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

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A production report submitted as per approved style and content

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Declaration

This report is a depiction of our original research work. Whenever contributions of others are incorporated, every effort has been undertaken to indicate this clearly, with reference to the literature, and acknowledgement of collaborative dissertations and discussions. This work is conducted under the guidance of Md. Asraf Ali Biswas, Professor,Department of Animal Science of Nutrition, Chattogram Veterinary and Animal Sciences University

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Chapter 01: Abstract

Methane emissions from livestock, particularly ruminants like goats, contribute significantly to greenhouse gas emissions and global warming. This report investigates the influence of beneficial organism on methane production on goats, utilizing two distinct groups, a control group and a treatment group where beneficial organism were introduced. The Chattogram Veterinary and Animal Sciences University's goat farm served as the study's site. We used Portable gas detector which is specifically designed to measure methane (CH_4) concentrations in the eructed air breath of goats and provides concentration values in parts per million (ppm). Statistical analysis revealed a significant decrease in methane emissions, suggesting that this organism has a positive impact on the rumen's microbial population and fermentation processes. Result showed a high decrease (122.09%) methane emission at 3 hours after morning feeding in the treatment group compared to the control group. Average methane emission from control group was 7.22 l/kg B.W. and from treatment group was 5.57 l/kg B.W.

Keyword: Methane, Goat, Beneficial organism, Gas detector

Chapter 02: Introduction

Methane (CH_4) is a colorless and odorless gas that arises from the microbial fermentation of grain within the gastrointestinal tracts of ruminant animals. These methane-producing microorganisms, known as methanogens, generate methane through the utilization of carbon dioxide and hydrogen, both of which are byproducts of microbial fermentation. Within the spectrum of greenhouse gases, which encompass carbon dioxide (CO_2), methane (CH_4), and nitrogen dioxide (N_2), methane stands out as a potent contributor. In fact, methane is approximately 20% more effective at trapping heat in the atmosphere than carbon dioxide. On a global scale, around 570 million tons of carbon dioxide equivalent (CO_2e) are annually released as methane, with ruminant enteric fermentation and manure management accounting for 31% and 6% of these emissions, respectively. Consequently, it is imperative for national greenhouse gas inventories to accurately assess ruminant emissions in accordance with established international guidelines, as underscored by Hoque et al. (2017).

The main source of anthropogenic CH_4 emissions is enteric methane (CH_4), which domestic animals' gastrointestinal tracts produce (Knapp et al., 2014). According to Hellwing et al. (2016), methane emissions cause a loss in gross energy intake of 3 to 14%.

According to research by Gerber et al. (2013), ruminants are accountable for a substantial 15% of the world's total methane emissions. The global warming potential of CH_4 is 28 times greater than that of carbon dioxide (Geneva: IPCC; 2014, p. 15).Consequently, there has been a rapid expansion of research dedicated to mitigating methane emissions over the past decade. Although respiration chambers have traditionally served as the primary method for quantifying CH_4 emissions from ruminants, they have limitations such as cost, capacity constraints, and inapplicability to grazing animals. In response to these challenges, innovative methane measurement techniques have emerged within the last decade, enabling measurements on farms and the simultaneous screening of numerous animals, as highlighted by Hammond et al.(2016).

It is worth noting that the rate of CH_4 emissions can fluctuate significantly throughout the day, varying by more than sixfold based on factors like diet, feed intake, and feeding schedules, as demonstrated by studies conducted by Müller et al. (1980) and Jonker et al. (2014). we collected cost-effective CH_4 gas using the face mask method, as described in Oss et al. (2016) and Silva et al. (2019). But most cost-effective method is spot sampling-based ABC method (Biswas et al.2022) which is used in this study. It is necessary to reduce the methane emission from animal. For this reason, we are trying to reduce methane emission from goat by using the beneficial organism in this context.

In light of these considerations, this study aims to quantify methane concentrations produced by goats, employing an economical methane detection system-ABC method. Additionally, we investigate the potential impact of beneficial microorganisms on methane production in this context.

Chapter 03: Methodology

The study was conducted at the CVASU Animal Farm, in the Chattogram Veterinary and Animal Sciences University (CVASU) in Khushi, Chattogram, Bangladesh.

