Effect of the Fermented Total Mixed Ration on *In-Vitro* Gas Production and Digestibility in Cattle

Md. Bashir Ahammed
Roll No.: 0118/02
Registration No.: 501
Session: 2018-2019

A thesis submitted in the partial fulfilment of the requirements for the degree of Masters of Science in Animal and Poultry Nutrition

Department of Animal Science and Nutrition
Faculty of Veterinary Medicine
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

JUNE 2020

Authorization

I hereby declare that I am the sole author of the thesis titled **Effect of the Fermented Total Mixed Ration on *In-Vitro* Gas Production and Digestibility in Cattle.** I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the electronic copy of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

The Author
June 2020

Effect of the Fermented Total Mixed Ration on *In-Vitro* Gas Production and Digestibility in Cattle

Md. Bashir Ahammed
Roll No.: 0118/02
Registration No.: 00501
Session: Jan-june/2018

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

(Prof. Dr. Ashraf Ali Biswas)
Supervisor
Department of Animal Science and Nutrition, CVASU

(Prof. Dr. Jahangir Alam)
Co-supervisor
Department of Animal Production and Management, SAU

(Prof. Md. Emran Hossain)
Chairman of the Examination Committee and
Head Department of Animal Science and Nutrition

Faculty of Veterinary Medicine
Chattogram Veterinary and Animal Sciences University
Khulshi, Chittagong-4225, Bangladesh

JUNE 2020

Acknowledgements

I sincerely appreciate The “Almighty Allah” for His graces, strength, sustenance and above all, His faithfulness and love all along the completion of my thesis. His benevolence has made me excel and successful in making my thesis possible.

My unalloyed appreciation goes to my amiable, ever supportive and humble supervisor, Professor **Dr. Md. Ashraf Ali Biswas** for his voluminous and invaluable contributions and instructions throughout my research and thesis period. His encouragement and high degree of freedom to me in the course of this study is highly appreciated. I am extremely privileged to convey my profound gratitude to the co-supervisor **Dr. Md. Jahangir Alam**, Professor, Department of Animal Production and Management, Sher-e-Bangla Agricultural University for his valuable advice, scholastic guidance, suggestions, contributions and instructions throughout my research.

It’s my pleasure to convey my profound gratitude to our Vice-Chancellor **Dr. Goutam Buddha Das**, Professor, Department of Animal Science and Nutrition, CVASU and It is my privilege to acknowledge **Dr**. **Md. Manirul Islam**, Professor, Department of Animal Science and Nutrition for their support, valuable advice and encouragement for the research work.

Furthermore I would also like to acknowledge with much appreciation the crucial role of all the staff ofthe **Department of Animal Science and Nutrition** in every step throughout the research period. I must not fail to sincerely appreciate and acknowledge the financial support from **National Science and Technology**, Ministry of Science and Technology, Bangladesh without which the research may not go smoothly.

I would like to thank **DR. Md. Ashaduzzaman Shubho, DR. Kona Adhikari**, **DR. Priunka Bhowmik and DR. Pronesh Dutta** their support during the whole experimental period. Finally, an honorable mention goes to my families and friends for their understandings and supports in completing this thesis.

The Author

June 2020

Table of Contents

List of Tables

List of Figures

List of abbreviations

Abbreviation		Elaboration
C	-	Control
CF	-	Crude fibre
CP	-	Methane
CH_4	-	Crude protein
CO_2	-	Carbon dioxide
DM	-	Dry Matter
DMD	-	Dry Matter Digestibility
DMI	-	Dry Matter Intake
EE	-	Ether extract
Ft	-	Feet
FTMR	-	Fermented Total Mixed Ration
gm	-	Gram
GE	-	Gross Energy
GHG	-	Green House Gas
GI	-	Gastro Intestinal
GIT	-	Gastro Intestinal Tract
H	-	Hour
H_2	-	Hydrogen
IU	-	International unit
IVOMD	-	*In-vitro* organic matter digestibility
Kcal/kg	-	Kilocalorie per kilogram
kg	-	Kilogram
Kg/ cow/d	-	Kilograms per cow per day
LY	-	Live Yeast
ME	-	Metabolizable energy
mg	-	Milligram
mmol/l	-	millimole per litre
N_2	-	Nitrogen
N_2O	-	Nitrous Oxide
NE	-	Net Energy
NH_3	-	Ammonia
NH_3-N	-	Ammonia Nitrogen
OM	-	Organic Matter
OMD	-	Organic Matter Digestibility/ Degradibility

SEM	-	Standard error of mean
TDN	-	Total Digestible Nutrients
TG	-	Total gas
VFA	-	Volatile Fatty Acid
Wt	-	Weight
<	-	Less than
>	-	Greater than
e.g	-	Example
et al	-	And his associates
%	-	Percentage
i.e.	-	That is
Sig.	-	Significance
Ref.	-	Reference

Abstract

In this study, Experiments were conducted to evaluate the fermented total mixed ration (FTMR) and total mixed ration (TMR) by rumen in *in vitro* fermentation technique and their effects on methane emission and digestibility measurement. Ruminal samples were collected from ruminal digesta and grind TMR feed used as a substrate. There were four diets, one was without molasses-yeast mixture (control), another was in addition of molasses at 0.1% inclusion rate (T_1) and the other two was in addition of molasses-yeast mixture at 0.1% (T_2) and 0.3% (T_3) inclusion rate. The present study indicated that there was significant ($p<0.05$) difference in pH among different treatment groups and decreasing pattern of gas production in treatment group than control group. In this study lowest total gas produced in T_3 (33.8 ml) group than C (40.4ml) group and CH_4 production considerably lowest in fermented group (26.2 ml in T_2 and 27.6 ml T_3) than C (31.6 ml) at 24 h of incubation period. The *in vitro* organic matter digestibility (IVOMD) was tended to higher in T3 (92.93%) diet than C (91.66) at 72 h intervals. It can be concluded from the present study that the FTMR at 0.3% (T_3) inclusion rate has better methane reducing capacity and higher digestibility than TMR.

Keywords: Fermented total mixed ration (FTMR), Total mixed ration (TMR), *in vitro* fermentation, Organic matter digestibility, Molasses-yeast.

CHAPTER-I: INTRODUCTION

Ruminants are an essential part of livestock sector, because 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 problem 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. Cattle industry has become one of the most important economic activities all over the world. But to get maximum production, a perfectly balanced nutrition supplement to the animal is inevitable. Regarding this situation, total mixed rations (TMR) can be an alternative solution to support the dairy cows for achieving maximum production by stall feeding without grazing indoor-housed system like dairy producing countries of the world. To ensure that, a total mixed ration (TMR) can be supplied to the animals which will avoids selective feeding. TMR feeding enhances feed intake, improves the ecology of the rumen leading to stimulated microbial activity to digest more feed and then finally increases productivity of the cows. 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 et al., 1984). TMR is a proper type of feed especially when agricultural by-products with high moisture are to be included (Li et al., 2003). Wachirapakorn et al., (1997) compared two feeding regimes (separate and TMR feeding) and found that TMR feeding increased dry matter intake (DMI) and milk production compared to separate feeding. It has also been experimentally confirmed in other studies 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). Fattening of cattle has become a very common practice all over the world. But most of time steroidal hormones such as

androgens and estrogens are used which may have human health consequences by consuming the beef and when released through excreta into the environment, it might pose chronic risk to wildlife (Raloff et al., 2009). Farmers raising homebred fattening cattle are showing increased interest in fibroid material assorted feed, such as the TMR allowance over concentrates because homebred fattening cattle (rapid growing) require more feed intake for rapid body weight gain (Kim et al., 2003). Again fermenting of total mixed ration (FTMR) is a simple method to potentially improve nutrient utilization and extend the shelf life of the feed. FTMR is made by mixing roughage with concentrate and then fermenting them in incubator for 72 hours. In dairy cows, Yuangklang et al., (2004) showed that FTMR increased feed intake and improved nutrient digestion. Vasupen et al., (2005, 2006) confirmed that FTMR improved the digestibility of dry matter (DM), organic matter (OM), fiber, and non-structural carbohydrate. If the coarse forage that is not suitable for feeding separately can be fermented and incorporated in TMR, it will reduce the wastage and improve feed quality. Including fermented feed in TMR may change its digestibility as well as feed efficiency and may be used widely in fattening. 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.

