INNOVATION OF LOW COST METHANE CONCENTRATION ESTIMATION METHOD & COMPARING METHANE CONCENTRATION OF SHEEP & GOAT

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The Author

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ABSTRACT

Ruminants are one of the main contributors to the release of greenhouse gases into the atmosphere. Large ruminants are capable of producing 250 to 500 Liters of methane (CH_4) daily. CH_4 is a by-product of feed fermentation that is released primarily through eructation by ruminants. Methanogenesis is carried out by anaerobic organisms called Archaea which are found in the rumen where the anaerobic conditions are optimal. There prevails several methods or technique to estimate the concentration of CH_4 eructed by the ruminants. Most of the techniques are too costly and requires highly skilled personnel. We used sensor based hand-held gas detector to detect the CH_4 concentration in exhaled air of sheep and goat. It is easy to operate and less expensive. We performed the experiment for two days at CVASU animal farm and recorded the PPM reading given by the gas detector. The data clearly demonstrate that CH_4 concentration in exhaled air of sheep and goat increase after feeding and reaches at the highest level in between 1.5 to 2 hours after feeding. 1 hour after feeding, significant variation between CH_4 concentration of sheep and goat was found in both days. The Mean CH_4 concentration of goats at 11:00 AM were 1077.42 PPM (1st day) and 1009 PPM (2nd day) which were higher than the mean CH_4 concentration of sheep 908.66 PPM (2nd day) and 856.96 PPM (2nd day).

Keyword: Methane, Fermentation, Gas detector, Sheep and Goat.

CHAPTER-I: INTRODUCTION

Methane is the important factor of global warming. It was reported that approximately 20% of greenhouse effect is due to methane (CH_4) (IPCC, 1990; 1992). Depletion of stratospheric Ozone is promoted by CH_4 (Blake & Rowland, 1988). Methane emission is responsible for approximately 3-14% loss in gross energy intake (Hellwing et al. 2016). So it is important to understand the global methane production and its sources.

Methane production by ruminant is one of the largest sources of global warming. Approximately 15% of global methane emissions are due to ruminants (Gerber et al. 2013). Ruminants yield methane by anaerobic fermentation of feed as by-product & emitted primarily by eructation (Janssen PH et al. 2008). About 87% of methane released from the animals mouth and nose originates from the forestomach through eructation and blood absorption (Murray et al. 1976). About 13% of the CH_4 is originated in the hindgut, where 89% is absorbed into the circulatory system and excreated via expiration (Ricci et al., 2014). The organisms responsible for methanogenesis are known as Archaea which located at the rumen that gives them ideal anaerobic condition. The methanogenic bacteria use two biochemical pathways to yield methane gas, such as the reduction of Carbon dioxide (CO_2) and decarboxylation of acetate. The reduction of CO_2 by Hydrogen (H_2) is the major one. The pattern of the methane production by ruminants is pulsatile, with high concentration of methane in exhaled breath coinciding with each eructation.

Open-circuit respiration chamber (Waghorn, 2014), Sulfur hexafluoride tracer technique (SF_6, Johnson et al., 2007) & the automated heat-chamber system (GreenFeed, Zimmerman & Zimmerman, 2012) are the most accepted methods for estimation of CH_4. Open-circuit breathing chambers for whole animals are the most popular right now, and they come in all shapes and sizes, from simple poly tunnels and shower curtains over stalls (Powell et al., 2007; Aguerre et al., 2011), to more complex and pricy dedicated calorimeters that require more time and money to set up (Global Research Alliance, 2012). These methods are too costly & require highly skilled labor which limits their utilization in developing country like ours. Some methods which are less accurate such as Laser based Methane estimation Device (LMD) is explored to measure CH_4 concentration in air emitted by the animals (Chagunda et al. 2009).

Recent research disclosed that there is variation in CH_4 production between individual animals when fed identical feed. Also the CH_4 emission rate is not constant & may vary time to time. Feed additives play an important part in rumen fermentation and CH_4 emission (Seon-Ho Kim et al., 2020). Consumption of lysozyme in the form of dietary supplements has been demonstrated to enhance rumen fermentation in vitro and to decrease CH_4 emissions (Ashraf A. Biswas et al., 2016). Genetic factors that affect the feeding behavior, live weight, age, type of feed etc are the possible factors for the variation.

The main objectives of this study are:

- To detect the methane concentration in exhaled air of goat & sheep.

