EFFECT OF BENEFICIAL ORGANISM ON SHEEP METHANE EMISSION ESTIMATED BY THE LOW COST ABC METHOD

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ABSTRACT

This study investigates the impact of beneficial organisms on sheep methane emissions using the Affordable Face Mask (ABC) technique. Methane, a potent greenhouse gas from ruminant microbial fermentation, has a high global warming potential. The research, conducted at Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh, involves four sheep (T_1, T_2, C_1, C_2) divided into treatment (T_1, T_2,) and control (C_1, C_2) groups. Acclimatizing the animals to the face masks ensures precise data collection. An inexpensive face mask was devised, linked to a plastic tank for gas collection. Blood samples were taken before and after the experiment to gauge Blood Urea (BU) and Blood Urea Nitrogen (BUN) levels. Gas capture occurred at specific intervals, with masks placed over the nose and mouth, analysing exhaled and inhaled breath for ten minutes. Data underwent STATA-13 analysis to unveil patterns. Results showed a slight decrease (6.5%) methane emissions at 3 hours after morning feeding in the treatment group compared to the control group that indicate those microorganisms have a positive impact on the rumen microbial population and fermentation. Average per animal methane emission from Treatment Group (69.45 l/d) which is lower than Control Group (77.54 l/d).

Key Words: Sheep, Methane Gas, ABC, BU, BUN, Probiotics & Feed Additives**.**

CHAPTER 1: INTRODUCTION

Methane (CH_4) is a colorless, odorless gas that results from the gastrointestinal tract's microbial fermentation of grain in ruminant animals. The term "methanogen" describes bacteria that create methane. Methanogens convert hydrogen and carbon dioxide into methane. Hydrogen and carbon dioxide are produced as a result of microbial fermentation (Morgavi et al., 2010). Compared to carbon dioxide, greenhouse gas (GHG) CH_4 has a 28-fold higher potential to contribute to global warming (IPCC, 2014). After carbon dioxide (CO_2), methane (CH_4) is thought to be the principal greenhouse gas (GHG) contributing to the phenomena of global warming (Tian et al., 2016). CH_4 is thought to account for about 20% of the greenhouse gas effect, although it is present in the atmosphere in much lower amounts than CO_2 (IPCC, 1990; 1992). Estimates vary by country and calculating method, but agriculture contributes significantly to global GHG production (Hristov et al., 2013). The stratospheric ozone layer's ozone depletion has been connected to methane (CH_4) (Blake and Rowland, 1988). The stratospheric emission of water vapour from the oxidation of CH_4 may act as a surface for ozone-degrading heterogeneous reactions. It is required by international agreements like the Kyoto Protocol that these emissions be reduced, or at the absolute least, stopped from rising (Howden and Reyenga, 1999). In particular, ruminants contribute to the build up of atmospheric CH_4, and enteric fermentation contributes to 17% of the world's sources of methane (Knapp et al., 2014). For this it causes global warming that may occur in the next 50 to 100 years is estimated to be slightly less than 2% based on the level of CH_4 production (Johnson et al., 1995). The amount of feed consumed, the kind of carbohydrates in the diet, the processing of the feed, the addition of lipids or ionophores to the diet, and changes in the ruminal microbiota are only a few of the variables that affect CH_4 emissions from cattle. These variables can be changed to lessen the amount of CH_4 that livestock emits (Johnson et al., 1995). Methane contributes to a significant energy loss in ruminant animals, with maintenance intake levels accounting for about 8% of gross energy and dropping to about 6% as intake levels increase. There are consequences for both effective animal production and worldwide environmental

protection as a result of increased knowledge and quantification of CH_4 formation in the rumen. There are numerous methods for measuring the CH_4 emissions from specific animals or groups of animals (Bhatta et al.,2007). Here we collect CH_4 gas by affordable face mask (ABC) method (Oss et al.,2016;Silveira et al.,2019). To reduce enteric methane emissions, a number of strategies have been tried, including the use of feed additives. Researchers have created substances like bromoethanesulphonate (BES) and bromopropanesulphonate (BPS) that specifically target the rumen's methanogenesis (Grawert et al. 2014). Since then, other nitrocompounds have been studied, including dimethyl-2-nitroglutarate, 2-nitro-methyl-propionate, 2-nitro-1-propanol, nitroethane, nitroethanol, and 3-nitro-1-propionic acid (Anderson et al. 2003, 2010). Those feed additives were not easily available and cost effective. So, the main goal of this study is to methane emission reduction estimation in Sheep by using beneficial microorganism in the concentrate feed.

