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Selection for reduced methane emission in Norwegian Red cows

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Abstract

Over the past years, methane production from dairy cattle gained a significant attraction because of its contribution to climate change and loss of dietary energy from the animals. This the first study to compare methane emission between high milk yielding (HL) and mastitis resistance (FL) lines of Norwegian Red cows, in level and development through lactation. The study also focussed on estimating the effects of genetic group, parity, lactation stage and calving season on methane emission. The data consisted of 5,012 observations of methane production measured with Greenfeed system from a herd of 47 Norwegian Red cows at the Center for Animal Experiments (SHF), NMBU. The data were analyzed using R software and least square means were calculated for methane production. The average methane production was 441g/d ranging from 115g/d to 497g/d. Overall, the average methane production was higher for HL in comparison to FL.

The analysis of methane production showed that the effect of genetic group on methane emission was not significant (p-value: 0.802993), whereas lactation number, calving month-year, lactation week and group * lactation number all had a significant effect (p-value <0.005). The difference in methane production between two groups was significant, indicating that indirect selection for high milk yield or mastitis resistance affects the methane production in these cows. The pairwise differences showed non-significant differences between the lactation weeks except for week 11. Cows in lactation 2 produced the highest methane (461g/d), and those in lactation 1 emitted the least amount (400g/d). There was a significant difference in methane emissions between lactation 1 and 2, and lactation 1 and 3 (p-value < 0.0001), however the difference was insignificant between lactation 2 and 3 (p-value: 0.4146). Cows calving in autumn months (September to November) produced more methane than those in winter months (December and January). There was a statistically significant difference between autumn months and winter months (p-value <0.05), however within months difference was insignificant (p-value >0.05). It is concluded that selection for mastitis resistance or milk yield provides basis for indirect selection for methane reduction, however, further experiments are required for building a solid conclusion.

Table of Contents

ABSTRACT	ii
1. INTRODUCTION	1
2. LITERATURE REVIEW	2
2.1 METHANOGENESIS IN CATTLE	2
2.2 IMPORTANCE OF REDUCING METHANE EMISSIONS IN CATTLE	3
2.3 BREEDING FOR ENTERIC METHANE EMISSION FROM COWS	3
2.3.1 <i>Direct Selection</i>	4
2.3.2 <i>Indirect Selection</i>	5
2.4 CORRELATION OF METHANE PRODUCTION WITH MILK PRODUCTION	5
2.5 CORRELATION OF METHANE PRODUCTION WITH MASTITIS RESISTANCE	6
2.6 VARIATION IN METHANE PRODUCTION ACROSS LACTATION	6
2.7 MEASUREMENT OF METHANE WITH GREENFEED SYSTEM	7
.....	8
3. MATERIAL AND METHOD	8
3.1 DATA	8
3.2 DESCRIPTIVE STATISTICS	9
3.2.1 VARIABLES	9
3.3 STATISTICAL MODEL	12
4. RESULTS	12
4.1 DESCRIPTIVE STATISTICS	12
4.2 FACTORS AFFECTING METHANE PRODUCTION	15
5. DISCUSSION	19
6. CONCLUSION	22
7. REFERENCES	23

1. Introduction

With the increase in global warming, greenhouse gas (GHG) emissions attracted significant attention as a potent source of climate change. Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide are major greenhouse gases responsible for the highest contribution to the greenhouse effect through the absorption of infrared radiation (Lashof & Ahuja, 1990). After CO₂, CH₄ is considered the second major culprit of climate change. CH₄ has a global warming index of 28 times than CO₂ (Myhre et al., 2014) and can remain in the atmosphere for ten years (Stocker, 2014). Dairy cattle contribute to 2.7% of anthropogenic greenhouse gas emissions, whereas 2.2% share comes solely from milk production (FAO, 2010). Dairy cow releases 250 to 400-gram average methane per day through breathing and burping (Bannink et al., 2011; Sejian et al., 2011) and 18.9 million metric tons (Tg) of methane per year globally (McMichael et al., 2007). In Norway, CH₄ from agriculture accounts for 9 % of total emissions from agriculture, out of which 60% comes directly from ruminants (Harstad & Volden, 2009).