-To determine the variation of concentration of methane in exhaled air before & after feeding.

-To establish a low cost methane concentration estimation method.

CHAPTER-II: MATERIALS & METHODS

To perform this experiment, we used SPD203 Methane gas detector. It can detect the gas and show the percentage concentration on digital display. This uses highly sensitive sensor for accurate measurement & detection.

The experiment was conducted in CVASU animal farm under the Department of Animal Science & Nutrition, Chattogram Veterinary & Animal Sciences University, Khulshi, Chattogram, Bangladesh.

To conduct the experiment, we formed two groups of 4 goats and sheep each. Body weight of all the animals was measured via electric weight machine.

Table-1: Body weight of sheep

Animal Id.	Body weight (Kg)
S-10	21.12
S-20	20.43
S-30	19.75
S-40	21.81

Table-2: Body weight of goat

Animal Id.	Body weight (Kg)
G-11	13.72
G-20	17.16
G-30	17.5
G-40	20.36

Each animal was given 400 grams of the same type of concentrate feed separately at 10:00 AM. Readings were taken from each animal in gas detector machine half an hour before giving feed. After that, readings were taken from each animal at intervals of half an hour after feeding until the readings reached the same level. We perform this activity two days in a row.

In this way, we measured and recorded the concentration of methane gas in the exhaled air of each goat and sheep respectively. And the recorded data was analyzed statistically by STATA software to compare methane concentration between sheep and goat.

CHAPTER-III: RESULTS AND DISCUSSION

On the basis of the data collected, it can be concluded that in both sheep and goat, the methane concentration in the breath air is low prior to consumption and increases post-consumption. Within 1.5 to 2 hours following ingestion, the methane concentration in the exhaled air reaches its highest level in both species. Subsequently, the methane concentration decreases gradually.