Four goats were selected for the experiment and divided into two groups: a control group and a treatment group, each consisting of two animals. The mature body weights of the control group were recorded as C_1(13.72 kg) and C_2(17.16 kg), respectively, while the treatment group consisted of C_3(17.5 kg) and C_4(20.36 kg) goats, which were identified accordingly.

Before measuring methane emissions from the treatment group, a feed mixture containing beneficial organism was provided to the animals the previous day. In contrast, the control group did not receive beneficial organism.Methane emissions were measured both before and after feeding on the same day from both group.

First Day: A new low cost spot sampling based ABC method (Biswas et al,2022) was used for methane emission estimation. Each goat received a daily ration of 400 grams of concentrate feed. Beneficial organism was mixed with the feed for the treatment group, while the control group received feed without beneficial organisms. We applied the face mask for a duration of ten minutes and during this period, we recorded the concentration of methane emission. Methane emissions were recorded before feeding and 1.5 hours, 3 hours, 6 hours, and 12 hours after feeding.

Second Day: On the second day, methane concentrations were measured from the control and treatment groups using the same procedure as the first day, both before and after feeding using ABC method.

Feeding of individual animal

Chapter 04: Results and Discussion

The data from the first and second days of the experiment reveal important insights into the impact of the treatment on methane emissions from goats at different time intervals after feeding

Table 1: Emission of methane concentration from goat at day 1

	Before Feeding (PPM)	After Feeding (PPM)			
Control group		1.5h	3h	6h	12h
C_1	1010	1520	2240	1905	1430
C_2	1420	1815	2330	1970	1467
Treatment group					
T_1	1557	1745	1930	1737	1564
T_2	1620	1887	2020	1820	1667

Table 2: Increased methane emission(%) after different time of morning feeding (Day 1)

		Mean±SD		T Value	P Value
		Control group	Treatment Group		
After Feeding	1.5h	39.16±16.04	14.28±3.12	2.15	0.16
	3h	92.93±40.80	24.33±0.52	2.38	0.14
	6h	63.67±35.27	11.96±0.56	2.07	0.17
	12h	22.45±27.06	1.99±2.17	1.07	0.40

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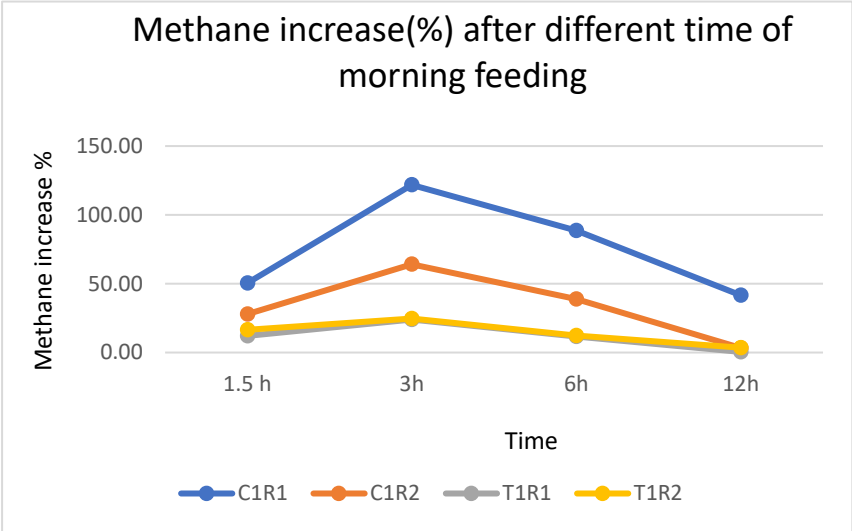
First Day:

- On the first day, there is a noticeable reduction in methane emissions in the treatment group compared to the control group across all time intervals.
- After 1.5 hours, the treatment group showed a slightly increased mean methane concentration (14.28%) compared to the control group (39.16%). The difference is not statistically significant.
- After 3 hours, the treatment group maintained a lower increased mean methane concentration (24.33%) compared to the control group (92.93%), again with no statistical significance at the 5% level.
- Similar trends were observed at 6 hours and 12 hours, not consider the result statistically significant at the 5 % significance level,although the differences were less pronounced at the 12-hour mark.