Objectives:

However, the present study is designed to investigate the following objective:

- To evaluate the effects of fermented TMR over simple TMR in ruminants.
- To evaluate the chemical composition of fermented and non-fermented TMR feed.
- To compare the gas production after *in-vitro* digestion of fermented and non-fermented TMR feed.
- To measure the DM and OM digestibility of fermented and non-fermented TMR feed.

Research Hypothesis:

Providing of fermented TMR feed in ruminant diet may improve the ruminal gastro-intestinal function, digestive performance, reducing gas production and helps in methane mitigation.

CHAPTER-II: REVIEW LITERATURE

Feeding a total mixed ration (TMR), a mixture of concentrate and roughage, is typically used in the dairy industry in developed countries. The advantage of TMR feeding is to avoid eating selection and to maintain rumen fermentation. TMR feeding enhances feed intake, improves the ecology of the rumen leading to stimulated microbial activity to digest more feed and then finally increases productivity of the cows.

Wachirapakorn et al., (1997) compared two feeding regimes (separate and TMR feeding) and found that TMR feeding increased dry matter intake (DMI) and milk production compared to separate feeding. The fermented total mixed ration (FTMR) is a simple method to potentially improve nutrient utilization and extend the shelf life of the feed. FTMR is made by mixing roughage with concentrate and then fermenting under anaerobic conditions (i.e. ensiling) in a sealed container for 21 days. In dairy cows, Yuangklang et al., (2004) showed that FTMR increased feed intake and improved nutrient digestion. Vasupen et al., (2005, 2006) confirmed that FTMR improved the digestibility of dry matter (DM), organic matter (OM), fiber and non-structural carbohydrate.

Wanapat et al., (1996) reported that addition of cottonseed in the diet can increase milk yield. Similar findings were reported by Smith et al., (1981) and Mena et al., (2001), which showed that cows fed a high whole cottonseed (WCS) diet had improved milk yield, milk fat and fat corrected milk (FCM). However, Sullivan et al., (1993) reported that cracked WCS (cWCS) improved animal performance better than WCS because the gossypolinc WCS bound with protein or another nutrient in the supplementation at 0.5 kg/h/d. The combination of FTMR and cWCS supplementation would be an alternative strategy to improve performance of lactating cows.

Randel et al., (1992) was to investigate the effect on intake, digestibility and milk production of processing WCS when used as a protein source in FTMR fed to dairy cows. Wachirapakorn et al., (1997) compared two feeding regimes (separate and TMR feeding) and found that TMR feeding increases dry matter intake (DMI) and milk production compared to separate feeding.

FTMR is a simple method to potentially improve nutrient utilisation and extend the shelf life of the feed. FTMR is made by mixing roughage with concentrate feed samples used in this study which have been tested for nutrient content through proximate analysis. In dairy cows, Yuangklang et al., (2004) showed that FTMR increased feed intake and improved nutrient digestion. Vasupen et al., (2005, 2006) confirmed that FTMR improved the digestibility of dry matter (DM), organic matter (OM), fibre and non-structural carbohydrate.

Sirohi et al., (2001) confirmed that The advantage of TMR feeding is to avoid eating selection and to maintain rumen fermentation. TMR feeding enhances feed intake, improves the ecology of the rumen leading to stimulated microbial activity to digest more feed which finally increases productivity of the cows. 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 et al., 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 et al., 2009). Including fermented feed in TMR may change its digestibility as well as feed efficiency.

Total mixed ration (TMR) enhances feed intake, improves the ecology of the rumen that leads to stimulated microbial activity to digest more feed, increase dry matter intake and milk production (Wachirapakorn et al., 1997) compared to separate feeding which ultimately increases productivity of the cattle. The rumen environment of the

ruminant's changes with the ingestion of feed stuffs which in turn affect the ruminal digestion, nutrient absorption and rate of passage. In conventional feeding system, animals consume a high proportion of concentrates which increase the risk of ruminal acidosis (Maekawa et al., 2002). TMR diets have often been attributed to a ruminal steady state condition, stabilize rumen fermentation pattern and improve energy and protein utilization in the rumen (Coppock et al., 1981). The merits of total mixed ration are related to the enhancement of utilization of low grade roughages, provides uniform feed intake and reduces feed wastage, a stable environment for rumen fermentation, minimal fermentation losses and fluctuation in release of ammonia (Rao et al., 2014).

Sarker et al., (2019) outlined a feeding effect of TMR on RCC cattle. They determined that efficient utilization of crop residues is an alternative way to overcome feed shortage for livestock feeding and indicated that rumen NH_3-N was positively correlated with TN intake of the animal. It can be concluded from the present study that the TMR provided better rumen environment at different hours of digestion could be used for better rumen fermentation. The best combination of roughage to concentrate ratio (30:70) was in T5 group for better N utilization to achieve maximum performance through proper feeding which might reflect the gross return of cattle.

Jahan et al., (2018) stated that TMR provided better rumen environment at different hours of digestion could be used for better rumen fermentation. The best combination of roughage to concentrate ratio (30:70) was better N utilization to achieve maximum performance through proper feeding which might reflect the gross return of cattle production. 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 et al., 2009). Including fermented feed in TMR change its digestibility as well as feed efficiency.

Kim et al., (2012) confirmed that FTMR-related treatment shows a superior performance to that of TMR during the ruminal fermentation period and demonstrated that the daily and total live weight gain and feed efficiency were higher ($p<0.05$) in the FTMR and TMR groups than in the control group. SGOT, SGPT and BUN ($p<0.05$) were reduced in FTMR relative to the control and TMR groups by 168 d which confirmed that FTMR shows better blood profiles than the TMR and control groups. Overall, these results appear to show that FTMR has better *in vitro* ruminal characteristics than those of TMR; growth performance and blood profiles were also found to be superior in FTMR than in the TMR and control groups.

Jahan et al., (2018) conducted a study to select the best combination of roughage and concentrate based on total mixed ration (TMR), to better rumen environment and determine the feeding effects of TMR on rumen metabolic profile in cattle. TMR provided better rumen environment at different hours of digestion. The best combination of roughage to concentrate ratio (30:70) for better N utilization to achieve maximum performance through proper feeding which might reflect the gross return of cattle production.

Kim et al., (2018) conducted an experiment on Hanwoo steers to assess the effects of fermented total mixed ration (FTMR) on the growth performance, carcass and meat quality traits. They stated that FTMR may not only improve the growth performance, biochemical metabolites and fatty and acetic acid profiles of steers, but may also enhance the carcass and meat quality characteristics of Hanwoo steers.

Li et al., (2003) demonstrated that TMR had higher ruminal NH3-N than those on CR. Feeding system did not alter VFA production but TMR feeding resulted in lower A/P ratio. TMR feeding tended to increase the number of bacteria and protozoa in the rumen fluid. TMR generally had higher fiber degrading enzyme activities, which might be the result of increased number of cellulolytic microbes in the rumen of

animals on TMR. Li et al., (2003) indicated that TMR may provide more favorable condition for nutrient digestion both in the rumen and in the total tract of steers.

Total mixed ration (TMR) has been used with a great interest by farmers because of its expected benefits in nutrition, management and production of ruminant animals by early researchers (Owen et al., 1979; Howard et al., 1986; Sirhi et al., 2001). Moseley et al., (1976); McGilliard et al., (1983) and Nock et al., (1985) reported that TMR system helped to maintain rumen pH and A/P ratio because TMR could provide more balanced ration with a uniform rate of roughage and concentrate and increased DM intake. For the high yielding lactating dairy cattle which require high concentrate feeds, TMR has been known to give benefits by increased meal frequency and feed intake, enhanced fiber digestion and nitrogen utilization and increased milk yield and milk fat production (Moseley et al., 1976; Owen et al., 1984). TMR is a proper type of feed especially when agricultural by-products with high moisture are to be included. Nutritive value of these by products has been reported by Givens et al., (1987) and Njie et al., (1995). Also Miron et al., (2002) showed an improved feed efficiency with partial replacement of corn by citrus pulp in TMR of high producing dairy cows. Although fair amount of information is available on the merit of TMR feeding system, its effects on rumen fermentation characteristics, especially microbial population and cellulolytic enzymes have not been clearly shown.

Meanongyai et al., (2017) conducted a trial on the effects of forage ensiling and ration fermentation on total mixed ration pH, ruminal fermentation and performance of growing Holstein-Zebu cross steers. They were determined the effect of forage ensiling and ration fermentation on total mixed ration pH, ruminal fermentation and animal performance.