CHAPTER 2: METHODS & METHODOLOGY

2.1. Study Area:

The research was carried out in the animal farm of Chattogram Veterinary and Animal Sciences University (CVASU), and the blood test was performed at the postgraduate laboratory of the Department of Physiology, Biochemistry, and Pharmacology at CVASU, Khulshi in Chattogram, Bangladesh.

2.2. Animal Preparation:

The four selected animals (T_1=27kg and T_2=24.60kg sheep for the treatment group and another C_1=27kg and C_2=25kg sheep for the control group) have to get used to wearing the face mask. Therefore, before the first week of gas collection, we regularly offer the animal the mask. So, the animal became accustomed to the mask. The mask is then put on properly restrained. Ten minutes were spent applying the mask. It draws oxygen during this period from the empty plastic tank.

2.3. Face Mask Preparation:

Using some readily available items, we create a facemask that is affordable. Rubber gloves, a water bottle, and a washbasin pipe were all utilized to make the mask.

2.4. Plastic Tank Preparation:

The 500-liter plastic tank and the mask were attached. Additionally, a plastic tank adapter and stainless steel lock nut were used for the connection. The sand and clay were thoroughly vacuumed in the plastic tank.

2.5. Blood Collection:

Blood is collected from all sheep before and after the experiment to analyse the blood to know the Blood Urea (BU) and Blood Urea Nitrogen (BUN) levels.

2.7. Gas Collection:

After three days of feeding the face mask is fitted with a plastic tank placed on the nose and mouth area of the animal. All exhaled and inhaled breath was collected into the plastic tank for ten minutes. We maintained a time protocol for collecting gas and collected gas five times in a day (before feeding and 1.5h, 3h, 6h, and 12h after feeding). Then the collected gas is counted by a gas detection machine.

2.8. Data analysis

All the data was analysed in STATA-13. The STATA analysis technique involves gathering and analysing vast amounts of data in order to spot trends and produce insightful conclusions.

CHAPTER3: RESULTS

The data of the experiment reveal important insights into the impact of the treatment of methane emissions from sheep at different time interval after feeding.

Table 1: Methane Emission concentration of sheep

Control Group	Before feeding	1.5h After feeding	3h After feeding	6h After feeding	12 After feeding
C1	680	1190	1530	1130	826
C2	770	1080	1675	1075	813
Treatment Group	Before feeding	1.5h After feeding	3h After feeding	6h After feeding	12h After feeding
T1	645	1078	1470	1008	710
T2	677	959	1365	959	775

The result of data analysis of the experiment is given below:-

Table 2: Methane increasing % after different times of feeding.

		Mean ± SD			
	Time	Control Group	Treatment Group	T Value	P Value
After Feeding	1.5h	57.63±24.86	54.39±18.02	0.15	0.55
	3h	121.27±5.28	114.77±18.59	0.48	0.66
	6h	52.9±18.79	48.97±10.34	0.26	0.59
	12h	13.53±11.24	12.34±2.9	0.17	0.56

On this day, there is a slightly reduction in methane emissions in the treatment group compared to the control group across all time of morning feeding.

After 1.5h hours of morning feeding, the treatment group showed a slight lower mean methane (54.39%) concentration compared to the control group (57.63%). The difference is not statistically significant because the P-value>0.05.

After 3h hours of morning feeding, the treatment group (114.77%) showed a slight lower mean methane concentration compared to the control group (121.27%). The difference is not statistically significant because the P-value>0.05.

After 6h hours of morning feeding, the treatment group (48.97%) showed a slight lower mean concentration compared to the control group(52.9%). The difference is not statistically significant because the P-value>0.05.

After 12h hours of morning feeding, the treatment group (12.34%) showed a slight lower mean concentration compared to the control group (13.53%). The difference is not statistically significant because the P-value>0.05.