The European Union committed to reduce its GHG emissions by 30 to 40% than 1990 levels by 2030 (UNFCCC, 2015). Norway has an international commitment to lower methane and Hydrofluorocarbons up to 40% by 2030, regulated under the Kyoto Protocol. The Norwegian government proposed reducing its GHG emissions by 5 Mt CO₂ equivalents by 2030 and emerging as a low-emission country by 2050 (Ministry of Climate and Environment, 2017). Norwegian Red cattle breeding company Geno is working on the project "Climate-smart agriculture" to monitor methane emissions in selected herds of Norwegian Red cows. In this project, 15 Green Feed machines are installed in university and commercial farms to collect individual methane production data from approximately 1,000 Norwegian Red dairy cows (Wright, 2021).

At the Livestock Production Research Centre (SHF), NMBU, two GreenFeed machines are used for measuring methane emissions from Norwegian Red cows. This herd of Norwegian Red cows has been under selection for milk production and mastitis resistance since 1978 (Heringstad et al., 2007). The first selection experiment (1978–1989) was carried out for high and low milk yield. The high yield line was further subjected to selection against protein yield and mastitis resistance. The second selection experiment resulted in large genetic differences in milk protein yield and mastitis resistance between the two groups (Heringstad et al., 2003).

Therefore, the herd is partitioned into two groups: high milk yield line (HL) and low mastitis frequency line (FL).

The objective of the current study is to investigate whether there are differences in levels of methane emissions between two genetic groups of cows. Since methane data is not sufficient to estimate genetic parameters, differences between the two genetic groups would be an indication of indirect selection responses after selection for milk yield or mastitis resistance. Additionally, the study is focused on estimation of average methane emission for the Norwegian Red breed, and effects of lactation stage and parity.

2. Literature Review

2.1 Methanogenesis in cattle

Methane is generated from cattle in two main ways (Figure 1): almost 90 percent CH₄ in the rumen and hindgut during microbial fermentation of feed and exhaled through belching and burping (Lan & Yang, 2019; Lines-Kelly, 2014), whereas the remaining 10% methane is released from the animal manure (McAuliffe et al., 2016) . The enteric CH₄ in ruminants is produced in the rumen through a metabolic pathway (Figure 2). The dietary polysaccharides are hydrolyzed into simple sugars, which undergo fermentation to produce volatile fatty acids (Lascano & Cárdenas, 2010). These volatile fatty acids (VFAs) provide a base for production of energy and protein in the ruminants. During fermentation, CO₂ and H₂ are generated. Methanogenic bacteria known as Archaea produce CH₄ under anaerobic conditions by utilizing CO₂ and H₂ (Bhatta & Enishi, 2007). Methanogens also play a critical role in removing H₂, thus facilitate the metabolism by ruminal microorganisms (Hook et al., 2010).

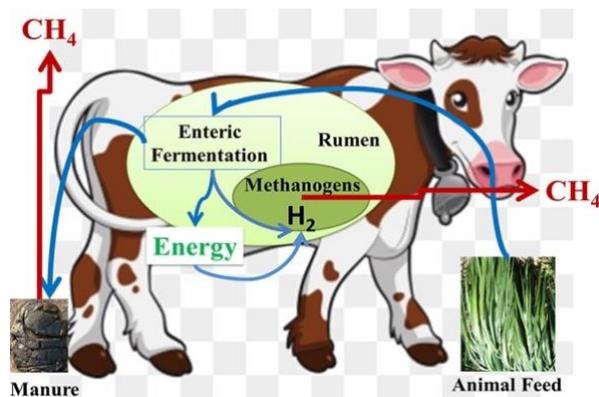


Figure 1. Methane emission in cattle (Kumari et al., 2020)