Lee et al., (2003) stated a study on effects of feeding system on rumen fermentation parameters and nutrient digestibility in holstein steers. In order to compare effects of feeding systems on rumen fermentation characteristics and nutrient digestion, steers were fed either total mixed ration (TMR) or separate concentrate-roughage ration (CR). Total tract digestibility of nutrients was higher in steers receiving TMR. Especially, DM, ADF and NDF in TMR were digested to a greater extent than those in CR. Rumen pH was not influenced by the feeding systems. Holstein steers on TMR had higher ruminal NH3-N than those on CR. Feeding system did not alter VFA production but TMR feeding resulted in lower A/P ratio. TMR feeding tended to

increase the number of bacteria and protozoa in the rumen fluid. Also steers fed TMR generally had higher fiber degrading enzyme activities, which might be the result of increased number of cellulolytic microbes in the rumen of animals on TMR. Our results indicate that TMR may provide more favorable condition for nutrient digestion both in the rumen and in the total tract of steers.

Arangs et al., (2017) determined the effect of feeding two fermented total mixed ration (FTMR) on methane production in dairy heifers and found that Feeding the FTMR differing in CH_4 potential did not affect DMI, digestibility, ruminal TVFA production or molar proportions of VFAs in dairy heifers.

In order to compare effects of feeding systems on rumen fermentation characteristics and nutrient digestion (Li et al., 2003) steers were fed either total mixed ration (TMR) or separate concentrate-roughage ration (CR). Total tract digestibility of nutrients was higher in steers receiving TMR. Especially DM, ADF and NDF in TMR were digested to a greater extent than those in CR. Rumen pH was not influenced by the feeding systems. Holstein steers on TMR had higher ruminal NH_3-N than those on CR. TMR feeding tended to increase the number of bacteria and protozoa in the rumen fluid. Also steers fed TMR generally had higher fiber degrading enzyme activities, which might be the result of increased number of cellulolytic microbes in the rumen of animals on TMR. Li et al., (2003) indicate that TMR provides more favorable condition for nutrient digestion both in the rumen and in the total tract of steers.

Coppock et al., (2008) outlined several advantages associated with this type of feeding system. In particular, the adlibitum feeding of TMR results in a ruminal steady state condition conducive to continuous rumen function and digesta flow. Maximum ruminal (microbial) benefit from feeding urea or rapidly degradable protein can be derived with this feeding strategy especially when soluble energy sources are provided.

TMR is a proper type of feed especially when agricultural by-products with high moisture are to be included (Li et al., 2003). Wachirapakorn et al., (1997) compared two feeding regimes (separate and TMR feeding) and found that TMR feeding increased dry matter intake (DMI) and milk production compared to separate feeding. It has also been experimentally confirmed in other studies 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)

Silage is a product based on fermentation, whereby lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSC) to organic acids under anaerobic conditions. The appropriate adjustment in density, moisture content, chopping length and the application of additives can significantly improve the fermentation quality, digestibility, and aerobic stability of silage. Total mixed ration (TMR) is a form of complete formula feed consisting of roughage, concentrate, minerals, vitamins, and other additives in certain proportions. It is widely used to provide ruminants with adequate and balanced nutrition, which can stabilize microbial function and enhance energy and protein utilization in the rumen (Meenongyai et al., 2017). Fresh TMR is also a highly deteriorative feed stuff that cannot be preserved for long periods. Ensiling can prevent the spoilage of TMR and improve its palatability by anaerobic fermentation. Balanced TMR silages can be transported to provide year-round nutritional balance feed for small-scale farms that lack labor. Silage is also common roughage in TMR, which has low pH and a large number of lactic acid bacteria attached to it. Fermented feedstuffs have been successfully used as raw materials for TMR silage in industry (Meenongyai et al., 2017). Nishino et al., (2017) found that the LAB species in TMR silages were selected during the ensiling process, and the bacterial community was unrelated to the ingredient crop silages. It was not conclusive as to whether the silage could directly stabilize the fermentation of TMR silage when the single silage composition accounted for more than half of the dry matter of the ingredients. Lactobacillus plantarum (LP) has been added to TMR silage and has proven to be effective in altering fermentation characteristics.

Xie et al., (2020) conducted the Application of Lactobacillus plantarum Inoculant and Potassium Sorbate on the Fermentation Quality, *In vitro* Digestibility and Aerobic Stability of Total Mixed Ration Silage Based on Alfalfa Silage and evaluate the effect of the application of an inoculant and a preservative on the fermentation quality, *in vitro* digestibility and aerobic stability of alfalfa silage-based fermented total mixed ration (TMR).

Agricultural policy in Egypt is aimed to increase the area cultivated by alternative crops such as berseem clover (BC). Rice straw (RS) in Egypt, is a potential feed during the fall and winter for many small-scale livestock owners when rotational BC

is unavailable. However, only ~25% of the total amount of RS is fed to livestock (Steele et al., 2009). Use of RS as an animal feed is limited by its low nutritive value, mainly attributable to the crystalline structure of the cellulose fibrils surrounded by hemicellulose and by the presence of lignin, which prevents enzymes penetration (Chahal et al., 1998).

Biological delignification of straws by white-rot fungi may be a promising way to improve their nutritive value (Fazaeli et al., 2002). The organisms predominantly responsible for lignocellulose degradation are fungi and the most effective are basidiomycetes (Rabinovich et al., 2004). Fungal lignocellulolytic enzymes break the polysaccharide-lignin complex. This would enhance the accessibility of enzymes to potentially digestible biomass resulting in higher degradation of the straw, which may create a more nutritious feed (Tawffek et al., 2011). Pleurotus fungi can grow on straw and decompose its structural carbohydrate (Fazaeli et al., 2002). The potential of Pleurotusostreatus to reduce indigestible cell wall components and increase cell wall digestibility of straw has been reported (Fazaeli et al., 2002).

Nocek et al., (1986) outlined a study on Performance of Dairy Cows Fed Forage and Grain Separately Versus a Total Mixed Ration. The finding is that Cows fed forage and grain separately had milk yields similar to those fed total mixed ration. Dry matter intake was lower for cows fed forage and grain separately from 22 through 49 due to reduce forage intake and was also lower from 50 through 77 due to decreased grain intake. Four percent fat-corrected milk production efficiency was higher for cows fed forage and grain separately. Abruptly changing cows from one feeding system to another did not influence milk yield, milk composition, or body weight gain. The computer controlled feeder system is an effective method to allot grain according to milk production requirements in free stall housing.

Chao et al., (2016) outlined a change in *in vitro* Rumen Fermentation Characteristics of Different Compositions of Total Mixed Rations (TMR) and the Ensiled TMRs. To evaluate the effects of the composition of total mixed rations (TMR) and ensiling of the TMR on rumen fermentation properties and methane production compared two types of TMRs, which were optimized for dairy cattle and beef cattle, and their ensiled TMRs (eTMR). To make eTMRs, TMRs were wrapped and fermented for 40 days. These eTMRs and TMRs were used for in vitro ruminal incubation experiment.

The type of TMR and ensiling both affected total short chain fatty acids, the amount of methane production, and relative proportions of acetate and butyrate in the *in vitro* rumen cultures of tested TMRs. The relative abundance methanogenic archaea in respective cultures determined by quantifying a gene involved in methane production (mcrA: α-subunit of methyl co-enzyme M reductase) was also affected by both the type and the ensiling, which was higher in the eTMRs than the TMRs ($p<0.001$). The results of the present study suggest that not only ensiling TMR but also the composition of TMR may affect in vitro rumen fermentation patterns, and that changes in the degree of methane generation due to ensiling TMR may also depend on the fermentation kinetics.

FTMR is made by mixing roughage with concentrate and then fermenting them in incubator for 72 hours. In dairy cows, Yuangklang et al., (2004) showed that FTMR increased feed intake and improved nutrient digestion. Vasupen et al., (2005, 2006) confirmed that FTMR improved the digestibility of dry matter (DM), organic matter (OM), fiber and non-structural carbohydrate.

Bharanidharan et al., (2018) have examined the effects of feeding total mixed ration (TMR) versus roughage and concentrate separately (SF) on ruminant methane production. They were compared differences in methane production, ruminal characteristics, total tract digestibility of nutrients and rumen microbiome between the two feeding methods in Holstein steers. These results indicated that SF reduces methane emissions from ruminants and increases propionate proportion of total VFA without affecting total tract digestion compared to TMR. There were no evidences that the response differed due to different major underlying microbial population.