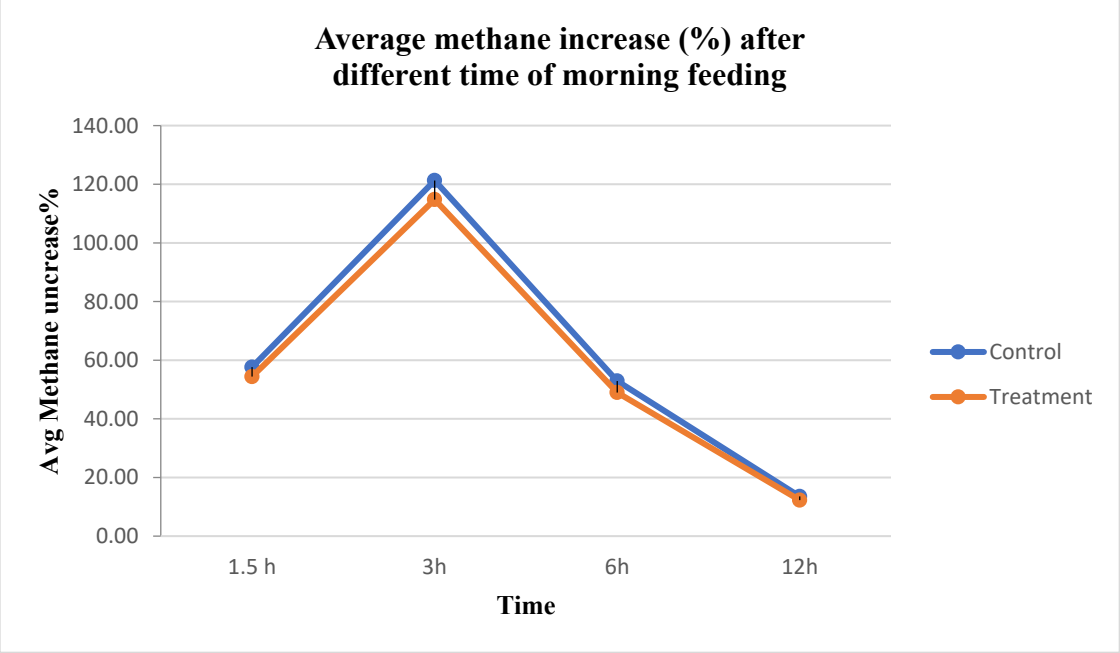

Fig 1: Average methane increase % after different times of feeding.