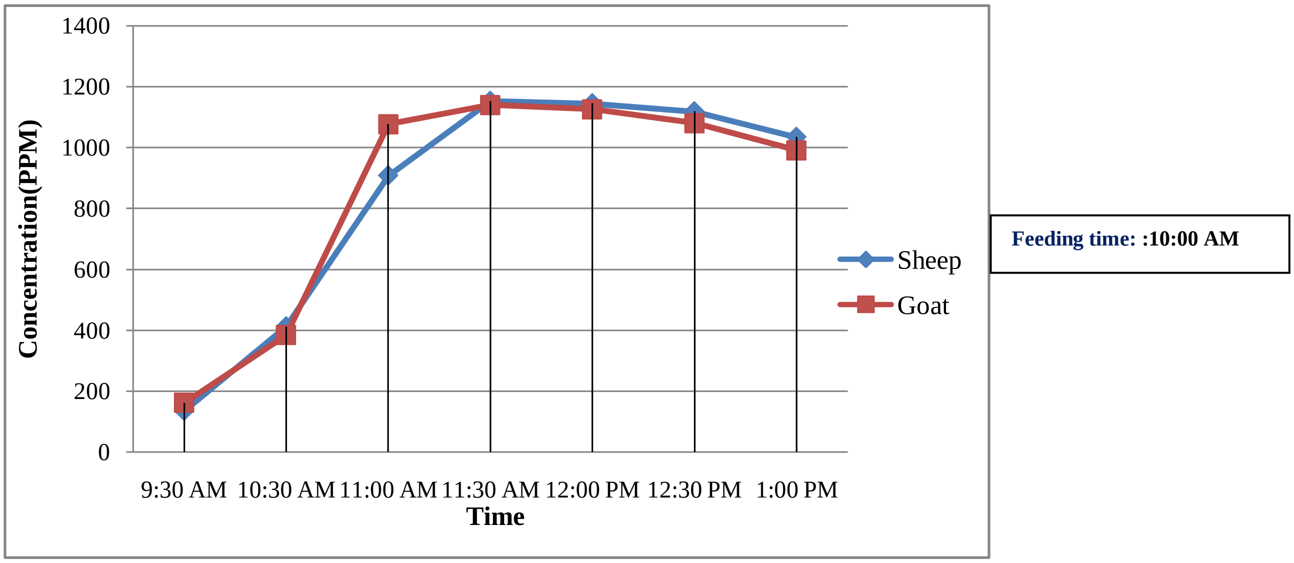

Fig-1: Methane emission concentration(PPM) of exhaled air on day-1

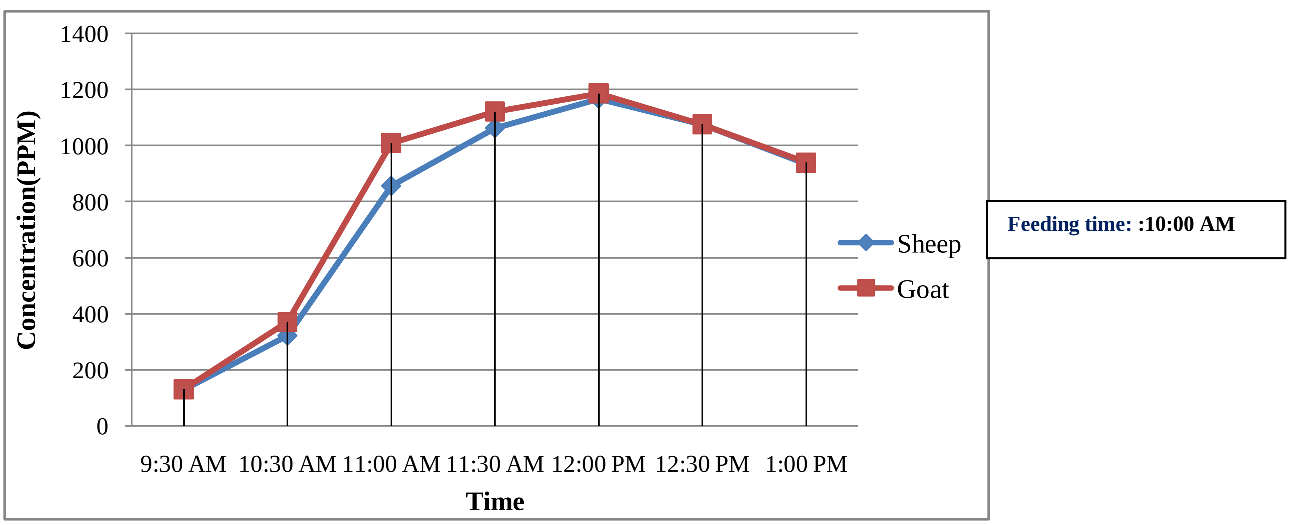

Fig-2: Methane emission concentration(PPM) of exhaled air on day-2

Table 3: Methane emission concentration Day 1

Time	Mean ± SD		SEM		T value	P value
	Sheep	Goat	Sheep	Goat		
9:30 AM	135.92 ± 6.88	162.25 ± 27.08	3.44	13.54	1.89	0.107
10:30 AM	412.83 ± 22.74	384.75 ± 52.74	11.37	26.37	0.98	0.365
11:00 AM	908.66 ± 42.59	1077.42 ± 33.51	21.29	16.75	6.23	0.001
11:30 AM	1153.25 ± 18.88	1140 ± 18.05	9.44	9.03	1.01	0.351
12:00 PM	1145.33 ± 35.43	1125.75 ± 33.85	17.71	16.93	0.799	0.455
12:30 PM	1119 ± 28.47	1080.75 ± 40.48	14.23	20.24	1.546	0.173
1:00 PM	1034.96 ± 42.82	990.54 ± 13.49	21.41	6.745	1.98	0.106

SD= Standard Deviation; SEM= Standard Error of Mean

Table 4: Methane emission concentration Day 2

Time	Mean ± SD		SEM		T value	P value
	Sheep	Goat	Sheep	Goat		
9:30 AM	129.08 ± 7.56	131.41 ± 28.65	3.78	14.32	0.16	0.878
10:30AM	322.37 ± 49.18	371.71 ± 53.53	24.59	26.765	1.36	0.223
11:00AM	856.96 ± 40.78	1009 ± 27.46	20.39	13.73	6.18	0.001
11:30AM	1063.08 ± 42.75	1121.5 ± 45.1	21.37	22.55	1.88	0.109
12:00PM	1167.5 ± 49.03	1186.75 ± 62.38	24.52	31.19	0.485	0.644
12:30PM	1076.17 ± 54.12	1076.92 ± 33.46	27.06	16.73	0.024	0.982
1:00 PM	935.25 ± 44.69	940.33 ± 42.37	22.34	21.18	0.165	0.874

SD= Standard Deviation; SEM= Standard Error of Mean

There was no significant difference ($P > 0.05$) between the CH_4 concentration of sheep and goat before feeding. After feeding, in case of both 1st and 2nd day at 10:30 AM, 11:30 AM, 12:00 PM, 12:30 PM and 1:00 PM, we found no significant difference ($P > 0.05$) of methane concentration in exhaled air of sheep and goat. But 1 hour interval after feeding at 11:00 AM we found significant difference ($P < 0.05$) between CH_4 concentration of sheep and goat in both days.