Johnson et al., (1995) outlined a finding on methane emission from cattle that Increasing atmospheric concentrations of methane have led scientists to examine its sources of origin. Ruminant livestock can produce 250 to 500 L of methane per day. This level of production results in estimates of the contribution by cattle to global warming that may occur in the next 50 to 100 yr to be a little less than 2%. 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.

Microorganisms that grow and reproduce in the fermentation processes in the rumen can pass into the later stages of digestion in the ruminant providing protein and

additional energy for growth. However, methane does represent a loss of energy from the animal production system with 6–12% of gross energy intake lost as methane. This can exceed the gross energy intake directed to live weight gain or wool production by as much as 3–4 times (Kurihara et al., 1999). Eckard et al., (2009) demonstrates the potential productivity gain from reducing methane emissions and it has been this objective of increasing efficiency of feed intake that has livestock category Rumen methane (Mt CO_2-e) Beef cattle 36.6, Feedlot cattle 2.1, Dairy cattle 6.8, Sheep 13.6.

Sayed et al., (2007) performed a study on *in vitro* evaluation of palm fronds as feedstuff on ruminal digestibility and gas production and carried out to evaluate using palm fronds only or supplemented with fibrolytic enzymes as alternative roughage on the ruminal nutrients digestibility and gas production. Finally concluded that adding fibrolytic enzymes improved the utilization of palm fronds as alternative roughage without negative effect on nutrients digestibility and reduced gas production which improve the environmental aspects of feeding ruminant animals.

In promoting the livestock industry, nutritional factors need to be taken seriously especially on ruminant farming. One of the efforts to increase dairy cattle productivity is by utilizing probiotics. Probiotics, which are currently being developed, have the potential of boosting milk production. Probiotics are substances that can alter intestinal microbes so that beneficial microbial balance can develop well (Fuller et al., 1992; Karpinska et al., 2001). Probiotics are non-digestible substances and give rise to increased bacterial activity in the colon (Roberfroid et al., 2000). The addition of *Bacillus spp.* probiotics to dairy cow rations can improve yield and quality of milk in the field (Supriyati et al., 2008). Probiotics is an additional product of live microbial feed that positively affects the livestock by maintaining the microbial balance of the digestive tract. There are several types of microbial feeds used as probiotics. Supriyati et al., (2008) show that some probiotics were able to increase milk yield. The addition of probiotics stimulates bacteria of the rumen which affects the increase of lactic acid resulting in the stabilisation of rumen pH. Increased microbial populations play a role in improving digestion of fibre materials to increase food intake and yield.

Yeast products based on Saccharomyces cerevisiae have been used as feed additives in the dairy industry for more than 20 yr with variable efficacy. Although several studies have observed increased milk production with live yeast supplementation in

lactating dairy cows (Desnoyers et al., 2009; Moallem et al., 2009; Ondarza et al., 2010), no effects were observed in others (Hasunuma et al., 2016; Ouellet and Chiquette et al., 2016). In meta-analytical or systematic literature summaries of effects of supplementation with live yeast (Desnoyers et al., 2009; Ondarza et al., 2010) milk production was increased but effects on DMI were inconsistent.

Yeast additives presumably exert beneficial effects via stabilizing ruminal pH by inhibiting lactate production (Durand et al., 2005) or increasing lactate utilization (Lynch and Martin, 2002; Fonty and Durand, 2006) along with increas-ing total-tract OM (Desnoyers et al., 2009; Ferraretto et al., 2012) and NDF digestibility (Ferraretto et al., 2012), partly via increasing the number of ruminal cellulolytic bacteria (Newbold et al., 1996; Mosoni et al., 2007). Live yeast may scavenge trace amounts of ruminal oxygen and, by increasing the redox potential of ruminal contents, increase the population of anaerobic bacteria and VFA production in the rumen (Fonty and Durand et al., 2006; Marden et al., 2008).

Beneficial effects of yeast cultures that contain no live yeast have been attributed to functional metabolites that include uncharacterized yeast growth factors, B vitamins, AA, organic acids and other fermentation products that stimulate bacterial growth and lead to increased microbial protein production, fiber digestion or increased utilization of fermentation end products (Miller et al., 2002; Moallem et al., 2009; Robinson et al., 2009). Like yeast cultures, inactivated or killed yeast may influence ruminal fermentation by supplying nutrients to autochthonous microorganisms (Oeztuerk, 2009; Opsi et al., 2012). Vyas et al., (2014) reported increased ruminal pH and greater relative abundance (RA) of Ruminococcus flavefaciens in the solid ruminal fraction of beef heifers in response to killed yeast supplementation. Yet very few studies on killed yeasts exist and to our knowledge, no previous study has simultaneously compared the effects of the dose and viability of the same yeast strain on ruminal fermentation, microbial diversity, diet digestibility and animal performance in lactating dairy cows. Such comparisons are needed to better understand the mode of action of yeast and to comprehend the specific roles of the dose and viability of yeast in improving the performance of lactating dairy cows. Jami et al., (2014) reported that the abundance of various rumen bacterial taxa and milk composition or feed efficiency are highly correlated, suggesting that the bacterial community plays an important role in regulating host physiological parameters. Several studies have

reported the effect of yeast supplementation on the abundance of cellulolytic bacteria (Callaway et al., 1997) and lactate-utilizing bacteria (Chaucheyras et al., 1996; Rossi et al., 2004), but none have examined associations between abundance of unknown and known bacterial taxa and performance measures in cows supplemented with or without yeast. The first objective of this study was to examine the effects of the dose and viability of a new S. cerevisiae yeast strain YE1496 on ruminal fermentation, digestibility and performance of lactating dairy cows. The second objective was to explore associations between animal performance measurements and ruminal bacteria abundance and hypothesized that yeast supplementation would improve rumen fermentation by increasing rumen pH or total VFA concentration, *in vivo* apparent digestibility, and milk yield; that the higher dose of live yeast would be the most effective treatment; and that killed yeast would be the least effective dietary treatment.
Cao et al., (2010) conducted a trial of adding lactic acid bacteria and molasses on fermentation quality and *in vitro* ruminal digestion of total mixed ration silage prepared with whole crop rice and examined the effects of molasses (M) and lactic acid bacteria (LAB) on the quality of total mixed ration (TMR) silages prepared with whole crop rice (WCR) and tofu cake (TC). Chemical composition, organic acids and *in vitro* ruminal digestion were determined. The results suggested that adding LAB increased the LA content of TMR silage and tended to decrease ruminal methane production, while adding M did not significantly increase the LA content of TMR silage and tended to increase ruminal methane production *in vitro.*
Molasses is a sticky dark by-product of processing sugar cane or sugar beets into sugar. Senthilkumar et al., (2004) discovered that molasses can be a source of quick energy and an excellent source of minerals for farm animals. It can also be a key ingredient for cost effective management of feeds and pastures. The calcium content of sugar cane molasses is relatively high (up to one percent), whereas the phosphorus content is low. Cane molasses is also high in other minerals like sodium, potassium, magnesium and sulphur but in beet molasses is higher in potassium and sodium but lower in calcium. Molasses also contains significant quantities of trace minerals such as copper, zinc, iron and manganese. Adding molasses with poor quality hay will increase feed intake and improve palatability.
Yeast products for ruminants based on Saccharomyces cerevisiae increase the number of cellulolytic bacteria (Wallace & Newbold et al., 1993; Alzahal et al., 2014) and are

associated with a higher rumen pH promoted by the yeast, which favours the growth of fibrolytic bacteria (Fibrobacter and Ruminococcus) and lactate-utilising bacteria (Pinloche et al., 2013). They have thus been regarded as rumen pH stabilisers (Chaucheyras et al., 2008; Desnoyers et al., 2009). In most *in vivo* evaluations of commercial products that contain *Saccharomyces cerevisiae*, researchers confirmed that the amounts of live cells were described by the commercial manufactures (Crosby et al., 2004; Pinloche et al., 2013; Ahmed et al., 2015; Pienaar et al., 2012).

In a few experiments, the colony-forming units (CFUs) were corroborated (Bitencourt et al., 2011; Vyas et al., 2014; Emmanuel et al., 2007). In contrast, data from Arcos-García et al. (2000) showed that the CFU value determined in the laboratory differed from that reported on the yeast product packaging. Opsi et al., (2012) demonstrated that live yeast affects ruminal fermentation slightly more than inactivated yeast. Several studies have been conducted to evaluate neutral detergent fibre (NDF) levels with yeast (Plata et al., 1994; Miranda et al., 1996; Wang et al., 2001) but information that compares forage sources is scarce.