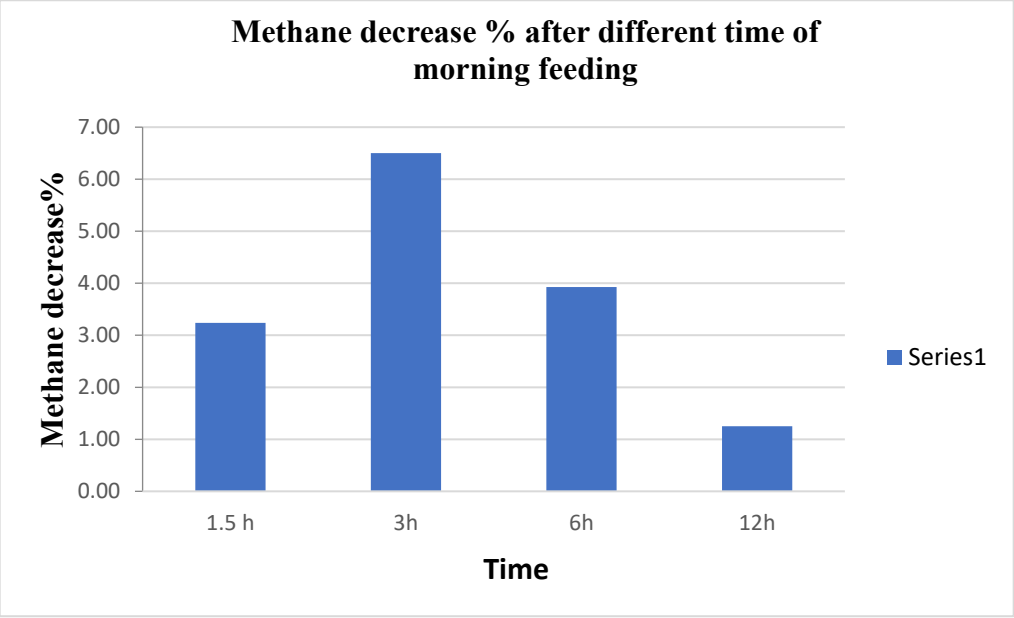

Fig 2: Histogram of methane decrease % after different times of feeding

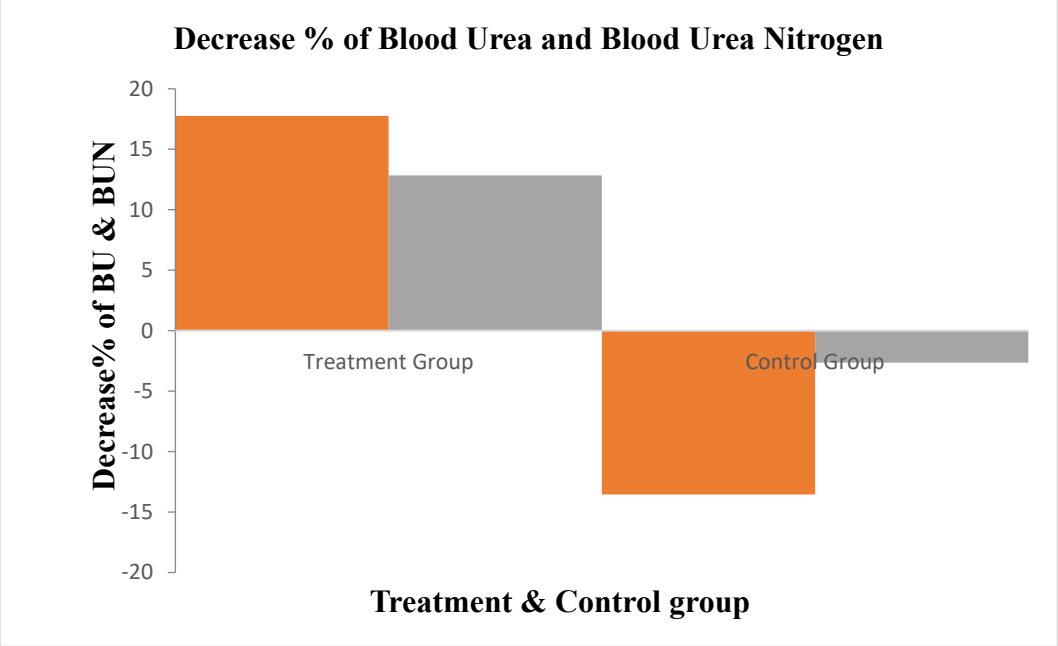

Fig 3: Decrease % of Blood Urea and Blood Urea Nitrogen.

In this study, the methane concentration was measured in parts per million, and for the conversion the applied formula is:

PPM (in L) =(PPM value/1000000)*Volume of the solution or medium (in liters)

The total methane emission on this day:

Control Group: 77.54 L/D/Animal

Treatment Group: 69.45 L/D/Animal

CHAPTER 4: DISCUSSION

Probiotics (direct-fed microorganisms) can enhance the growth performance of ruminants by increasing dry matter intake and fiber digestibility (Gado et al., 2011, Salem et al., 2013).In this study we use beneficial microorganism which is a probiotics. When given to animals, probiotics are non-pathogenic live microorganisms that have not been found to cause drug resistance or drug residues. By considering the drug resistance we used beneficial microorganism in this study. Probiotics have been utilized extensively in the food and feed sectors over the past few decades and have the potential to replace antibiotics (McAllister et al., 2011). The selection of feed additives should first be investigated in a ruminal environment because outcomes from utilizing feed additives have generally been inconsistent and are assumed to be partially attributable to enzyme properties, the makeup of the target forage, and ruminal circumstances (temperature and pH) (Colombatto et al., 2007).In this study we were used probiotics for the study of ruminal function of sheep. For ruminants, particularly dairy cows, there are many commercially available probiotic supplements. These products can be classified as either bacterial or yeast (fungi) products, and reactions to yeast probiotics are typically correlated with stimulation of cellulolytic and lactate-utilizing bacteria in the rumen (McAllister et al., 2011) all of those are not easily available and also not cost effective so, in this study we used beneficial fungi which is easily available and also cost effective which is upported by this author. Exogenous enzymes boosted microbial adhesion to diets and the overall quantity of viable rumen bacteria, according to Colombatto et al. (2007) which is similar to our study. The decrease in methane generation may be caused by an enzyme-driven alteration in the micro-flora of the methanogenium (Zhou et al., 2011) Which is Support our study results, after 1.5h hours of morning feeding, the treatment group showed a slight lower mean methane (54.39%) concentration compared to the control group (57.63%), after 3h hours of morning feeding, the treatment group (114.77%) showed a slight lower mean methane concentration compared to the control group (121.27%), after 6h hours of morning feeding, the treatment group (48.97%) showed a slight lower mean concentration compared to the control group(52.9%), after