The mean CH_4 concentration of goats were 1077.42 PPM (1st day) and 1009 PPM (2nd day) which is higher than the mean CH_4 concentration of sheep 908.66 PPM (2nd day) and 856.96 PPM (2nd day). The fig-3 indicates also a considerable difference among sheep (882.205 PPM) and goats (1041.335PPM) at 11:00 AM in the total mean CH_4 concentration.

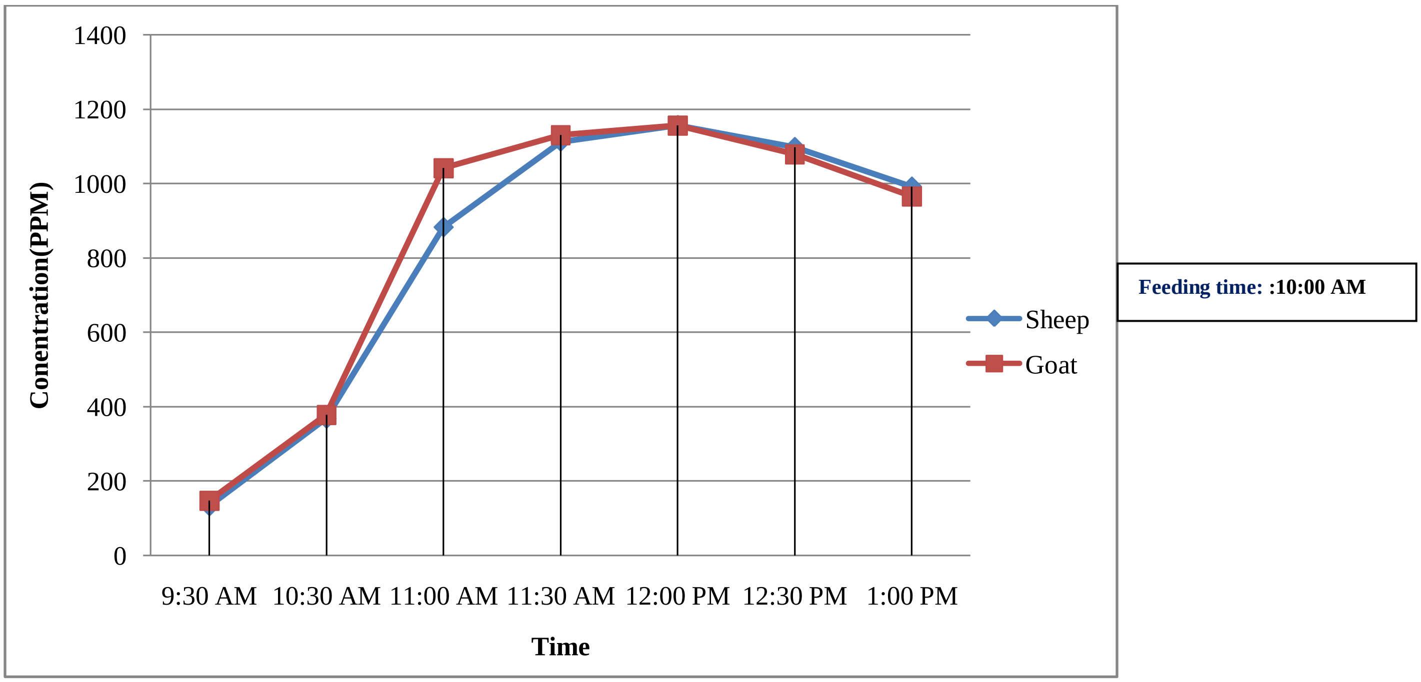

Fig-3: Total mean methane emission concentration(PPM) of exhaled air

Methane concentration variation between sheep and goats can be attributed to differences in their digestive systems. Both animals are ruminants, but their diets and digestive processes vary. Sheep typically consume more fibrous plants, leading to a slower fermentation process in their stomachs and thus lower methane production. Goats, on the other hand, have more diverse diet that includes a broader range of plants, leading to faster fermentation and potentially higher methane emissions. Other factors like body weight, gut microbiota composition and metabolic differences can also contribute to the variation in methane concentrations between these two animals.