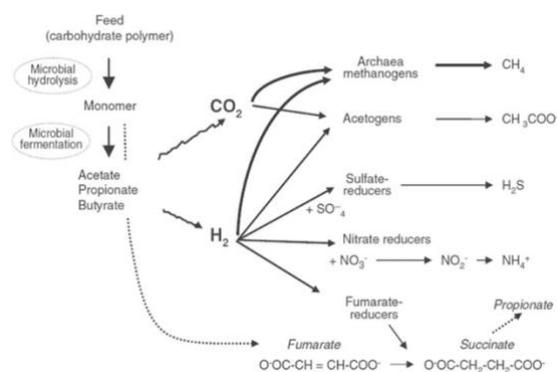


Figure 2. Schematic microbial fermentation and methanogenesis in the rumen (Morgavi et al., 2010)

2.2 Importance of reducing methane emissions in cattle

In addition to the environmental footprint, methane gas is also responsible for the energy loss from ruminants, affecting animals' feed efficiency and overall productivity. According to (Liu et al., 2017), energy loss for ruminants due to methane is considered to be between 2 % to 12 % of gross energy intake. CH₄ is being synthesized from H₂ and CO₂, both of which otherwise can be used for formation of VFAs (Ungerfeld, 2018). If the loss of dietary energy in the form of CH₄ is reduced, it can increase the feed efficiency and ultimately lead to an increase in milk production. (Esmail, 2020) reported that reducing 20% of methane can synthesize 0.6 kg/d of milk in lactating cattle. A considerable reduction in methane emissions would not only benefit the cattle in terms of environment-friendliness, but also feed efficiency and increased production (de Haas et al., 2017)

2.3 Breeding for enteric methane emission from cows

Although dietary interventions proved to be successful in reducing enteric methane emissions from ruminants (Beauchemin et al., 2009), their impacts are short-term and reversible (Cottle et al., 2011). Researches showed that methane production in ruminants has a genetic component and a heritability of 0.25 (Zetouni et al., 2018), thus forming the foundation for genomic selection. Genetic selection can be carried out through either direct selection of low emitter cows (Cavanagh et al., 2008) or by indirect selection for traits that positively affect reduced emissions (Amer et al., 2018). Genomic selection requires many generations to have a successful outcome (Lassen & Difford, 2020) and large number of cows in the reference population to increase the accuracy of genomic breeding values of bulls (Hayes et al., 2009).

However, the benefits overpass the limitations because the changes through genetic selection are permanent and cumulative (Wall et al., 2010).

(De Haas et al., 2011) used genome-wide association studies (GWAS) to identify single nucleotide polymorphisms (SNPs) associated with methane production in Holstein, thus providing the evidence of genetic influence on methane production. (Pszczola et al., 2017) estimated heritability for CH₄ production ranging from 0.23 to 0.30 over one complete lactation in dairy cows. Since methane emission is partly under genetic control, several researches suggested to incorporate methane emission as an economic trait into the breeding goal. (González-Recio et al., 2020; Wall et al., 2010). (Bell et al., 2016) suggested to calculate the economic value of methane before its addition into the breeding program. However, routinely recording of methane at dairy farms, and genomic selection with a large female reference population are required for including this trait into breeding schemes (López-Paredes et al., 2020).

2.3.1 Direct Selection

The cattle display a huge variation in methane generation, and a part of this variation is caused by genetics, providing the basis for direct selection (Garnsworthy et al., 2012b). (Grainger et al., 2007) reported variation in methane levels between the dairy cows, despite providing the same feed and environmental conditions. The between-cow variation gives an opportunity to breed low-emitting cows with 10% to 20% methane reduction per generation (Waghorn & Woodward, 2006). Based on this additive genetic variance, cows emitting low levels of methane can be selected for future breeding.

