestibility%	Sample	pH	Total gas(ml)	Digestibility%
6TR1	6.45	30	26.16	6FR1	6.58	15	32.6
6TR2	6.37	25	25.52	6FR2	6.58	18	36.34
6TR3	6.13	30	23.28	6FR3	6.62	19	27.52
6TR4	6.41	29	27.88	6FR4	6.60	18	36.5
6TR5	6.34	25	24.2	6FR5	6.63	17	29.48

Table 3.5: At 12 hr incubation period pH, Total gas(ml), Digestibility%:

Sample	pH	Total gas(ml)	Digestibility%	Sample	pH	Total gas(ml)	Digestibility%
12TR1	6.29	35	27.88	12FR1	6.46	28	41.9
12TR2	6.27	35	31.46	12FR2	6.44	30	39.1
12TR3	6.23	38	30.48	12FR3	6.44	29	39.14
12TR4	6.28	34	33.5	12FR4	6.48	27	41.34
12TR5	6.22	37	29.34	12FR5	6.45	30	38.58

Table 3.6: At 24 hr incubation period pH, Total gas (ml), Digestibility%:

Sample	pH	Total gas(ml)	Digestibility%	Sample	pH	Total gas(ml)	Digestibility%
24TR1	5.78	63	31.42	24FR1	5.79	46	46.1
24TR2	6.02	50	32.78	24FR2	5.89	45	43.3
24TR3	6.00	52	36.96	24FR3	5.75	45	42.4
24TR4	5.87	55	32.12	24FR4	6.00	47	43.08
24TR5	5.90	54	34.40	24FR5	5.76	43	40.82

Table 3.7: At 48 hr incubation period pH, Total gas (ml), Digestibility%:

Sample	pH	Total gas(ml)	Digestibility%	Sample	pH	Total gas(ml)	Digestibility%
48TR1	5.73	74	34.16	48FR1	5.57	60	47.94
48TR2	5.59	72	37.62	48FR2	5.69	61	49.08
48TR3	5.59	72	31.1	48FR3	5.63	56	43.68
48TR4	5.57	73	33.32	48FR4	5.66	58	44.82
48TR5	5.56	78	37.72	48FR5	5.67	60	44.04

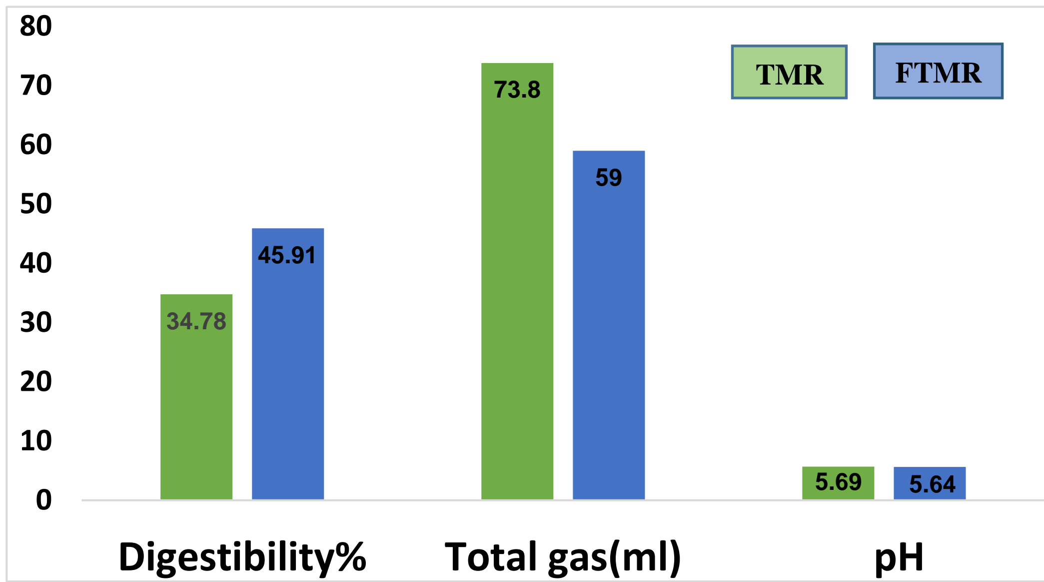

Fig. 3.1: Digestibility%, Total gas (ml), pH value at 48hr *In-vitro* trial.