12h hours of morning feeding, the treatment group (12.34%) showed a slight lower mean concentration compared to the control group (13.53%). The all differences is not statistically significant because the P-value>0.05. According to Duin et al. (2016) the current meta-analysis study, 3-NOP is a useful feed addition for reducing ruminant enteric methane emissions and we found the same result that nitrogenous compound helps to reduce the CH_4 emissions but there is a dissimilarity with this author they also found using 3-NOP at 100 mg/kg DMI would result in reductions in CH_4/BW, CH_4/DMI, CH_4/milk generated, CH_4/DOM, and CH_4/GEI of 19.3%, 19.2%, 21.1%, 17.8%, and 16.7% from control respectively which is the absence in this study. But Gado et al., (2017) described that Exogenous enzyme addition's positive effects are influenced by a number of variables, including the type of enzyme preparation, particular enzyme activity, enzyme stability, amount administered, food composition, and application technique considering this our study result is acceptable. Gado et al., (2008) demonstrated that probiotic products made from *Ruminococcus flavefaciens* anaerobic fermentation boost sheep and goat live weight gain and feed conversion of wheat straw supported by this study which also found the same result in our study. A study by Salem et al. (2013) found that steers treated with multi-enzymes derived from the same probiotic product as this study's probiotic product consumed more DM. By lowering the amount of NH_3-N in the rumen liquor and incorporating NH_3-N into microbial protein, probiotic products can boost the stimulation of neuronal microorganism activity (Gado et al., 2011). A similar study was described by Salem et al. (2015) reported that the probiotics product used in the current study increased the amount of microbial protein available for animal metabolism, suggesting that it may be more effective at improving fiber digestibility and consequently providing more nutrients for ruminal microorganisms that are good for microbial growth. Because rumen microbial N_2 production was increased, which may be partially attributable to more fiber digestion or an improved capacity of rumen bacteria to digest feed, feeding the enzyme preparation may have stimulated or raised total viable rumen bacterial numbers, or both which is support to our study because we used beneficial microorganism. Here found

blood urea N_2 and blood urea were decreased in the treatment group (3.59% and 17.76%) from the control group (-2.64% and -13.54%), a similar study was described by Singh et al. (2001). In my experiment overall 8.09L/D/animal more methane decrease in treatment group. So I think those beneficial organism are helpful to reduce the methane emission.

LIMITATION

As the methane collection device was not fully accurate, there was a chance of contamination with other atmospheric gasses. The author couldn't eat enough food here and the equipment was old. The sample size was small due to which the result was not significant. If the sample size could have been increased and the amount of food had been given, then a very good result would have been obtained.

CONCLUSION

Based on the results and the subsequent discussion, it can be concluded that the treatment involving the beneficial fungus led to marginal reductions in methane emissions at different time intervals after feeding. While these reductions were not statistically significant for most time points, they suggest a potential trend toward lowered methane emissions. The experiment underscores the intricate interplay of factors in determining the effectiveness of probiotics or feed additives and the need for a thorough understanding of ruminal conditions and microbial interactions. The results also emphasize the variability that can exist between different studies and treatments, highlighting the need for further research and investigation into optimizing the use of such additives for methane reduction and improved ruminant performance.

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LITERATURE REVIEW

Since they contribute to greenhouse gas emissions and have an effect on climate change, livestock, particularly sheep, have attracted a lot of attention for their methane (CH_4) emissions. For the purpose of comprehending and reducing methane emissions from ruminant animals, in vitro studies, which simulate the digestive functions of sheep in a controlled laboratory setting, have become an essential tool. The research on in vitro methane emissions from sheep is summarised in this review of the literature, with particular attention paid to methodology, key discoveries, and the state of the science at the time. Methane is a by product of the microbial fermentation that takes place inside the rumen of ruminant animals, and it has a much larger potential to cause global warming than carbon dioxide (CO_2). Sheep are a significant source of methane emissions into the atmosphere, mostly due to a process known as enteric fermentation in livestock. A study from the Intergovernmental Panel on Climate Change (IPCC) in 2007 states that enteric fermentation is responsible for roughly 14% of the world's anthropogenic methane emissions.(IPCC, 2007).