C1R1, C1R2 = Control Group

T1R1, T1R2 =Treatment Group

Figure 1: Methane increase (%) from before feeding on different time of morning feeding (Day 1)

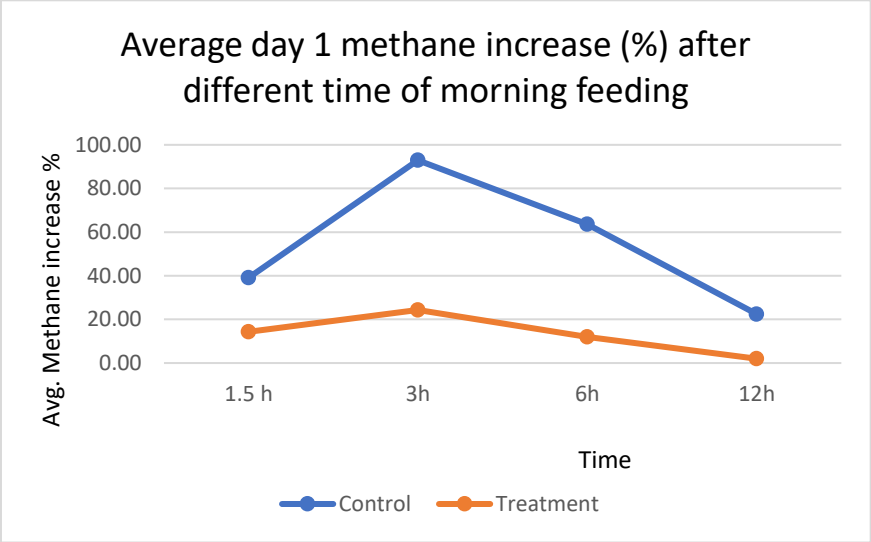


Figure 2: Average methane increase (%) from before feeding on different time of morning feeding (Day 1)

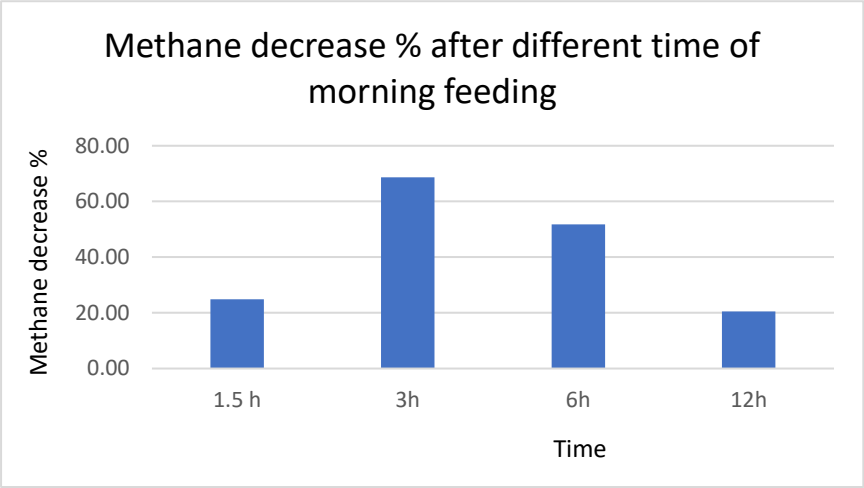


Figure 3: Methane decrease (%) from before feeding on different time of morning feeding (Day 1)

Table 3: Emission of methane concentration from goat at 2nd day

	Before Feeding (PPM)	After Feeding (PPM)			
Control group		1.5h	3h	6h	12h
C_1	840	1590	2113	1854	1311
C_2	765	1262	1789	1520	1017
Treatment group					
T_1	1010	1203	1217	1120	1093
T_2	1090	1257	1316	1240	1097

Table 4: Increased methane emission (%) after different time of morning feeding (Day 2)