CHAPTER-IV: CONCLUSIONS

The results demonstrate that the CH_4 concentration values prior to feeding did not vary significantly between the sheep and goat. There is also no significant difference in sheep and goat for the same type and the same amount of feed consumed in a given period of interval. But in between 1 to 1.5 hour interval after feeding there is significant variation in methane concentration of sheep and goat. Confidently, more correct values could be obtained if the concentration of CH_4 measured in more precise way and the data were compared using a most recognized and accepted method. However, it’s important to consider factors such as calibration, sensor accuracy, and maintenance to ensure reliable results.

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CHAPTER-VI: LITERATURE REVIEW

Methanogenic bacteria under anaerobic conditions form methane in the rumen of ruminants. This process allows ruminants to use energy in low calorie feed such as grass and fodder that has a high content of cellulose. The level of feed intake and animal's digestibility are determining factors for methane emissions from ruminants (Blaxter et al. 1965). Feed additives play an important part in rumen fermentation and CH_4 emission (Seon-Ho Kim et al., 2020). Consumption of lysozyme in the form of dietary supplements has been demonstrated to enhance rumen fermentation in vitro and to decrease CH_4 emissions (Ashraf A. Biswas et al., 2016). For calculating emissions at a specific level the relation that they have identified is used.

Since 1950, there has been an increase in the number of domesticated animals in the world. Due to an increase in number of ruminants and growing milk production, GHG emissions from ruminants are increasing. Between 1966 and 1986, the methane emissions from domestic animals were 0.6 Tg/year or 0.75 per year (Crutzen et al. 1986). Lerner et al. (1988) compiled a global database of methane emissions from livestock and found that only five countries account for half of global emissions: India, Brazil, The former Soviet Union, USA and China. For 2010, Kelliher and Clark (2010) estimated that methane emissions from ruminants would amount to approximately 100 Tg per year, in which 14 Tg from China, 12 Tg from Brazil, 11 Tg from India, 5.5 Tg from the USA, 3 Tg from Australia, 3 Tg from Pakistan, 3 Tg from Argentina, 2.5 Tg from Russia, 2.3 Tg from Mexico and 2 Tg from Ethiopia. According to a study by Das et al., (2020), livestock GHG emissions from enteric fermentation in Bangladesh were estimated to be 30,124 gigagram.

In animals, there is no universally superior method for CH_4 quantification. Some methods are better suited to small scale scenarios, mainly due to the fact that they have been developed with this in mind and others were built from scratch as a result of their conception for wide scale use. However, for the measurement of enteringic methane emissions from ruminants is carried out with a wide range of techniques and methods. In the past ten years, a number of novel CH_4 measurement techniques have been developed which can be applied to a wide range of animals and are suitable for use on farms (Hammond et al., 2016, Biswas et al., 2019).

The respiration chamber is a well established and documented as a reliable CH_4 measurement system (Hammond et al. 2011). Open-circuit breathing chambers for whole animals are the most popular right now, and they come in all shapes and sizes, from simple poly tunnels and shower curtains over stalls (Powell et al., 2007; Aguerre et al., 2011), to more complex and pricy dedicated calorimeters that require more time and money to set up (Global Research Alliance, 2012). An animal is held in a containment chamber large enough for them to be comfortably accommodated, and kept under somewhat negative atmospheric pressure. This prevents any foreseeable gaseous leaks coming in instead of out (Johnson et al. 1995; storm et al. 2012).

Zimmerman (1993) developed the SF_6 technique, and Johnson et al. (1994) were the first to report use of this method for estimating ruminant CH_4 emissions. This method is suitable for confined or free range and grazing animals, in which a permeation tube with the known rate of SF_6 gas leakage into the reticulorumen has been placed inside the animal. In general, average CH_4 emissions are provided by the SF_6 technique. For a given animal, it may differ from that obtained in respiration chambers. Despite the fact that a mean CH_4 emission cannot differ, within and the SF_6 technique used in sheep has considerably increased variation between animals (Pinares-Patino et al., 2011) and dairy cattle (Grainger et al., 2007) relative to the respiration chamber technique.