Key Findings from In Vitro Studies:

Numerous in vitro studies have yielded essential insights into methane emissions from sheep. Some key findings include:

1. ***Dietary Interventions****:* Through modifying the rumen microbial community, in vitro investigations have shown that dietary adjustments such as adding tannins, oils, or particular feed additives can drastically lower methane generation.
2. **Microbial Community Dynamics**: In vitro studies have provided valuable information on the composition and dynamics of rumen microbial communities responsible for methane production, aiding in the development of targeted mitigation strategies.

3. **Genetic Factors**: Research has indicated that there may be genetic differences among sheep breeds in their methane emissions, highlighting the potential for selective breeding to reduce emissions.
4. **Methanogen Inhibitors**: In vitro studies have explored the efficacy of various chemical inhibitors in reducing methane production by selectively inhibiting methane-producing microbes.

Current State of Knowledge and Future Directions:

Research into sheep-derived in vitro methane emissions is advancing quickly. Our knowledge of the complexity involved in methane production within the rumen has been expanded by recent investigations. Additionally, advances in omics technologies, such as metagenomics and metatranscriptomics, have enabled a deeper exploration of the microbial communities responsible for methane emissions.

Future research in this area should focus on:

1. **Integration of Multi-Omic Approaches**: Combining metagenomics, metatranscriptomics, and metabolomics to gain a comprehensive understanding of microbial functions and interactions within the rumen.
2. **Development of Sustainable Feeding Strategies**: Investigating sustainable dietary interventions and their long-term impacts on methane emissions while ensuring animal health and productivity.
3. **Selective Breeding**: Exploring the potential for selective breeding to develop low-methane-emission sheep breeds.
4. **Methane Capture Technologies**: Investigating innovative technologies for capturing and utilizing methane emissions from livestock.

The development of mitigation measures and the advancement of our understanding of sheep methane emissions require the use of in vitro research, which have become crucial instruments in this regard. In vitro research plays a key role in tackling the global challenge of methane emissions from livestock, particularly sheep, because of the combination of precise control, ethical considerations, and efficiency.

Background and Importance: Methane is a powerful greenhouse gas with a far higher potential for global warming than carbon dioxide (CO_2). (IPCC, 2007). Due to the fact that sheep have a special digestive system that involves fermentation in the rumen, livestock, particularly sheep, are known to be substantial sources of methane emissions. In order to create ways to reduce these emissions while guaranteeing sustainable livestock production, it is essential to understand the mechanisms controlling methane production in sheep.

In vitro Models for Methane Emission Studies: Because they enable exact control of the experimental settings and repeated observations, in vitro models have grown in popularity for researching methane emissions from sheep. The rumen simulation technique (RUSITEC) and the gas production technique are the two most popular in vitro systems (Hristov et al., 2011). These models imitate the intricate digestive procedures that take place in the rumen of a sheep, making them useful tools for determining how dietary modifications, feed additives, and microbial populations affect the production of methane.

Factors Influencing Methane Emission:

Diet Composition: In vitro models have been used in numerous research to examine the effect of diet composition on methane emissions from sheep. According to research, nutritional elements like the kind and quality of forage, the addition of grains, and the presence of additives like lipids and tannins can all have a big impact on methane generation. High-quality forage diets with reduced fiber content typically result in lower

methane emissions, but diets based on grains can result in higher emissions (Van Zijderveld et al., 2011; Patra, 2012).

Microbial Populations: Methane generation heavily depends on the rumen's microbial population. Studies conducted in vitro have looked into how various microbial populations and their activities may impact methane emissions. Probiotics have been investigated as rumen microbial manipulation techniques to lower methane emission. (Hook et al., 2011).

Feed Additives: The effectiveness of several feed additives, including as tannins, lipids, and essential oils, in lowering methane emissions has also been researched. These additives frequently function by changing the microbial populations or metabolic processes that result in the production of methane. (McGinn et al., 2004).

Mitigation Strategies: Studies conducted in vitro have shed light on potential mitigating tactics for lowering sheep methane emissions. Among these tactics are the creation of methane inhibitors, vaccinations, and genetic selection for sheep with minimal methane production. Many of these strategies, nevertheless, are still in the experimental stage and need more verification.

Limitations and Future Directions: While in vitro research has greatly advanced our knowledge of sheep methane emissions, there are several drawbacks to take into account. These models don't accurately represent the rumen environment's complexity, and their findings might not always be applicable to real-world situations. Future studies should therefore concentrate on enhancing the precision and applicability of in vitro models and confirming results in real sheep herds.