		Mean±SD		T Value	P Value
		Control group	Treatment Group		
After Feeding	1.5h	77.13±17.20	17.22±2.68	4.87	0.04
	3h	142.71±12.51	20.62±0.16	13.80	0.005
	6h	109.7±15.57	12.33±2.03	8.77	0.01
	12h	44.51±16.36	4.43±5.36	3.29	0.08

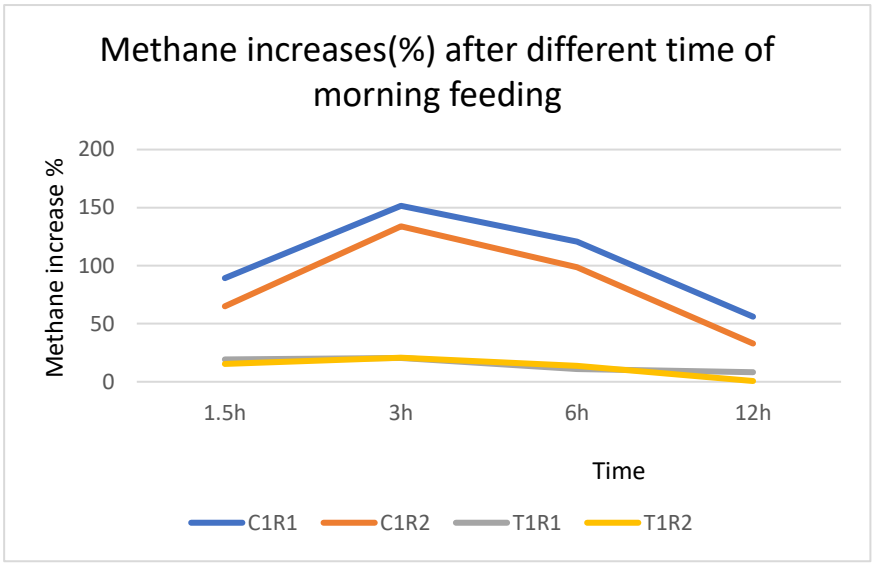
Second Day:

- On the second day, the treatment group consistently exhibited significantly lower methane concentrations compared to the control group across all time intervals.
- After 1.5 hours, the treatment group had a increased mean methane concentration of (17.22%), while the control group had a higher concentration of (77.13%). The difference is statistically significant.
- After 3 hours, the treatment group continued to display a lower increased mean methane concentration (20.62 %) compared to the control group (142.71%). The difference in increased mean methane concentrations between the treatment and control groups after 3 hours is considered statistically significant.

- After 6 hours in treatment group, exhibited low increased mean methane concentrations compared to the control group and considered statistically significant.

On the second day, the treatment group demonstrated statistically significant reductions from increased methane percentage emissions at the 1.5 hour and 3 hour and 6 hours marks.These results indicate that the treatment had a substantial and statistically significant impact on reducing methane emissions from goats at specific time intervals.

However, it's essential to note that the significance of the results varied depending on the time point and the day of the experiment. Additionally, at some time points, although the treatment group had lower methane concentrations, the differences were not statistically significant. This suggests that the effectiveness of the treatment may vary depending on the specific conditions and time elapsed since feeding.



C1R1, C1R2 = Control Group

T1R1, T1R2 = Treatment Group

Figure 4: Methane increase(%) from before feeding on after different time of morning feeding (Day 2)