For the purpose of obtaining CH_4 emission measurements over a 24 hour period, the use of respiratory chambers and SF_6 technique is generally employed. But short term measurement methods offer the ability to detect the presence of CH_4 at specific time points. These methods are typically automated, minimally invasive and non-tempering, thus enabling a high animal throughput. Repeated short-term measurements using facemasks have been used in previous studies (Washburn and brody, 1937).

The GreenFeed system uses a static, short term measurement device to measure CH_4 emissions from individual animals by combining air flow, gas level and head position detection during each animals stay in the unit (Huhtanen et al., 2015). In this system, the collected air is blended, filtered and the airflow is measured by means of an anemometer with hot film coating. The CH_4 concentration in the sample is determined by non-disperse infrared analysis.

Estimation of methane emissions from the animal is based on the background corrected ratio of CH_4 to CO_2 and the predicted CO_2 emissions, as provided by Madsen et al. (2010). The precision of the CH_4 emission estimation based on the CH_4 to CO_2 ratio will be contingent upon a number of variables, including the origin of gases present in air sample, as well as the diurnal variations in the CH_4:CO_2 ratio due to variations in animal behavior and fermentation rate related to meal size and feeding frequency (Madsen et al., 2010).

A measurement of CH_4 concentration in air eructed from cattle at milking has been reported for the first time by Garnsworthy et al., 2012. A sample inlet is inserted into the feed collector of an automated milking system. The gas concentration in the air in the feed manger are taken at 1 second intervals, analyzed and recorded. The method excludes CH_4 in the inhaled air from all eructation's, as well as any flatulence (Garnsworthy et al., 2012). The fact that the distance between the head of the animal and the point of sampling has a significant influence on CH_4 and CO_2 concentration, which is not a factor in the overall air sampling, is a concern for the techniques used to measure gas concentrations in exhaled air samples (Hegarty, 2013).

The use of handheld laser detectors for measuring CH_4 concentration in air between animal's nose or mouth and LMD is a further option to monitor the exhaled level of CH_4 (Ricci et al., 2014). The analysis only relies on peak values reflecting CH_4 concentration increases due to inhalation or eructation. The average values of CH_4 concentration can be readily provided by the LMD. It has the potential to generate accurate estimation of methane from ruminants, thus making it a useful tool for on-field monitoring and decision making for GHG mitigation measures (Chagunda et al., 2013).

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CHAPTER-VII: APPENDIX

Table 5: Methane emission concentration Day 1(Sheep)

	Before feeding	**After feeding**					
Time	**9:30Am**	**10:30AM**	**11:00AM**	**11:30AM**	**12:00PM**	**12:30PM**	**1:00PM**
	Avg. (PPM)	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**
Sheep-10	139.33	379.67	851.33	1168.00	1174.67	1144.33	1083.33
Sheep-20	125.67	421.00	931.00	1169.00	1172.33	1139.67	1058.67
Sheep-30	138.33	419.33	903.33	1129.67	1134.33	1108.33	999.50
Sheep-40	140.33	431.33	949.00	1146.33	1100.00	1083.67	998.33

Feeding time: 10:00 AM

Table 6: Methane emission concentration Day 1(Goat)

	Before feeding	**After feeding**					
Time	**9:30Am**	**10:30AM**	**11:00AM**	**11:30AM**	**12:00PM**	**12:30PM**	**1:00PM**
	Avg. (PPM)	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**
Goat-11	171.67	411.33	1049.33	1127.33	1123.00	1039.67	971.67
Goat-20	131.00	374.67	1053.67	1145.67	1153.67	1113.67	1001.00
Goat-30	194.33	315.67	1085.00	1124.00	1147.33	1117.33	999.50
Goat-40	152.00	437.33	1121.67	1163.00	1079.00	1052.33	990.00

Feeding time: 10:00 AM

Table 7: Methane emission concentration Day 2(Sheep)

	Before feeding	After feeding					
Time	**9:30Am**	**10:30AM**	**11:00AM**	**11:30AM**	**12:00PM**	**12:30PM**	**1:00PM**
	Avg. (PPM)	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**
Sheep-10	131.67	257.33	803.50	1087.50	1202.00	1126.33	984.50
Sheep-20	118.33	352.00	855.33	1103.33	1214.33	1100.00	961.00
Sheep-30	130.33	312.50	867.00	1006.50	1143.00	1077.67	904.50
Sheep-40	136.00	367.67	902.00	1055.00	1110.67	1000.67	891.00