Roa et al., (1997) compared lucerne and coffee hull and cornstalk with or without *Saccharomyces cerevisiae* on *in situ* digestion and rumen fermentation, and did not find forage/yeast interactions with differences among forages. However, a legume and a lignocellulosic residue differ greatly in nutritional value and the response to yeast addition in digestibility can be different. Therefore, the objective of this study was to evaluate the effects of two commercial yeast products on *in vitro* fermentation kinetic parameters, as determined by gas production, of lucerne- and oat-based diets, dosed at the same CFU levels of *Saccharomyces cerevisiae*.

Liu et al., (2019) conducted a study on the Effects of active dry yeast on growth performance, rumen fermentation characteristics and slaughter performance in beef cattle and investigated the effects of active dry yeast (ADY) on rumen microbial composition and slaughter performance of beef cattle.

Elmasry et al., (2016) conducted a trial of the 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. Two yeast products formulated with *Saccharomyces cerevisiae* were evaluated at the same colony-forming units (CFUs) per gram of substrate. Samples of maize, lucerne and oat hays were mixed (0.5 kg) to a proportion of 80% forage (lucerne or oat) with 20% maize (DM basis) and combined with each

yeast to obtain 1.5x107or 3.0x107CFU/g DM. There was also a control without yeast. The two yeast products showed the same effects on the dynamics of gas production and in vitro digestibility when dosed at the same number of viable cells or CFUs, and there was no interaction with forage quality.

In most *in vitro* evaluations of commercial products that contain *Saccharomyces cerevisiae*, researchers confirmed that the amounts of live cells were described by the commercial manufactures (Crosby et al., 2004; Pinloche et al., 2013; Ahmed et al., 2015; Pienaar et al., 2012).

Several studies have been conducted to evaluate neutral detergent fibre (NDF) levels with yeast (Plata et al., 1994; Miranda et al., 1996; Wang et al., 2001) but information that compares forage sources is scarce.

Roa et al., (1997) compared lucerne and coffee hull and cornstalk with or without *Saccharomyces cerevisiae* on in situ digestion and rumen fermentation, and did not find forage/yeast interactions with differences among forages. *In vitro* research with mixed ruminal microorganisms likewise has been inconsistent regarding the effects of direct-fed microbials. Several researchers observed that direct-fed microbials increased cellulolytic bacterial numbers in the rumen and stimulated the production of some fermentation end products.

Pinloche et al., (2013) Compared to the control diet supplementation of probiotic yeast maintained a healthy fermentation in the rumen of lactating cattle (higher VFA concentration higher rumen pH and lower Eh and lactate). Methane (CH_4) in ruminants is produced along with volatile fatty acids (VFA) in the rumen as end products of fermentation (Johnson and Johnson et al., 1995). The substrates for methanogenesis are hydrogen (H_2) produced during fermentation of fibrous carbohydrates to acetate and butyrate (Moss et al., 2000) but propionate produced during fermentation of non-fibrous carbohydrate (NFC) is hydrogen sink product. Methanogens use H_2 to reduce carbon dioxide (CO_2) to CH_4 (Benchaar et al., 2001) representing a loss of energy in the range of 2 to 12% of gross energy intake (GEI) and between 3.9 to 7.4% GEI for the dairy cow (Johnson and Johnson, 1995; Kebread et al., 2008).

Methane production per animal are affected by dry matter intake (DMI), feed composition, feed quality and production level besides individual animal variation (Ramin and Huhtanen et al., 2013). Feeding a fermented total mixed ration (FTMR) to

sheep was reported to reduce methane emission and increase digestibility (Cao et al., 2010). Similarly, Cao et al., (2012) reported that FTMR supported higher in vitro DM digestibility.

The *in vitro* techniques have been developed to overcome the shortcoming of the *in vivo* technique. In this technique the bags are extracted and weighed at fixed times for measuring the disappearance of feed from the bags, providing information about rate and extent of feed digestion (Kitessa et al., 1999). The technique has been largely employed to evaluate rumen degradability of feeds and found to predict well the *in vivo* digestibility of the feed (Damiran et al., 2008). However, the technique is criticized for the need of rumen fistulated ruminants.

The *in vitro* gas production technique measures the appearance of fermentation products (gases, volatile fatty acids, NH_3) when feed samples are incubated in rumen liquor. When a feed is incubated with buffered rumen liquor, it is degraded, and the degraded matter is partitioned to yield gases (mainly CO_2 and CH_4) and microbial biomass. It is assumed that gas production is related to the rate and extent of feed digestion.

CHAPTER-III: MATERIALS AND METHODS

The study was conducted in postgraduate laboratory under the Department of Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University (CVASU) Khulshi, Chattogram. The chemicals and most of the instruments were provided by Animal Science & Nutrition department laboratory and most of the experiments were performed in Department of Physiology, Biochemistry and Pharmacology and PRTC laboratories of CVASU.

3.1 Study period:

The overall research was conducted from July, 2018 to January, 2019.

3.2 Collection of Feed:

The concentrate and roughage type feed materials of the cattle were collected from Chittagong Veterinary and Animal Science University (CVASU) Bangladesh. Feed powder of less than 1mm (<1mm) was prepared using mortar.

3.3 Chemical composition of feed

The chemical composition of the Total Mixed Ration and Fermented Total Mixed Ration are presented in Table 3.1.

Table 3.1: Chemical composition of the experimental fermented and non-fermented feeds.

Parameter	Dietary treatment			
%	C	T_1	T_2	T_3
DM	95.3	94.3	94.3	94.2
Ash	7.9	7.8	7.9	7.8
OM	92.0	92.2	92.0	92.1
CP	12.9	13.5	13.6	13.7
CF	16.8	17.2	17.2	17.1

3.4 Optimization of yeast concentration:

The adequate amount of sugar molasses solution was taken in fermentation flask and the pH and temperature were maintained at 4.0 and 35^{o}C and kept in a constant temperature shaker. The quantities of yeast like 2.0g were added. An anaerobic condition was maintained for four days and during this period, the strain converts sugar into bio-ethanol with the evolution of CO_2 and the fermented solution was analyzed at every 48 h and 72 h intervals (Periyasamy et al., 2009). After 72 h of incubation period, were count the yeast cell was 4.4×10^{8} cells/ml in Neubauer chamber at direct 1: 10-fold dilution method.

3.5 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 cheesecloths and poured in an airtight flask. The usual temperature for rumen 6 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 slaughter house.

3.6 Buffer for Rumen Fluid:

The buffer medium was prepared according to the method described by Asanuma et al., (1999) with the following composition in mg/L: dipotassium phosphate (K_2HPO_4), 450; monopotassium phosphate (KH_2PO_4), 450; magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H2O$), 190; calcium chloride dehydrate ($CaCl_2{\cdot}2H_2O$), 120;

Sodium chloride (NaCl), 900; cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl$), 600; ammonium sulfate ($(NH_4)2SO_4$), 900; Trypticase peptone (BBL; Becton Dickinson, Cockeysville MD), 1000; and, Yeast extract (Difco Laboratories, Detroit, MI), 1000. 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. 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. The pH was balanced by adding one to two drops of Sodim Hydroxide (NaOH) (Base) and Hydrochloric Acid (HCL) (Acid). 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. However, the rumen fluid was mixed with the buffer the next day after collection of freshly slaughtered cow and rumen fluid. The upper residue of the rumen fluid was removed while the middle portion was collected and used in the experiment. The pooled and particle-free rumen fluid was transferred to a buffer medium bearing pH 6.9 (Hino et al., 1992) in a 1:3 rumen fluid:buffer ratio. 4000 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.

3.7 Serum Bottles Preparation:

Fifty ml of buffered rumen fluid was anaerobically transferred under a constant flow of N_2 gas atmosphere in order to make it oxygen free as per suggested by Asanuma *et al*., (1999) to 100 ml serum bottles containing the 0.5g TMR feed added with molasses and molasses containing yeast at different concentrations. Finally, the rumen fluid buffer was prepared to be poured in 80 different serum bottles for the ultimate *in-vitro* experiment. Sealing with rubber septum stopper and aluminum cap (Asanuma and Hino, 2000) of the bottles containing the mixed substrate and buffered rumen fluid will follow which will then be incubated subsequently at 39°C for 6, 24, 48, and 72 h in a shaking incubator with 120 rpm (Hattori and Matsui, 2008).