CHAPTER IV

Discussion

This experiment was designed to analyse the effect of total mixed ration and fermented total mixed ration on *in vitro* rumen fermentation. The current *in vitro* experiment indicated that better digestibility and less gas production with FTMR feed & decreasing tendency of pH at each 6 h, 12 h, 24 h and 48 hr incubation period. The results of the experiment confirmed that gas production increased with the advancing incubation period. But fermented ration feed produced significantly less gas production then total mixed ration in each incubation period. Mao *et al* (2007) also noted that the total gas production would increase with advancing rumen fermentation period. This consistency illustrates the similarity between present and previous research results. Better digestibility found at every 6hr, 12hr, 24hr & 24hr in-vitro incubation trial with fermented total mixed feed. Cao *et al* (2012) reported increased digestibility of FTMR compared with fresh TMR. Effect of FTMR on diet digestibility have good improvement (Yuangklang *et al.*, 2004) The positive effects on digestibility have been confirmed by Desnoyers *et al.,* 2009 and Poppy *et al.,* 2012 which is also proved with this study .In this study *Saccharomyces Cerevisiae* used for the fermentation .The higher digestibility values could be explained by a higher population of cellulolytic bacteria, which is one of the most consistent effects of yeast (Martin & Nisbet, 1992; Wallace & Newbold, 1993).Total gas production are significantly decreased in case of fermented feed than non-fermented mixed feed showed in table 3.2. Less gas production occurred with fermented feed also supported by different reports such as Arangsri *et al.,* 2017, Cao *et al.,* 2010, Kim *et al.*, 2012, Chao *et al.*, 2016.The pH values of the present experiment did not differ according to the effects of different TMR and FTMR, but all gradually decreased with the time period which is also supported by Kim *et al.*, (2012). Ruminal pH was nearly 6.0 and not affected by TMR & FTMR ($P<.01$ at 6h & 12h, Table 3.3) ($P>0.05$ at 24h & 48h, Table 3.3). Vasupen *et al.*, 2006 reported that ruminal pH was not affected by feeding FTMR. Meenongyai *et al.*, 2017 also reported that Utilizing silage or total ration fermentation did not negatively impact on ruminal pH.

CHAPTER V

Conclusion

FTMR has potential effect to decrease total gas production & increase digestibility. So we can conclude that FTMR is better than TMR in terms of productivity & environment friendly livestock production.

CHAPTER VI

References

Ahmed, M.H., Salem, A.Z.M., Olafadehan, O.A., Kholif, A.E., Rivero, N., Mariezcurrena, M.A., Camacho, L.M., Elghandour, M.M.Y., Alonso, M.U., Almaz, A.H.A. (2016). Effect of pre- and post-partum dietary crude protein level on the performance of ewes and their lambs. *Small Rumin Research. 136:221–226.*

Arangsri, M., Pattarajinda, V., Duangjinda, M., Mungkalasiri, J., Angthong, W., and Bernard, J.K. (2017). Impact of fermented total mixed rations on intake, VFA and methane production of dairy heifers.*Indian Journal Of Animal Research Article. ID: B-749. DOI:10.18805/ijar.B-749*

Asanuma, N., Iwamoto, M., and Hino, T. (1999). Effect of the addition of fumarate on methane production by ruminal microorganisms *in vitro*. *Journal of Dairy Science*, 82(4): 780-787.