Feeding time: 10:00 AM

Table 8: Methane emission concentration Day 2(Goat)

	Before feeding	After feeding					
Time	**9:30Am**	**10:30AM**	**11:00AM**	**11:30AM**	**12:00PM**	**12:30PM**	**1:00PM**
	Avg. (PPM)	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**	**Avg. (PPM)**
Goat-11	112.33	378.67	992.00	1056.33	1233.33	1080.00	946.00
Goat-20	102.00	374.50	982.50	1156.67	1175.33	1086.67	912.00
Goat-30	161.00	301.67	1018.00	1127.33	1235.33	1110.33	998.00
Goat-40	150.33	432.00	1043.50	1145.67	1103.00	1030.67	905.33

Feeding time: 10:00 AM

Statistical Analysis

```
. ttest zerohour , by (Animal)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4     135.915    3.439316    6.878631    124.9696    146.8604
       1 |       4      162.25    13.53849    27.07698    119.1645    205.3355
---------+--------------------------------------------------------------------
combined |       8    149.0825    8.159678    23.07905    129.7879    168.3771
---------+--------------------------------------------------------------------
    diff |            -26.335    13.96852               -60.51474    7.844736
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.8853
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0542         Pr(|T| > |t|) = 0.1084          Pr(T > t) = 0.9458

. ttest firsthour , by (Animal)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4    412.8325    11.36821    22.73642    376.6538    449.0112
       1 |       4      384.75    26.37045    52.74089    300.8275    468.6725
---------+--------------------------------------------------------------------
combined |       8    398.7912    14.31339    40.48438    364.9455     432.637
---------+--------------------------------------------------------------------
    diff |            28.0825    28.71649               -42.18421    98.34921
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.9779
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8171         Pr(|T| > |t|) = 0.3659          Pr(T > t) = 0.1829

. ttest Firsthalfanhour , by (Animal)
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. ttest Firsthalfanhour , by (Animal)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4     908.665    21.29462    42.58924     840.896     976.434
       1 |       4    1077.418    16.75467    33.50935    1024.097    1130.738
---------+--------------------------------------------------------------------
combined |       8    993.0412    34.26915    96.92779    912.0076    1074.075
---------+--------------------------------------------------------------------
    diff |          -168.7525    27.09575               -235.0534   -102.4516
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -6.2280
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0004         Pr(|T| > |t|) = 0.0008          Pr(T > t) = 0.9996

. ttest secondhour , by (Animal)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4     1153.25    9.440726    18.88145    1123.205    1183.295
       1 |       4        1140    9.026248     18.0525    1111.274    1168.726
---------+--------------------------------------------------------------------
combined |       8    1146.625    6.544259    18.50996     1131.15      1162.1
---------+--------------------------------------------------------------------
    diff |              13.25    13.06141               -18.71012    45.21012
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   1.0144
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8252         Pr(|T| > |t|) = 0.3495          Pr(T > t) = 0.1748

. ttest Secondhalfanhour , by (Animal)
```

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. ttest Secondhalfanhour , by (Animal)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	4	1145.333	17.71451	35.42901	1088.957	1201.708
1	4	1125.75	16.92717	33.85433	1071.88	1179.62
combined	8	1135.541	11.93056	33.74472	1107.33	1163.753
diff		19.5825	24.50169		-40.37097	79.53597

```
    diff = mean(0) - mean(1)                                      t =   0.7992
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7727         Pr(|T| > |t|) = 0.4546          Pr(T > t) = 0.2273
```

. ttest Thirdhour , by (Animal)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	4	1119	14.23293	28.46586	1073.704	1164.296
1	4	1080.75	20.24246	40.48492	1016.329	1145.171
combined	8	1099.875	13.54498	38.31099	1067.846	1131.904
diff		38.25	24.74537		-22.29974	98.79974

```
    diff = mean(0) - mean(1)                                      t =   1.5457
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9134         Pr(|T| > |t|) = 0.1731          Pr(T > t) = 0.0866
```

. ttest Thirdhalfanhour , by (Animal)