3.8 Serum Bottle Setup:

The final bottle setup was made according to the following treatments were: non-addition, 0.1% Molasses, 0.1% and 0.3% Yeast culture and, hereafter referred to as control, treatment 1 (T_1), treatment 2 (T_2), treatment 3 (T_3) and keeping five replications of each treatment. Thereby, the incubation times were 6 hour, 24 hour, 48 hour and 72 hour. As for bottles, four types of bottles were made, where 20 bottles for each control and treatments. There were 5 bottles fixed for every 6 hour, 24 hour, 48 hour, 72 hour at both control and treatments group. Finally, all the bottles of both control and treatments group were put into shaking incubator at 39℃ temperature for *in vitro* gas production with 120 rpm (Hattori and Matsui, 2008).

3.9 Collection of Total Gas:

Calibrated gas syringe made of glass was used to collect the gas produced in the in vitro test. Fermentation parameters were monitored at the end of each incubation time set. A needle channel connected to the syringe was extended into the sealed fermentation bottle to measure the positive pressure created by the gas build up in the headspace of the syringe at room temperature and allowing the gas to flow inside a syringe barrel. The plunger was pulled gradually until the pressure the volume of gas trapped inside the barrel was recorded as the TG produced in ml.

3.10 pH Measurement:

The pH meter used to determine the pH value after opening each serum bottles.

3.11 CO_2 and CH_4 measurement:

Lime-water were prepared for the measurement of CH_4 and CO_2. The TG contained gas syringe sink into the lime-water jar and backward pressure of syringe take the lime water into the syringe tube where the CO_2 itself reacts with the lime and disappear. The rest of the gas in the syringe tube indicates the amount CH_4 production in ml. Rest of this CH_4 amount subtracted from measured TG and this result indicates CO_2 production in ml (M. Mel *et al.*, 2014).

3.12 Determination of *in vitro* dry matter and organic matter digestibility:

Earlier to the *in vitro* rumen fermentation, the DM and organic matter (OM) of

concentrate feed was determined by drying at 105℃ for 16 h and ashing at 550℃ for 12 h, respectively. The resulting percent DM and percent OM was used to compute the initial DM (DMi) and initial OM (OMi) of the substrate in grams. Fermenta samples from each serum bottle after the specified incubation period were drained in dried, pre-weighed nylon bags and knotted using nylon thread, then splashed with flowing water for 15 minutes or until the turbidity of water resulting from washing disappeared. The final DM (DMf) and OM (OMf) of the feed were determined using the same conditions applied when determining the initial values (DMi and OMi). The DM and OM digestibility (%) were calculated as ([DMi −DMf]/DMi) × 100 and ([OMi− OMf]/OMi) × 100, respectively.

3.13 Layout of the experiment

Table-3.2: Layout of the experiment showing treatment and replication:

Hours	C	T_1	T_2	T_3
6 h	1	1	1	1
	2	2	2	2
	3	3	3	3
	4	4	4	4
	5	5	5	5
24 h	6	6	6	6
	7	7	7	7
	8	8	8	8
	9	9	9	9
	10	10	10	10
48 h	11	11	11	11
	12	12	12	12
	13	13	13	13
	14	14	14	14
	15	15	15	15
72 h	16	16	16	16
	17	17	17	17
	18	18	18	18
	19	19	19	19
	20	20	20	20

C = Diet without molasses-yeast mixture, T1 = Diet containing molasses at 0.1% of TMR DM, T2 = Diet containing molasses-yeast mixture at 0.1% of TMR DM and T3 = Diet containing molasses-yeast mixture at 0.3% of TMR DM.

CHAPTER-IV: RESULTS

4.1 *In vitro* fermentation parameters

4.1.1. pH

Decreasing tendency of pH value with increasing incubation period where significant difference was noticed in TMR and FTMR feed at 24 h, 48 h and 72 h respectively ($p<0.05$).

Table 4.1: pH from *in vitro* rumen fermentation of the experimental fermented and non-fermented feeds.

Incubation Period	Treatment				P value
	C	T_1	T_2	T_3	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
6 h	5.64 ± 0.05	5.57 ± 0.02	5.61 ± 0.01	5.61 ± 0	0.07
24 h	5.46 ± 0.01	5.41 ± 0.01	5.45 ± 0.03	5.43 ± 0.01	0.02
48 h	5.33 ± 0.01	5.27 ± 0.01	5.31 ± 0.01	5.32 ± 0.01	0.00
72 h	5.29 ± 0.01	5.22 ± 0.03	5.27 ± 0.01	5.24 ± 0.04	0.03

C= Diet without molasses-yeast mixture, T_1= Diet containing molasses at 0.1% of TMR DM, T_2= Diet containing molasses-yeast mixture at 0.1% of TMR DM and T_3= Diet containing molasses-yeast mixture at 0.3% of TMR DM.

4.1.2 Total Gas

In case of total gas, significant ($p<0.05$) difference was observed after 24 h of incubation period. Though there was no significant difference was observed at 6, 48 and 72 h respectively ($p>0.05$). But tended to lowest total gas was in T_3 (45.1 ml) after 72h than the C (48.4 ml). Significantly lowest total gas was observed in T_3 (33.8 ml) in group and highest was in C (40.4 ml) group at 24 h of incubation period (Table 4.2).

Table 4.2: Total gas (ml) production from *in vitro* rumen fermentation of the experimental fermented and non-fermented feeds.

Incubation period	Treatment				P value
	C	T_1	T_2	T_3	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
6 h	12.2 ± 1.1	12.8 ± 0.8	13.2 ± 1.3	11.6 ± 0.5	0.09
24 h	40.4 ± 2.5	37.8 ± 4.2	34.8 ± 4.0	33.8 ± 2.0	0.02
48 h	45.2 ± 3.0	39.2 ± 5.2	42.6 ± 3.2	42.6 ± 2.4	0.11
72 h	48.4 ± 1.1	46.2 ± 5.2	46.4 ± 4.3	45.1 ± 1.4	0.50

C= Diet without molasses-yeast mixture, T_1= Diet containing molasses at 0.1% of TMR DM, T_2= Diet containing molasses-yeast mixture at 0.1% of TMR DM and T_3= Diet containing molasses-yeast mixture at 0.3% of TMR DM.

4.1.3 Methane (CH_4) production

In vitro CH_4 production there was no significant ($p>0.05$) difference observed after 6, 24, 48 and 72h of incubation period. But Methane production decreased at 24 h of incubation period in T_3 (26.2 ml) group than C (31.6 ml) group (fig 4.1).

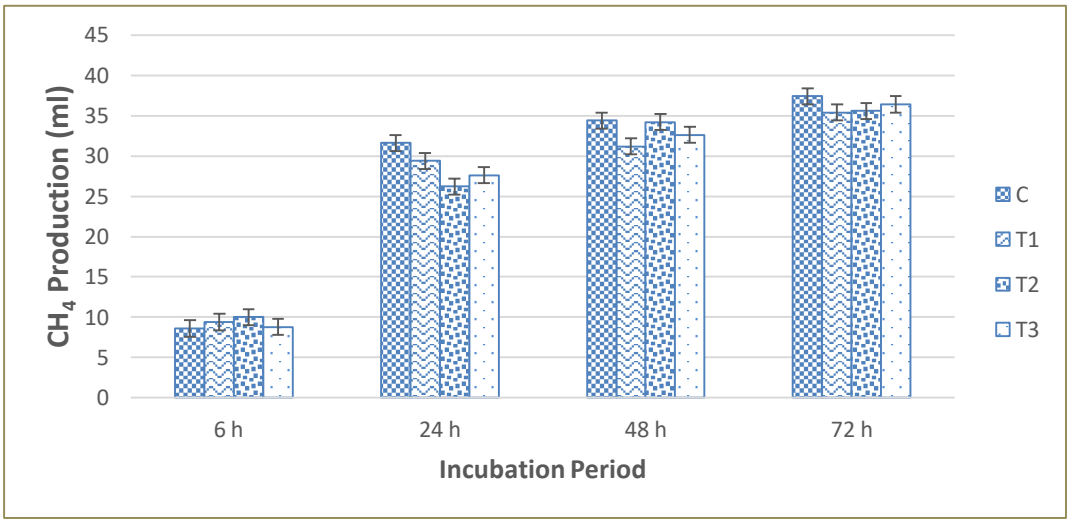

Fig 4.1: CH_4 production (ml/.5g DM) from in vitro rumen fermentation from fermented and non-fermented feeds.

4.1.4 CO_2 production

In case of CO_2 production there was significant ($p<0.05$) difference was noticed at 48h of incubation period. Whereas there was no significant difference was observed at 6, 24 and 72h respectively. Highest CO_2 production was observed in C (11.2 ml)

group and lowest was in T3 (9.6 ml) group at 72 h of incubation period (fig 4.2).