Conclusion: Our understanding of the elements driving these emissions and potential mitigation techniques has improved as a result of in vitro investigations on methane emissions from sheep. The importance of dietary components, microbial populations, and feed additives has been established, and study in these fields is ongoing. Reduced

methane emissions from sheep remain a key topic of research as the world looks for solutions to combat climate change, with in vitro research being crucial in guiding sustainable livestock production methods. Developing successful methane reduction measures while guaranteeing the wellbeing and productivity of sheep requires further study and innovation in this area.

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The Author

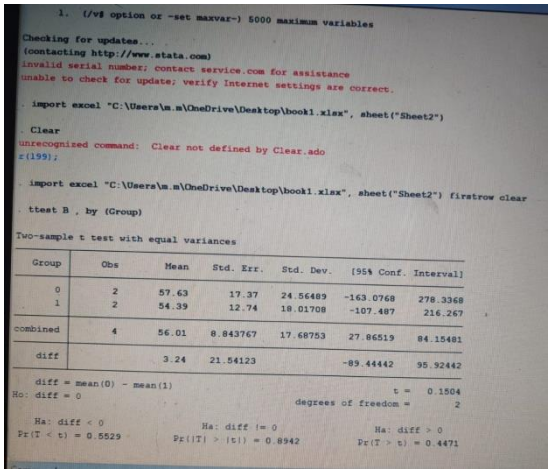
BIOGRAPHY

I am Mohammad Robaidul Islam, son of Mohammad Rafiqul Islam and Kohinur Akter. I successfully completed my Secondary School Certificate exams at Bakalia Govt. High School, Chattogram, in 2014, and my Higher Secondary Certificate exams at HajeraTaju Degree College, Chattogram, in 2016. I am currently pursuing a Doctor of Veterinary Medicine (DVM) degree at Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. My aspirations entail becoming a veterinary practitioner and actively contributing to the progress of the nation.