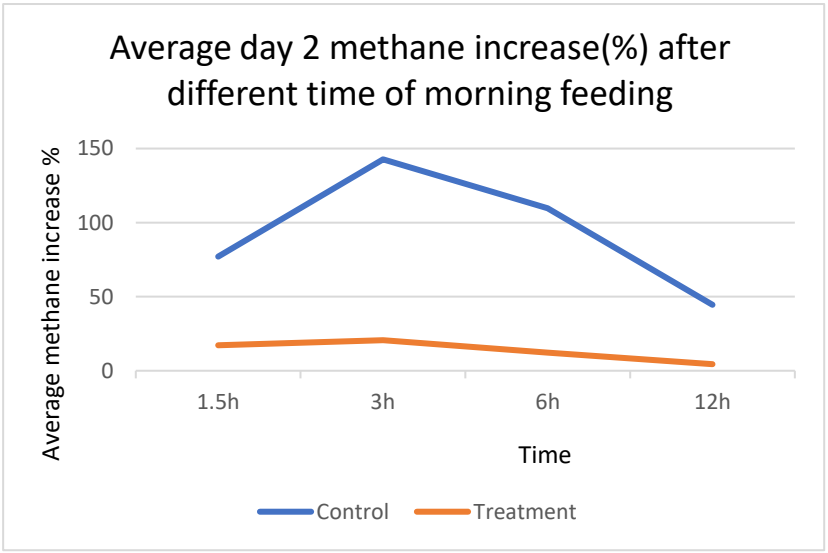


Figure 5: Average methane increase (%) from before feeding on after different time of morning feeding (Day 2)

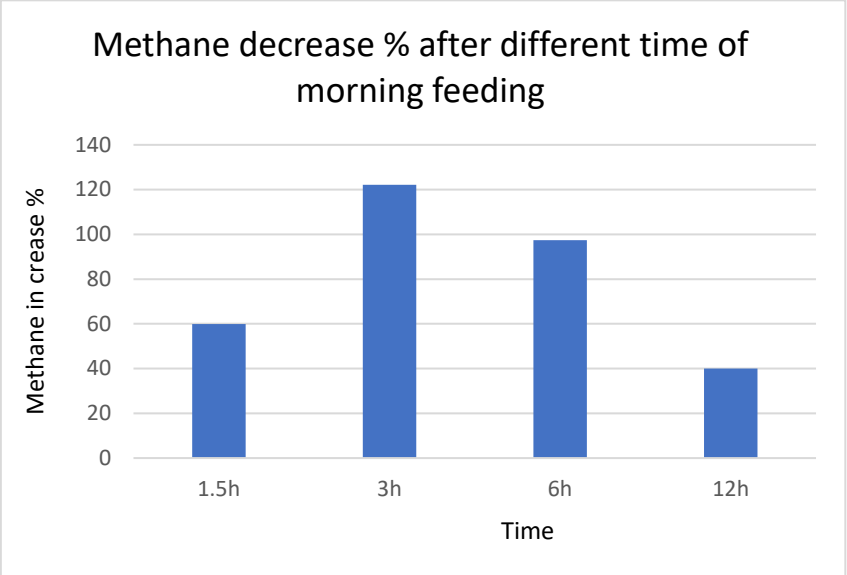


Figure 6: Methane decrease (%) from before feeding on after different time of morning feeding (Day 2)

We measured methane concentrations in parts per million (ppm), and we applied the following formula for conversion:

PPM (in L) = (PPM value / 1,000,000) * Volume of the solution or medium (in liters)

Table 5: Methane emission from goat on the basis of body weight

Day		Methane emission (l/kg B.W.)
Day 1		
	Control group	8.03
	Treatment group	6.70
Day 2	Control group	6.71
	Treatment group	4.44

Average methane emission from control group was 7.37 l/kg B.W. and from treatment group was 5.57 l/kg B.W

When we look at the average emissions over the two days, we see that, on average, the control group emitted more methane than the treatment group. This suggests that the treatment, which involves the use of beneficial organisms, might have a positive effect in reducing methane emissions compared to the control group.

Chapter 05: Conclusions

In conclusion, the treatment showed significant promise in reducing methane emissions from goats, particularly at specific time intervals. The results highlight the potential for implementing this treatment as part of a strategy to mitigate greenhouse gas emissions in livestock, but further research is needed to understand the variations in effectiveness and optimize its application in practical livestock management.

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Literature review

Ruminant rumen methane production is predominantly facilitated by anaerobic methanogenic bacteria, enabling ruminants to extract energy from low-quality forage such as grass and high-cellulose fodder. It's worth noting that pseudo-ruminants like pigs and horses also produce methane, albeit in smaller quantities. Additionally, the synthesis of methane through fermentation in insects, particularly termites, has gained global recognition.

Approximately 18% of anthropogenic greenhouse gas emissions are attributed to the global livestock industry. In Bangladesh, Das et al. (2020) estimated that livestock contribute a substantial 30,124 gigagrams of greenhouse gases through enteric fermentation.