Cao,Y., Takahashi, T., Horiguchi, K., Yoshida, N., and Zhou, D. (2012). In vitro ruminal dry matter digestibility and methane production of fermented total mixed ration containing whole-crop rice and rice bran. *Grassland Sci.,58: 130-139.*

Cao,Y., Takahashi,T., Horiguchi, K., Yoshida, N.(2010). Effect of adding lactic acid bacteria and molasses on fermentation quality and *in vitro* ruminal digestion of total mixed ration**.** *Grassland Sci.,56: 19-25.*

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A. (2007). Invited review: Essential oils as modifiers of rumen microbial fermentation. *J Dairy Sci. 90(6):2580–2595.*

Chao,W., Yeno,Y.U., Yanegara, A.J., Kondo1, M.,Ban-tokuda,T., Matsui, H .(2016).Changes in in vitro Rumen Fermentation Characteristics of Different Compositions of Total Mixed Rations (TMR) and the Ensiled TMRs. *Advances in Animal and Veterinary Sciences.DOI: 10.14737/journal.aavs/2016/4.4.178.182.*

Chumpawadee, S., and Pimpa., O. (2009). Effects of non forage fiber sources in total mixed ration on feed intake, nutrient digestibility, chewing behavior and ruminal fermentation in beef cattle. *J. Anim. Vet. Adv. 8:2038-2044.*

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., & Sauvant, D. (2009). Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. *J. Dairy Sci. 92, 1620-1632.*

Elmasry, A.M.A., Mendoza, G.D., Miranda, L.A., Vázquez, G., Salem, A.Z.M., Hernández, P.A. (2016). Effects of types and doses of yeast on gas production and in vitro digestibility of diets containing maize (Zea mays) and lucerne (Medicago sativa) or oat hay. *South African Journal of Animal Science. Journal Home.Vol 46, No 4.*

Goering, H.K., and Van Soest, P.J. (1970). Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). *Agric. Handbook No. 379. Agricultural Research Service, United States Department of Agriculture. Washington, DC.*

Harrison, J.H., Riley, R.E., and Long, K.A. (1989). Effect of type and amount of buffer addition to grass silage-based total mixed rations on milk production and composition. *J. Dairy. Sci. 72:1824-1830.*

Hattori, K., and Matsui, H. (2008). Diversity of fumarate reducing bacteria in the bovine rumen revealed by culture dependent and independent approaches. *Anaerobe,* 14(2): 87-93.

Henry, B., and Eckard, R. (2009). Greenhouse gas emissions in livestock production systems. *Tropical Grasslands Volume 43, 232–238.*

Howard, K.,Takusari, N., and Yamagishi, N. (1986). Effect of TMR feeding frequency on eating behavior of lactating cow. *J. Dairy Sci. 69:692-694.*

Johnson, K.A., Johnson, D.E. (1995). Methane emissions from cattle. *J Anim Sci. 73(8):2483-92.*

Kellems, R., Jones, O. R., Andrus, D., and Wallentine, M. V. (1991). Effect of moisture in total mixed rations on feed consumption and milk production and composition in Holstein cows. *J. Dairy Sci. 74:929-932*

Kholif, A.E., Khattab, H.M., El-Shewy, A.A., Salem, A.Z.M., Kholif, A.M., El-Sayed, M.M., Gado, H.M., Mariezcurrena, M.D. (2014). Nutrient Digestibility, Ruminal Fermentation Activities, Serum Parameters and Milk Production and Composition of Lactating Goats Fed Diets Containing Rice Straw Treated with *Pleurotus ostreatus. Asian-Australas J Anim Sci. 27(3): 357–364.doi: 10.5713/ajas.2013.13405.*

Kim, K.H., Kim, K.S., Lee, S.C., Oh, Y.G., Chung, C.S., and Kim, K.J. (2003). Effects of total mixed rations on ruminal characteristics, digestibility and beef production of Hanwoo. *J. Anim. Sci. Technol. (Kor). 45:387-396.*

Kim, S.H., Alam, M.J., Gu1, M.J., Park, K.W., Jeon, C.O., Jong, K.H., Cho, K.K., and Lee, S.S. (2012) .Effect of Total Mixed Ration with Fermented Feed on Ruminal In vitro Fermentation, Growth Performance and Blood Characteristics of Hanwoo Steers. *Asian-Aust. J. Anim. Sci. Vol. 25, No. 2: 213 – 223.*

Li, D.Y., Ko, J.Y., Choi, N.J., Lee, S.S., Song, J.Y., Lee, S.Y., Park, S.H., Sung, H.G., and Ha, J.K. (2003). Effects of types of TMR on rumen fermentation characteristics and nutrients digestibility in sheep. *J. Anim. Sci. Technol. (Kor). 45:805-812.*

Martin, S.A., & Nisbet, D.J. (1992). Effect of direct fed microbial on rumen microbial fermentation. *J. Dairy Sci. 75, 1736-1744*.