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. ttest Thirdhalfanhour , by (Animal)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	4	1034.958	21.41065	42.82129	966.8193	1103.096
1	4	990.5425	6.74575	13.4915	969.0745	1012.01
combined	8	1012.75	13.35801	37.78215	981.1633	1044.337
diff		44.415	22.44818		-10.51373	99.34373

```
    diff = mean(0) - mean(1)                                      t =   1.9786
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9524         Pr(|T| > |t|) = 0.0952          Pr(T > t) = 0.0476
```

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. ttest zerohour , by (Animal)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	4	129.0825	3.78285	7.565701	117.0438	141.1212
1	4	131.415	14.32518	28.65036	85.82589	177.0041
combined	8	130.2488	6.872733	19.43902	113.9973	146.5002
diff		-2.3325	14.81623		-38.58651	33.92151

```
    diff = mean(0) - mean(1)                                      t =  -0.1574
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4400         Pr(|T| > |t|) = 0.8801          Pr(T > t) = 0.5600
```

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. ttest firsthour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4     322.375    24.59276    49.18552    244.1099    400.6401
       1 |       4      371.71    26.76551    53.53102    286.5302    456.8898
---------+--------------------------------------------------------------------
combined |       8    347.0425    19.23642    54.40882    301.5556    392.5294
---------+--------------------------------------------------------------------
    diff |            -49.335    36.34827                -138.276      39.606
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.3573
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1118         Pr(|T| > |t|) = 0.2235          Pr(T > t) = 0.8882

. ttest Firsthalfanhour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4    856.9575    20.39206    40.78411    792.0609    921.8541
       1 |       4        1009    13.73105     27.4621    965.3017    1052.698
---------+--------------------------------------------------------------------
combined |       8    932.9787    30.90492     87.4123    859.9002    1006.057
---------+--------------------------------------------------------------------
    diff |          -152.0425    24.58409               -212.1976   -91.88739
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -6.1846
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0004         Pr(|T| > |t|) = 0.0008          Pr(T > t) = 0.9996

. ttest secondhour , by (Animal)
```

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. ttest secondhour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4    1063.083    21.37557    42.75115    995.0559    1131.109
       1 |       4      1121.5    22.55038    45.10076    1049.735    1193.265
---------+--------------------------------------------------------------------
combined |       8    1092.291    18.13167    51.28412    1049.417    1135.166
---------+--------------------------------------------------------------------
    diff |          -58.4175    31.07145               -134.4466    17.61159
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.8801
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0546         Pr(|T| > |t|) = 0.1091          Pr(T > t) = 0.9454

. ttest Secondhalfanhour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4      1167.5    24.51734    49.03467    1089.475    1245.525
       1 |       4    1186.747    31.19053    62.38106    1087.485     1286.01
---------+--------------------------------------------------------------------
combined |       8    1177.124     18.7218    52.95324    1132.854    1221.394
---------+--------------------------------------------------------------------
    diff |          -19.2475    39.67303               -116.3239     77.8289
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -0.4852
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.3224         Pr(|T| > |t|) = 0.6448          Pr(T > t) = 0.6776

. ttest Thirdhour , by (Animal)
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. ttest Thirdhour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4    1076.168    27.05919    54.11837    990.0531    1162.282
       1 |       4    1076.918     16.7328     33.4656    1023.666    1130.169
---------+--------------------------------------------------------------------
combined |       8    1076.543    14.72811    41.65738    1041.716    1111.369
---------+--------------------------------------------------------------------
    diff |                -.75    31.81487               -78.59819    77.09819
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -0.0236
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4910         Pr(|T| > |t|) = 0.9820          Pr(T > t) = 0.5090

. ttest Thirdhalfanhour , by (Animal)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |       4      935.25    22.34623    44.69247    864.1343    1006.366
       1 |       4    940.3325    21.18483    42.36967    872.9129    1007.752
---------+--------------------------------------------------------------------
combined |       8    937.7912    14.28628    40.40771    904.0096    971.5729
---------+--------------------------------------------------------------------
    diff |             -5.0825    30.79207               -80.42797    70.26297
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -0.1651
Ho: diff = 0                                     degrees of freedom =        6

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4372         Pr(|T| > |t|) = 0.8743          Pr(T > t) = 0.5628

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BIOGRAPHY

I am Md. Sayem Abdullah, son of Abu Ahmed & Momtaz Begum. I passed my Secondary School Certificate examination from Bandarban Govt. High School in 2014 & Higher Secondary Certificate examination from Bandarban Cantonment Public School & College in 2016. I enrolled for Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. In future, I would like to work as a veterinary practitioner.