Pioneering work by Ritzman and Benedict (1938) provided insights into methane emissions from various species, including cows, sheep, goats, horses, and even elephants. Their research indicated that methane emissions typically account for 4–7% of ruminant gross energy intake at maintenance feeding levels. Building upon this Baxter and Clapperton (1965) further explored the relationship between methane emissions, feeding quantity, and digestibility. Subsequently, this relationship has served as a fundamental basis for precise emission calculations. In underdeveloped countries where livestock feed largely comprises low-quality straw and fodder, Indian research identified methane production at 9% in cattle fed at maintenance levels.

Another method for quantifying methane in exhaled air involves handheld laser methane detectors (LMD). These portable devices utilize infrared-absorption spectroscopy to measure methane levels between the animal's nose or mouth and the LMD. Data collection occurs in brief 2-4 minutes intervals, generating a series of peaks synchronized with the animal's breathing cycle. The analysis focuses on peaks displaying an increase in methane content resulting from exhalation or eructation.

Because it can be used right out of the box (taking into account the calibration recommendations) and is portable from animal to animal and farm to farm, the idea of using an off-the-shelf hand-held methane gas detector to measure CH_4 concentrations from animals is very appealing and innovative.

Strategies that increase animal production efficiency, decrease the amount of feed fermented per unit of product, or alter the rumen's fermentation rhythm can all effectively mitigate CH_4 emissions. This comprehensive review discusses the significance of methane emissions in ruminants, including goats. It covers various measurement techniques and highlights the importance of mitigating methane production for environmental and animal productivity reasons.

Chemical substances with a particular inhibitory impact on rumen archaea have been the focus of research in this field. Chloroform, 2-bromo-ethane sulfonate, bromochloromethane (BCM), and cyclodextrin were among the substances that performed best when tested in vivo. When used in sheep, goats, and cattle, these CH_4 inhibitors decreased CH_4 synthesis by up to 50% in vivo (Immig et al., 1996; Lila et al., 2004; Mitsumori et al., 2011; Knight et al., 2011).

It is worth noting that the rate of CH_4 emissions can fluctuate significantly throughout the day, varying by more than sixfold based on factors like diet, feed intake, and feeding schedules, as demonstrated by studies conducted by Müller et al. (1980) and Jonker et al. (2014).

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The Author

August 31

Appendix

Table: Methane increase percentage at day 1

Methane increase (%)			1.5 h	3h	6h	12h
	Control animal	C_1	50.50	121.78	88.61	41.58
		C_2	27.82	64.08	38.73	3.31
	Treatment animal	T_1	12.07	23.96	11.56	0.45
		T_2	16.48	24.69	12.35	3.52

Table: Methane increase percentage at day 2

Methane increase%			1.5h	3h	6h	12h
	Control animal	C_1	89.29	151.55	120.71	56.07
		C_2	64.97	133.86	98.69	32.94
	Treatment animal	T_1	19.11	20.5	10.89	8.22
		T_2	15.32	20.73	13.76	0.64

Table: Average methane increase % in a day 1

Average methane increase (%) day 1	1.5 h	3h	6h	12h
Control Group	39.16	92.93	63.67	22.45
Treatment Group	14.28	24.32	11.95	1.98

Table: Average methane increase % in a day 2

Average methane increases(%) Day 2	1.5h	3h	6h	12h
Control	77.13	142.71	109.7	44.51
Treatment	17.22	20.62	12.33	4.43

Table: Methane decrease % in treatment group from control group at Day 1

	1.5 h	3h	6h	12h
Methane decrease(%)	24.88	68.61	51.72	20.46

Table: Methane decrease % in treatment group from control group at Day 2

	1.5h	3h	6h	12h
Methane decrease(%)	59.91	122.09	97.37	40.08

Figure: Statistical analysis in Stata13 of methane increase (%) and t value and p value of goat at day 1(both treatment and control group)

After 1.5hours:

```
. ttest B, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	39.16	11.34	16.03718	-104.9284	183.2484
1	2	14.275	2.205	3.118341	-13.74218	42.29218
combined	4	26.7175	8.593498	17.187	-.6308457	54.06585
diff		24.885	11.55239		-24.82091	74.59091