Mao, S.Y., Zhang, G., and Zhu., W.Y., (2007). Effect of disodium fumarate on *in-vitro* rumen fermentation of different substrates and rumen bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA. *Asian-Aust. J. Anim. Sci. 20:543-549.*

Meenongyai, W., Pattarajinda, V., Stelzleni, A.M., Sethakul, J., Duangjinda, M.(2017). Effects of forage ensiling and ration fermentation on total mixed ration pH, ruminal fermentation and performance of growing Holstein-Zebu cross steers. *Anim. Sci. J: 88(9):1372-1379. doi: 10.1111/asj.12797.*

Netsanet, B., Kapoor, V., Tewatia, B.S. (2015). Effect of roughage to concentrate ratio in the diet on milk production and fatty acid profile of milk in crossbred cows. *Indian Journal of Animal Nutrition. Volume: 32, Issue: 4.*

Nock, J.E., Steele, R.L., and Braund, D.G. (1986). Performance of dairy cows fed forage and grain separately versus a total mixed ration. *J. Dairy Sci. 69:2140-2147.*

Owen, J.B. (1984). Complete-diet feeding for cattle. *Livest. Prod. Sci. 11:269-285.*

Periyasamy, S., Venkatachalam, S., Ramasamy, S., Srinivasan, V. (2009). Production of Bio-ethanol from Sugar Molasses Using *Saccharomyces Cerevisiae.* Modern Applied Science *Vol.3 No.8 pp.32-37 ref.19*

Pinloche, E., McEwan, N., Marden, J., Bayourthe, C., Auclair, E., & Newbold, C.J. (2013). The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLoS ONE 8, e67824.*

Poppy, G.D., Rabiee, A.R., Lean, I.J., Sanchez, W.K., Dorton, K.L., Morley, P.S. (2012). A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. *J Dairy Sci. 95, 6027-6041.*

Sirohi, S.K., Raman, M., and Walli, T.K. (2001). Development and evaluation of protected fat in wheat straw based total mixed ration. *Asian-Aust. J. Anim. Sci. 14:1405-1408.*

Vasupen, K., Yuangklang,C., Witayakun, S., and Srinanuan, P. (2005). Effect of difference moisture on quality of fermented total mixed ration. *In: Proceedings of the Technical Conference of 43rd Kasetsart University, Bangkok, Thailand.*

Wallace, R.J., and Newbold, C.J. (1993). Rumen fermentation and its manipulation: the development of yeast cultures as feed additives. In: Biotechnology in the Feed Industry. *Ed: Lyous, T.P., Alltech Technical Publications, Nicholasville, Kentucky. pp. 173-192.*

Yuangklang, C., Vasupen, K., Wittyakun, S., Srinanaun, P., and Sukho, C. (2004). Effect of total mixed ration and fermented total mixed ration on feed intake, ruminal

fermentation, nutrient digestibility and blood metabolites in dairy cows. In: Proceeding of the 11th AAAP Congress "New Dimensions and challenges for sustainable livestock farming" *The Asian-Australasian Association of Animal Production Societies, Kuala Lumpur, Malaysia.*

CHAPTER VII

Biography

Ovirup Bhushan Paul, son of Parimal Kanti Paul and Shilu Rani Paul at present is an intern veterinarian under the Faculty of Veterinary Medicine in Chittagong Veterinary And Animal Sciences University (CVASU). He passed the Secondary School Certificate (SSC) examination in 2009 followed by Higher Secondary Certificate (HSC) examination in 2011. In future he wants to be a Veterinary Microbiologist & provide proper services to the people by doing the right services for animals in Bangladesh.