The variation in the emission level can be attributed to various animal factors such as difference in retention time or passage rate of digesta, rumen volume, and microbial population between low and high emitters (Cabezas-Garcia et al., 2017). The genetics of the animals affect the feed efficiency and dry matter intake, leading to the between-animal difference (Arthur et al., 2001). However, measurement of methane directly from large number of animals is arduous at-present and may cause hindrance in direct selection on lower methane emissions. The advancement in the approaches for measuring methane, and selection strategies can facilitate large-scale recording on individual cows and inclusion of the trait into breeding planning (De Haas et al., 2011).

2.3.2 Indirect Selection

Various studies showed a considerable correlation between the methane production and other selection traits (Amer et al., 2018; López-Paredes et al., 2020; Zetouni et al., 2018). Selecting the cows indirectly for production traits, health traits, fitness traits, and fertility traits could help mitigate methane emissions (De Haas et al., 2011; Garnsworthy, 2004). A significant increase in net income, and reduced emissions per cow and per kilogram of milk solids was observed in Australian dairy herd by (Bell et al., 2013) when cows were selected for an increase in milk solids, health, fertility, and survival rate, and decrease in milk volume, live weight, and dry matter intake. Similar to methane production in dairy cows, a proportion of variation in these traits is also genetic, thus leading to indirect selection (Bell et al., 2016)

2.4 Correlation of Methane production with milk production

The general notion that high milk yielding cows produce more methane was supported by (Garnsworthy et al., 2012a), who derived a positive phenotypic correlation ($r = 0.71$) between methane emission rate and daily milk production. Nevertheless, (Bell et al., 2016) computed high genetic correlation of methane with fat and protein corrected milk, signifying that selection for increased milk production will reduce methane. (Bell et al., 2010) mentioned 1.1% reduction per year and 19% abatement per kilogram milk for selection on increased milk fat and protein content. Similarly, (Pryce & Bell, 2017) estimated 1% decline in total methane emissions from the Australian dairy industry per year because of decrease in number of cows and increase in milk production per cow.

An increase in milk yield per cow results in a decrease in emissions per liter of milk, particularly in those countries where greater improvement in milk production through breeding has been observed (Capper & Bauman, 2013). For instance, USA dairy systems in 2007 produced 43% of the total CH₄ per billion kg of milk reported in 1944 (Capper et al., 2009); whereas, milk production increased four times in the same period (Britt et al., 2018). Using 'economic allocation method', (Zehetmeier et al., 2012) calculated decrease in methane emissions from 1.06 kg CO₂eq to 0.89 kg CO₂eq, and increase in milk production from 6000 kg to 10,000 kg per cow per year. In Canadian dairy, estimation intensity was reduced by -0.001 kg CO₂eq per kg milk solids per cow annually (Richardson et al., 2021). (De Haas et al., 2011) reported that the heritability for predicted methane emission per milk production was 0.35, hence, selection can potentially decrease methane per unit milk production.

2.5 Correlation of Methane production with mastitis resistance

Mastitis, a bacterial infection of the mammary glands, is also associated with increased methane emissions in dairy cows (Gülzari et al., 2018; Mostert et al., 2019). Methane eructation of cows with clinical mastitis appeared to be elevated by 6.2% compared with cows without clinical mastitis in a simulation study by (Mostert et al., 2019). (Gülzari et al., 2018) showed that GHG emissions intensity can be reduced by 3.7% if the level of somatic cell count declines from 800,000 cells/mL to 50,000 cells/mL. The reduction in mastitis cases increases the feed efficiency and milk production, reduces the waste and requirement for more replacement heifers, thus indirectly reducing methane emission per unit product. Moreover, the economic value for enteric methane was -£1.55 per % incidence, similar to the mastitis traits (Bell et al., 2016).