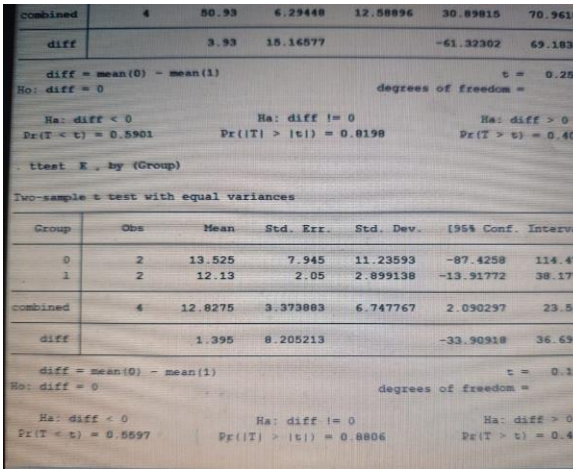
APPENDIX

Table: Raw data of Blood Urea and Blood Urea

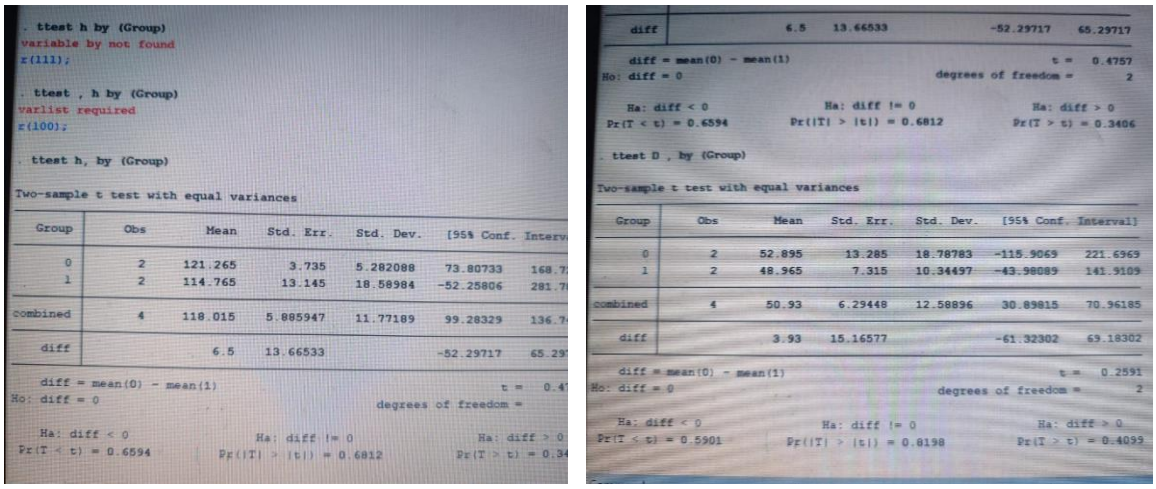
ID	BU Before Experiment	BUN Before Experiment	BU After Experiment	BUN After Experiment
C_1	44.5	23	42.6	19.8
C_2	38.2	18.7	51.3	23
T_1	42.4	21.5	25.4	12
T_2	35.5	17.5	38.5	18.8



```
. (/v# option or -set maxvar-) 5000 maximum variables

Checking for updates...
(contacting http://www.stata.com)
invalid serial number; contact service.com for assistance
unable to check for update; verify Internet settings are correct.

. import excel "C:\Users\m.m\OneDrive\Desktop\book1.xlsx", sheet("Sheet2")

. Clear
unrecognized command:  Clear not defined by Clear.ado
r(199);

. import excel "C:\Users\m.m\OneDrive\Desktop\book1.xlsx", sheet("Sheet2") firstrow clear

. ttest B , by (Group)

Two-sample t test with equal variances

   Group |  Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |    2       57.63       17.37    24.56489   -163.0768    278.3368
       1 |    2       54.39       12.74    18.01708    -107.487     216.267
---------+--------------------------------------------------------------------
combined |    4       56.01    8.843767    17.68753    27.86519     84.15481
---------+--------------------------------------------------------------------
    diff |            3.24    21.54123               -89.44442     95.92442
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.1504
Ho: diff = 0                                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5529         Pr(|T| > |t|) = 0.8942          Pr(T > t) = 0.4471
```



```
combined |    4       50.93    6.29448    12.58896    30.89815     70.961
---------+--------------------------------------------------------------------
    diff |            3.93   15.16577               -61.32302     69.183
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.25
Ho: diff = 0                                     degrees of freedom =

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5901         Pr(|T| > |t|) = 0.8198          Pr(T > t) = 0.41

. ttest E , by (Group)

Two-sample t test with equal variances

   Group |  Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interv
---------+--------------------------------------------------------------------
       0 |    2      13.525       7.945    11.23593    -87.4258    114.4
       1 |    2       12.13        2.05    2.899138   -13.91772    38.17
---------+--------------------------------------------------------------------
combined |    4     12.8275    3.373883    6.747767    2.090297    23.5
---------+--------------------------------------------------------------------
    diff |            1.395    8.205213               -33.90918    36.69
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.1
Ho: diff = 0                                     degrees of freedom =

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5597         Pr(|T| > |t|) = 0.8806          Pr(T > t) = 0.4
```



```
. ttest h by (Group)
variable by not found
r(111);

. ttest , h by (Group)
varlist required
r(100);

. ttest h, by (Group)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interv
---------+--------------------------------------------------------------------
       0 |       2     121.265       3.735    5.282088    73.80733    168.7
       1 |       2     114.765      13.145    18.58984   -52.25806    281.7
---------+--------------------------------------------------------------------
combined |       4     118.015    5.885947    11.77189    99.28329    136.7
---------+--------------------------------------------------------------------
    diff |                 6.5    13.66533                -52.29717    65.29

    diff = mean(0) - mean(1)                                      t =   0.47
Ho: diff = 0                                     degrees of freedom =

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6594         Pr(|T| > |t|) = 0.6812          Pr(T > t) = 0.34
```

```
    diff |                 6.5    13.66533                -52.29717    65.29717

    diff = mean(0) - mean(1)                                      t =   0.4757
Ho: diff = 0                                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6594         Pr(|T| > |t|) = 0.6812          Pr(T > t) = 0.3406

. ttest D , by (Group)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       2      52.895      13.285    18.78783   -115.9069    221.6969
       1 |       2      48.965       7.315    10.34497   -43.98089    141.9109
---------+--------------------------------------------------------------------
combined |       4       50.93     6.29448    12.58896    30.89815    70.96185
---------+--------------------------------------------------------------------
    diff |                3.93    15.16577                -61.32302    69.18302

    diff = mean(0) - mean(1)                                      t =   0.2591
Ho: diff = 0                                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5901         Pr(|T| > |t|) = 0.8198          Pr(T > t) = 0.4099
```

Fig : Statistical analysis in Stata 13 of t & p value

Fig: Feeding of Sheep

Fig: Blood Collection from Sheep

Fig: Plastic Tank Preparation