```
    diff = mean(0) - mean(1)                                      t =   2.1541
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9180         Pr(|T| > |t|) = 0.1641          Pr(T > t) = 0.0820
```

After 3hours:

```
. ttest h, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	92.93	28.85	40.80006	-273.644	459.504
1	2	24.325	.365	.516188	19.68724	28.96276
combined	4	58.6275	23.04264	46.08527	-14.70446	131.9595
diff		68.605	28.85231		-55.53647	192.7465

```
    diff = mean(0) - mean(1)                                      t =   2.3778
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9297         Pr(|T| > |t|) = 0.1405          Pr(T > t) = 0.0703
```

After 6hours:

```
. ttest D, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	63.67	24.94	35.27049	-253.2227	380.5627
1	2	11.955	.395	.5586144	6.936049	16.97395
combined	4	37.8125	18.07106	36.14213	-19.69769	95.32269
diff		51.715	24.94313		-55.60662	159.0366

```
    diff = mean(0) - mean(1)                                      t =   2.0733
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9131         Pr(|T| > |t|) = 0.1739          Pr(T > t) = 0.0869
```

After 12hours:

```
. ttest E, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	22.445	19.135	27.06098	-220.6882	265.5782
1	2	1.985	1.535	2.170818	-17.51902	21.48902
combined	4	12.215	9.813343	19.62669	-19.01544	43.44544
diff		20.46	19.19647		-62.13574	103.0557

```
    diff = mean(0) - mean(1)                                      t =   1.0658
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8009         Pr(|T| > |t|) = 0.3981          Pr(T > t) = 0.1991
```

Figure: Statistical analysis in Stata13 of methane increase (%) and t value and p value of goat at day 2(both treatment and control group)

After 1.5 hours:

```
. ttest B, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	39.16	11.34	16.03718	-104.9284	183.2484
1	2	14.275	2.205	3.118341	-13.74218	42.29218
combined	4	26.7175	8.593498	17.187	-.6308457	54.06585
diff		24.885	11.55239		-24.82091	74.59091

```
    diff = mean(0) - mean(1)                                      t =   2.1541
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9180         Pr(|T| > |t|) = 0.1641          Pr(T > t) = 0.0820
```

After 3hours:

```
. ttest h, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	142.705	8.845	12.50872	30.31862	255.0914
1	2	20.615	.115	.1626346	19.15379	22.07621
combined	4	81.66	35.42888	70.85775	-31.09049	194.4105
diff		122.09	8.845748		84.02982	160.1502

```
    diff = mean(0) - mean(1)                                      t =  13.8021
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9974         Pr(|T| > |t|) = 0.0052          Pr(T > t) = 0.0026
```

After 6 hours:

```
. ttest D, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	109.7	11.01	15.57049	-30.19531	249.5953
1	2	12.325	1.435	2.029396	-5.908404	30.5584
combined	4	61.0125	28.47287	56.94573	-29.60087	151.6259
diff		97.375	11.10312		49.60212	145.1479

```
    diff = mean(0) - mean(1)                                      t =   8.7701
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9936         Pr(|T| > |t|) = 0.0128          Pr(T > t) = 0.0064
```

After 12 hours:

```
. ttest E, by(Treated)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	2	44.505	11.565	16.35538	-102.4423	191.4523
1	2	4.43	3.79	5.359869	-43.72652	52.58652
combined	4	24.4675	12.59045	25.18089	-15.60092	64.53592
diff		40.075	12.17018		-12.28907	92.43907

```
    diff = mean(0) - mean(1)                                      t =   3.2929
Ho: diff = 0                     degrees of freedom =        2

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9594         Pr(|T| > |t|) = 0.0812          Pr(T > t) = 0.0406
```

Biography

Muhammad Newas Hossain son of Mohammad Nurul Amin, was born on January 25. He successfully completed his secondary school certificate examination at Govt. Muslim High School, Chattogram, in 2014. He subsequently accomplished his Higher Secondary Certificate examination at Hazera Taju Degree College, Chattogram. Currently, he is engaged in a year-long internship program as a part of his pursuit of a Doctor of Veterinary Medicine (DVM) degree at Chattogram Veterinary and Animal Sciences University.