Limited research has been found related to the genetic relationship between the udder health of the animal and the methane production. In a GWAS study by (Pszczola et al., 2018), QTLs associated with the udder health of the cow and QTLs indicating susceptibility to illness were also detected in the QTLs regions linked to the methane production. The genetic correlation between methane production and udder health was 0.06 and the heritability was 0.02 for udder health, calculated by (Zetouni et al., 2018). This low correlation could be biased because of only one observation per lactation for each animal (Zetouni et al., 2018). (Kandel et al., 2018) computed a positive correlation 0.22 between udder health and predicted methane emission. The positive correlation indicates that selection for mastitis resistance in dairy cows can abate methane to some extent.

2.6 Variation in methane production across lactation

The rate of methane emission in cows show a notable variation during different stages of lactation and from one lactation to the next (Lassen & Difford, 2020). Therefore, it is important to consider the methane emissions recorded at different points during an animal's productive life to understand the implications for selection based on methane emissions. (Vanlierde et al., 2015) suggested to take into consideration number and stage of lactation while calculating predicted methane emissions. In their experiment, the average coefficients were 444, 467, and 471 CH₄ g/d for cows in first, second, and third lactation, depicting a rising pattern.

Furthermore, methane production showed an increasing trend between 0 and 100 days in milk (DIM) and decreasing thereafter, following the lactation curve for milk production.

Methane emission is a longitudinal trait, hence, the changes in mean and genetic variance must be investigated over full lactation period (Pszczola et al., 2017). (Rischewski et al., 2017) reported a remarkable difference in mean daily CH₄ production (g/d) between the start, mid and end of the lactation, showing a continuous increase. Genetic variance for methane displayed an irregular increasing pattern with the highest level at the end of the lactation, whereas heritability increased to its maximum value upto 212 DIM and decreased slightly at the end of lactation (Pszczola et al., 2017).

2.7 Measurement of methane with GreenFeed system

The GreenFeed system (C-Lock Inc., Rapid City, South Dakota, USA) is a head-chamber system that is used for measuring short-term methane and carbon dioxide emissions from individual animals during each animal's visit to GreenFeed unit (Lind, 2020). The emission measurements using a GreenFeed unit are usually carried out over short periods (3-7 minutes) and are repeated several times a day, being highly dependent on each animal's voluntary visit to the unit (Lind, 2020). Major limitation of the GreenFeed system is the use of bait feed, which could represent up to 5% of the animal's dry matter intake during a measurement (Hristov et al., 2016). However, (Hammond et al., 2013) concluded that emission rates estimated by the GreenFeed are comparable to respiration chambers or the SF₆ technique.

The system is constructed around an automatic feeder and consist of an animal Radio-Frequency Identification system (RFID), a feed baiting system, an air handling and measurement system, a gas tracer system, electronics and communication system, and a data handling system (Figure 3). The animals voluntarily visit the unit upon being attracted to the palletized feed bait. The function of RFID reader is to read the RFID tag, identify the animal and deliver the correct amount of bait. The start and end time of visit is automatically recorded by infrared sensors. The system uses extractor fan and sensors, which direct air over the animal's head and sweep the emitted CH₄ and CO₂ into an air intake manifold (Hristov et al., 2016). The gases are measured by integrating measurements of airflow, gas concentration, and detection of head position of each animal (Huhtanen et al, 2015).