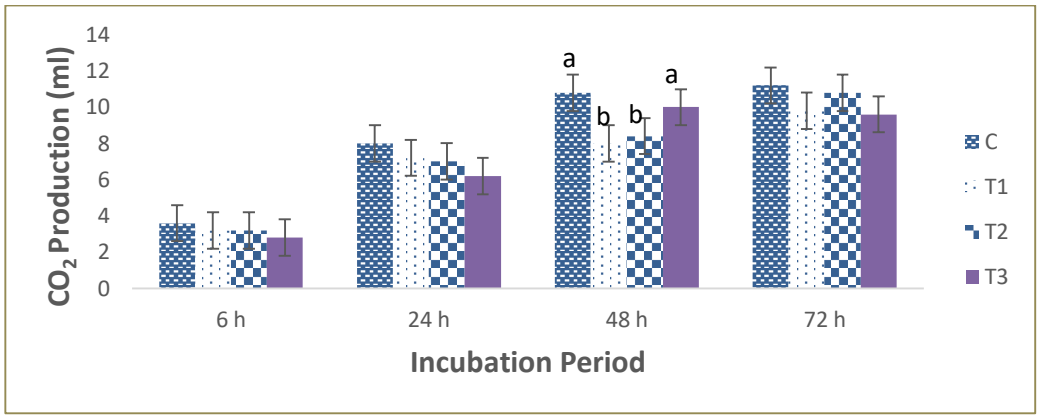

Fig 4.2: CO_2 production (ml/.5g DM) from in vitro rumen fermentation from fermented and non-fermented feeds.

4.1.5 Dry Matter and Organic Matter digestibility

There was no significant ($p<0.05$) difference observed at 6h, 48h, and 72h of incubation period. Significantly highest DM digestibility was observed in T3 (37.45%) group and lowest DM digestibility was observed in C (33.23%) after 24 h incubation (fig. 4.3).

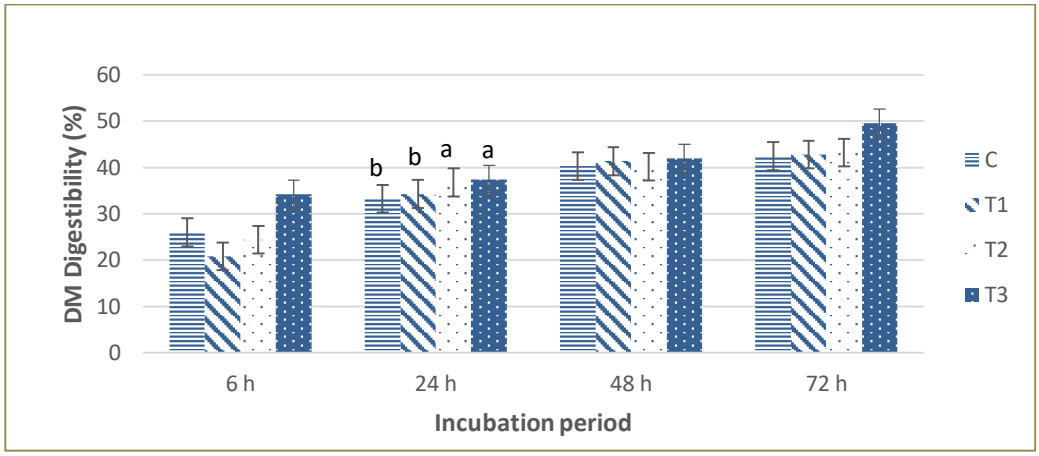

Fig.4.3: Dry matter (DM) digestibility of different fermented and non-fermented feeds.

On the other hand, there was no significant difference on OM digestibility after 6h, 24h and 48h of incubation period. Significantly highest OM digestibility was observed in T3 (92.93 %) group after 72 h of incubation than the C (91.66 %) group (fig 4.4).

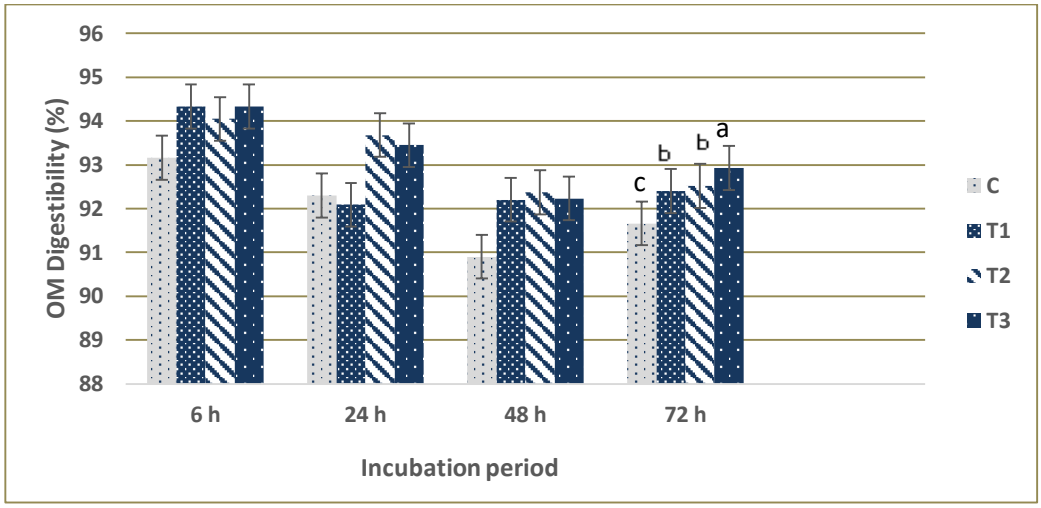

Fig. 4.4: Organic matter (OM) digestibility of different fermented and non-fermented feeds.

CHAPTER V: DISCUSSION

This experiment was designed to analyze the effect of TMR and Fermented TMR 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, 24 h, 48 h and 72 h 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.

pH:

Ruminant animals solely depend on cellulolytic ruminal microorganisms to digest cellulose. In the rumen fermentation process, pH is considered a leading factor affecting rumen microbiome, fermentation and CH_4 production. It has also been experimentally confirmed in other studies 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). The DM digestibility decreased with pH declining, this may relate to a negative effect of acid condition on microbial activity, particularly that of fibrolytic bacteria (Russell et al., 1996). In this study, significant difference was noticed in TMR and FTMR feed at 24 h, 48 h and 72 h respectively ($p<0.05$) which is supported by Kim et al., (2012). Ruminal pH affects ruminal bacteria that is neutral pH contribute to ruminal bacteria to digest feed sample and produce high total VFA. The low pH seems to contribute to the conversion of lactic to propionic acid in the rumen. The addition of probiotics stimulates bacteria of the rumen which affects the increase of lactic acid resulting in the stabilization of rumen pH. In additions yeast increases the production of organic acid. These organic acids reduce the pH of the rumen. This illustrates the similarity between the present and previous study.

Total gas:

The higher total gas production observed in high proportion of non-fermented TMR than fermented TMR feed. The results of the experiment confirmed that gas

production increased with the advancing incubation period. But fermented TMR feed produced significantly less gas production than non-fermented feeds in each incubation period. Significantly lowest total gas was observed in T_3 (33.8 ml) in group and highest was in C (40.4 ml) group at 24h of incubation period. Less gas production occurred with fermented TMR feed supported by different reports such as Arangsri et al., (2017); Cao et al., (2010); Kim et al., (2012) and Chao et al., (2016).

CH_4:

Methane production per animal are affected by dry matter intake (DMI), feed composition, feed quality and production level besides individual animal variation (Ramin et al., 2013). Feeding a fermented total mixed ration (FTMR) to sheep was reported to reduce methane emission and increase digestibility (Cao et al., 2010). There was no significance difference observed between FTMR and TMR feed in present study but tended to reduce methane production among treatment group is supported by Chao et al., (2016). Rumen methane production is generally higher when more fibrous feed is applied to cattle (Dehority et al., 2003). A remarkable decrease in methane generation in response to ensiling TMR was reported in a previous *in vitro* study (Cao et al., 2012). Yeast has the ability to shift H_2 utilization from methanogenesis to reductive acetogenesis through the homoacetogenic bacteria that can produce acetate from CO_2 and H_2 (Mwenya et al., 2004). *In vitro* studies have shown beneficial effects of feeding live yeast strain on growth and H_2 utilization and acetate production by acetogenic bacteria isolated from a rumen of lambs, even in the presence of methanogens (Chaucheyras-Durand et al., 1997). Martin et al., (2010) reported a 20% reduction in CH_4 production after a 48 hours incubation of alfalfa supplemented with a live yeast product. CH_4 production is significantly decreased in case of fermented feed than non- fermented mixed feed. Less methane production occurred with fermented feed also supported by Cao et al., (2010) and Kim et al., (2012).