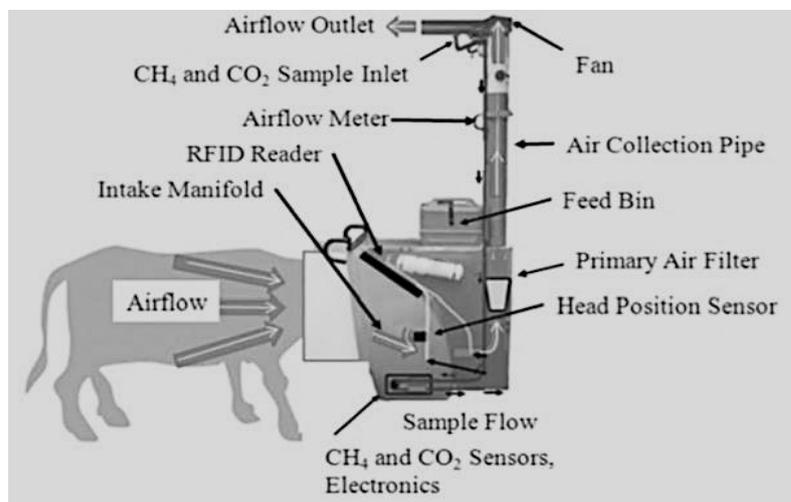


Figure 3. Layout of the GreenFeed system (C-Lock Inc., Rapid City, SD, USA).

Source: (Huhtanen et al., 2015)

3. Material and Method

3.1 Data

The data material was obtained from the herd of Norwegian Red cows at the Center for Animal Experiments (SHF), NMBU. The cows at SHF belong to two different genetic groups: high milk yield line (HL) and low mastitis frequency line (FL). Methane was measured from the cows by GreenFeed (C-Lock Inc., Rapid City, South Dakota, USA) from February 2020 to May 2020. The first dataset consisted of records of methane production, CO₂, date of measurement, start time and end time of measurement, and total time spent by the cow in the GreenFeed unit. The second dataset included information about the cows' birth date, calving date, calving age, lactation number, and the group to which each cow belonged. Both the datasets were merged, and the final dataset consisted of 59 cows with 5,622 observations.

In the data set, some observations did not have information for either calving date, lactation number, or calving age. These observations with missing information were removed so that those used in the calculations had all these variables recorded. Furthermore, only data from cows having at least ten observations and only days with at least ten observations for methane were included. After the restrictions, the data set consisted of 5,012 observations from 47 cows measured on 80 days in total. Lactation week for each cow was calculated and added to the final dataset. Moreover, observations from lactation number 3 and higher were merged into one group, named as lactation number 3.

The total number of cows and observations in each group and lactation are given in Table 1. Out of 47 cows, 18 cows belonged to group 1 (high milk yield line) with 2197 observations, and

29 cows belonged to group 2 (low mastitis frequency line) with 2815 observations. Group 1 consisted of 1127, 973, and 97 observations, whereas group 2 comprised of 1799, 480, and 536 observations in lactation number 1, 2, and 3, respectively. Of group 1, 7 cows belonged to lactation number 1, 9 cows to lactation number 2, and only 2 cows to lactation number 3. From group 2, the number of cows in lactation number 1, 2, and 3 were 16, 7, and 6 respectively.

Table 1. Number of cows (in the brackets) and observations per group and lactation number

	Number of cows	Number of (cows) observations per lactation			Total number of Observations
		Lactation 1	Lactation 2	Lactation 3	
Group 1	18	(7) 1127	(9) 973	(2) 97	2197
Group 2	29	(16) 1799	(7) 480	(6) 536	2815
Total	47	(23) 2926	(16) 1453	(8) 633	5012

3.2 Descriptive Statistics

Rstudio was used for data editing, descriptive statistics and statistical analyses (Verzani, 2011).

3.2.1 Variables

The variables in the dataset, along with their units and description, are mentioned in Table 2.

Table 2. Variables in the dataset along with their units and description

Variable	Unit	Description
CH ₄	grams/day	Methane production
Group	no.	Genetic group to which cow belonged (1 for HL & 2 for FL)
Date	y-m-d	Date of methane emission
Time	h-m-s	Total time spent by the cow in the Green Feed unit
Lactation	no.	Cow's lactation number (1,2,3)
Lactation Week	Week	Cow's number of weeks since calving
CalvingMonth-Year	y-m	Year and month of the last calving before the start of methane measurement

The total number of observations per cow, per day, and lactation week for both groups of cows were calculated and plotted in the figures. As shown in Figure 4, the total number of observations per cow ranges from 13 to 188.

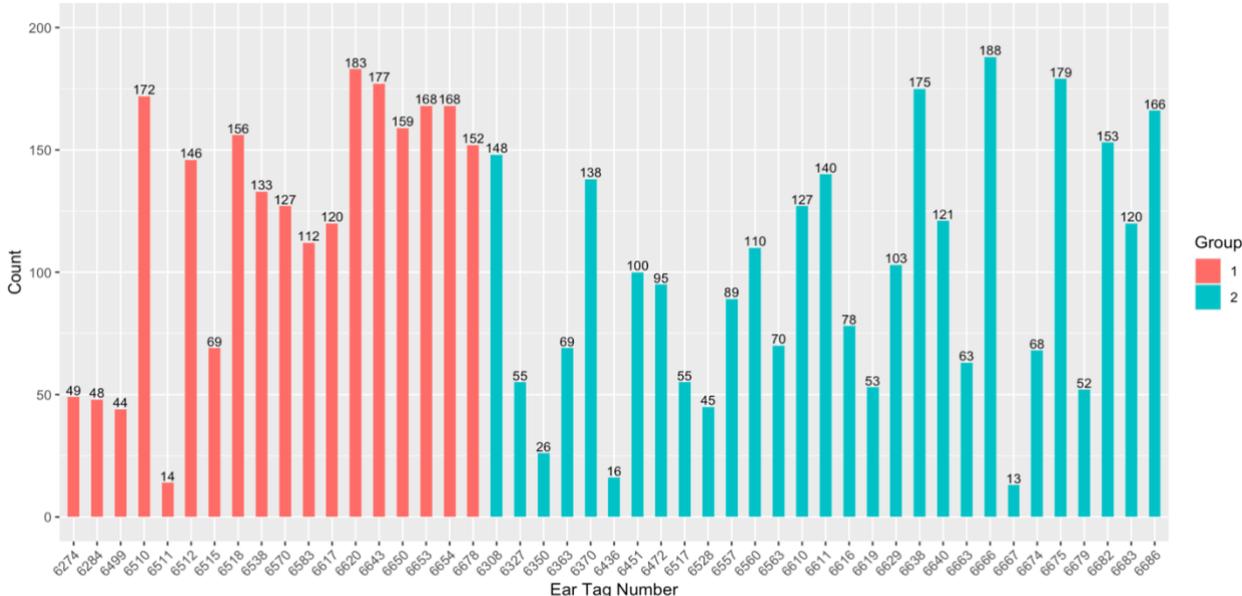


Figure 4. Number of observations per cow ranging from 13 to 188

Figure 5 displays a large variation in the number of observations per day throughout the sampling duration. Group 2 has a higher number of records in comparison to group 1.