CO_2:

In this study there were significant difference was observed in CO_2 production. CO_2 production consistently decreased in treatment group that supported the results of Kim et al., (2012) where they observed the effect on CO_2 production as a result of yeast

addition at different doses in treatment group.

Dry matter and organic matter digestibility:

In present study, there was significant difference was observed in DM digestibility at 24h of incubation period at 0.1% fermented TMR diet treatment, which indicated that fermented TMR diet could improve nutrient digestibility. Cao et al., (2012) reported that FTMR supported higher in vitro DM digestibility which is revealed with the present study. The OM digestibility resulted for the C and T diet were not different and consistent over time, whereas OM digestibility was reduced by both the diet from 6 hours onwards. Tended to highest OM digestibility was observed in T_3 (92.93%) group after 72 h of incubation than the C (91.66%) group. Cao et al., (2012) reported increased OM digestibility of fermented ration compared with fresh ration which agrees with the present study.

CHAPTER-VI: CONCLUSION

In summary, the range of technical options available at present to farmers to reduce CH_4 emissions in cattle is limited and no single option appears to provide a simple solution. The result of this *in vitro* study stated that increased amount of Fermented TMR feed lower the total gas production. In addition, significantly highest DM digestibility was observed in FTMR feed. Not only did the FTMR increase *in vitro* DM digestibility, it also reduced methane production. Regardless of the TMR used, DM digestibility and methane decreased with pH declining. Based on the findings of this study, it may be concluded that, FTMR has significant effect to decrease CH_4 production and increase digestibility.

CHAPTER-VII: RECOMMENDATIONS

Many factors such as pH, temperature etc. having effects on ruminal digestion remain undetected. Advanced studies with better technological supports are required to detect those factors. Further studies are recommended to find the VFAs production. Further studies with extended time and sufficient fund are required to extend the number of samples and quality fermented feed. Furthermore, researches are recommended to explain the in vitro digestion techniques.

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., and Almaz, A.H.A. 2016. Effect of pre- and post-partum dietary crude protein level on the performance of ewes and their lambs. Small Ruminant Research. 136:221–226.

Arambel, M. J., and B. A. Kent. 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early to mid-lactation dairy cows. Journal of Dairy Science. 73:1560–1563.

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.

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

Bargo F, LD Muller, JE Delahoy and TW Cassidy. 2002. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. Journal of Dairy Science. 85: 2960-2975.

Bennett, T. 2004. Effect of Diamond V yeast culture on milk yield andcomposition of dairy cows from early to late lactation. Diamond V field trial no. DA019-s. Diamond V, Mona Vale, NSW, Australia.

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

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 Science. 58: 130-139.

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

Chao,W., Yeno,Y.U., Yanegara, A.J., Kondo1, M.,Ban-tokuda,T., and 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.

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. Journal of Animal and Veterinary Advances.8:2038–2044.

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

Elmasry, A.M.A., Mendoza, G.D., Miranda, L.A., Vázquez, G., Salem, A.Z.M., and 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.Volume 46: No 4.

Girdhar N and N Balaraman. 2005. Feeding of maize fodder based total mixed ration having different levels of energy and protein to lactating crossbred cows. Indian Journal of Dairy Science. 61: 435-440.

Goad DW, Goad CL, and Nagaraja TG. 1998.Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. Journal of Animal Science.76: 234–241.

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. Journal of Dairy Science. 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. Journal of Dairy Science: 69:692-694.

Hundal JS, RP Gupta, M Wadhwa and MPS Bakshi. 2004. Effect of feeding total mixed ration on the productive performance of dairy cattle. Animal Nutrition and Feed Technology. 4: 179-186.

Johnson, K.A., and Johnson, D.E. 1995. Methane emissions from cattle. Journal of Animal Science. 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. Journal of Dairy Science. 74:929-932.

Khan SR, SK Singh and V Mudgal. 2010. Effect of feeding complete rations on the performance of lactating crossbred cow. Indian Journal of Animal Nutrition. 27: 261-264.

Kholif, A.E., Khattab, H.M., El-Shewy, A.A., Salem, A.Z.M., Kholif, A.M., El-Sayed, M.M., Gado, H.M., and 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 pleurotusostreatus. Asian-Australas Journal of Animal Science. 27(3): 357–364.

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. Journal of Animal Science and Technology (Korea). 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-Australas Journal of Animal Science. Volume 25. No. 2:

213 – 223.

Lascano, G.J., andHeinrichs A.J. 2007. Yeast culture *(Saccharomyces cerevisiae)* supplementation in growing animals in the dairy industry. CAB Reviews. 2, 1-13.

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. Journal of Animal Science Technology (Korea). 45:805-812.

Mao, S.Y., Zhang, G., and Zhu. 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-Australas Journal of Animal Science. 20:543-549.

Martin, S.A., and Nisbet, D.J. 1992. Effect of direct fed microbial on rumen microbial fermentation. Journal of Dairy Science. 75: 1736-1744.

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. Journal of Animal Science: 88(9):1372-1379.

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. Journal of Dairy Science. 69:2140-2147.

Opsi, F., Fortina, R., Tassone, S., Bodas, R. and López, S. 2012. Effects of inactivated and live cells of Saccharomyces cerevisiae on *in vitro* ruminal fermentation of diets with different forage: concentrate ratio. Journal of Agricultural Science. 150: 271-283.

Owen, J.B. 1984. Complete-diet feeding for cattle. Livestock Production Science. 11:269-285.

Patra, A.K. 2012.The use of live yeast products as microbial feed additives in ruminant nutrition. Asian Journal of Animal Veterinary Advances. 7: 366-375.

Periyasamy, S., Venkatachalam, S., Ramasamy, and S., Srinivasan, V. 2009. Production of Bio-ethanol from Sugar Molasses Using *Saccharomyces cerevisiae*. Modern Applied Science. Volume 3. No.8 :32-37.

Pienaar, G.H., Einkamerer, O.B., Van der Merwe, H.J., Hugo, A. and Fair, M.D. 2012. The effect of an active live yeast product on the digestibility of finishing diets for lambs. Small Ruminant Research. 123: 8-12.

Pinloche, E., McEwan, N., Marden, J., Bayourthe, C., Auclair, E., and Newbold, C.J. 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle.

Poppy, G.D., Rabiee, A.R., Lean, I.J., Sanchez, W.K., Dorton, K.L., and 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. Journal of Dairy Science. 95: 6027-6041.

Rao KA, R Kishorek and DS Kumar. 2014. Growth performances ram lambs fed different roughage based total mixed rations. Indian journal of Animal Nutrition. 2:121-127.

Russell, J. B. and P. J. Van Soest. 1984. *In vitro* ruminal fermentation of organic acids common in forage. Applied Environmental Microbiology. 47:155-159.

Sirohi, S.K., Raman, M., and Walli, T.K. 2001. Development and evaluation of protected fat in wheat straw based total mixed ration. Asian-Australian Journal of Animal Science. 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.

Wachirapakorn, C, T Puramongkol and V Seepuang. 1997. Total mixed ration (TMR) or complete ration (CR) for dairy cows. Journal of Dairy Cows 5:53.

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. 173-192.

Wongnen, C., Wachirapakorn C., Patipan C., Panpong D., Kongweha K. Namsaen N., Gunun P. and YuangklangC. 2009. Effects of fermented total mixed ration andcracked cottonseed on milk yield and milk composition in dairy cows.

Asian-Australian Journal of Animal Science. 22(12): 1625-1632.

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.

BIOGRAPHY

I am DR. Md. Bashir Ahammed, was born in Kushtia. I completed my DVM degree from Chattogram Veterinary and Animal Sciences University. Before that, I completed my HSC from Kushtia Govt. College, Kushtia in 2010 and SSC from United High School Banshgram. During my completion of graduation, I received clinical training from Tamil Nadu Veterinary and Animal Sciences University, India and University Putra Malaysia, Malaysia. Currently, I am serving as a Livestock Extension Officer under Livestock and Dairy Development Project (LDDP). I have a keen interest to research on Trans baoundary animal diseases and their protective measure.