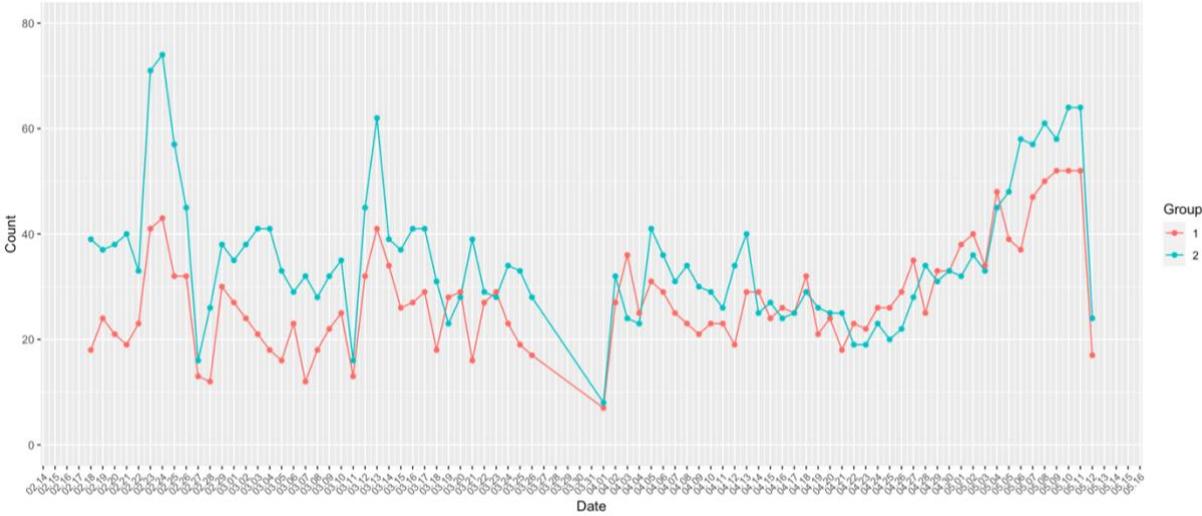


Figure 5. Number of observations per day.

Similarly, there is an unequal distribution of observations across the lactation weeks 8 to 33, ranging from a few to about 200 records (Figure 6). Overall, group 2 has a higher number of

records across the lactation period than group 1 ($N_2= 2815$, $N_1=2197$). Moreover, about 90% observations were from lactation week 13 to 32.

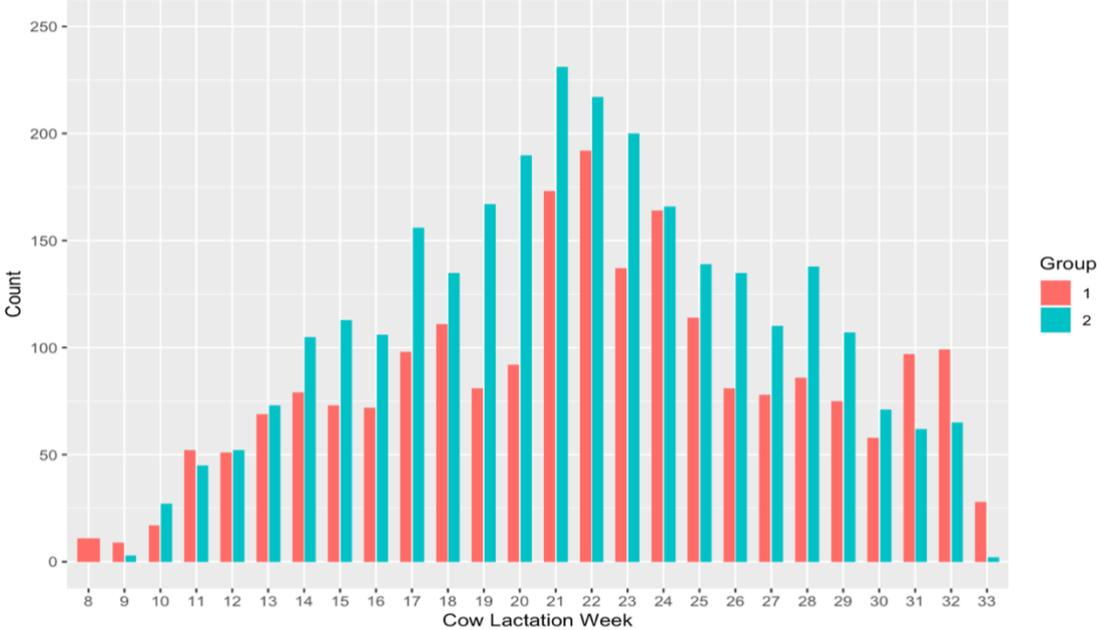


Figure 6. Number of observations per lactation week

Each cow was supposed to spend at least two minutes in the GreenFeed unit at every measurement. Some cows stayed for slightly more time in our experiment than the others, with the average time per cow ranging from two minutes and thirty seconds to three minutes and eighteen seconds. The average time spent by each cow in the unit during the whole sampling period is presented in Figure 7.

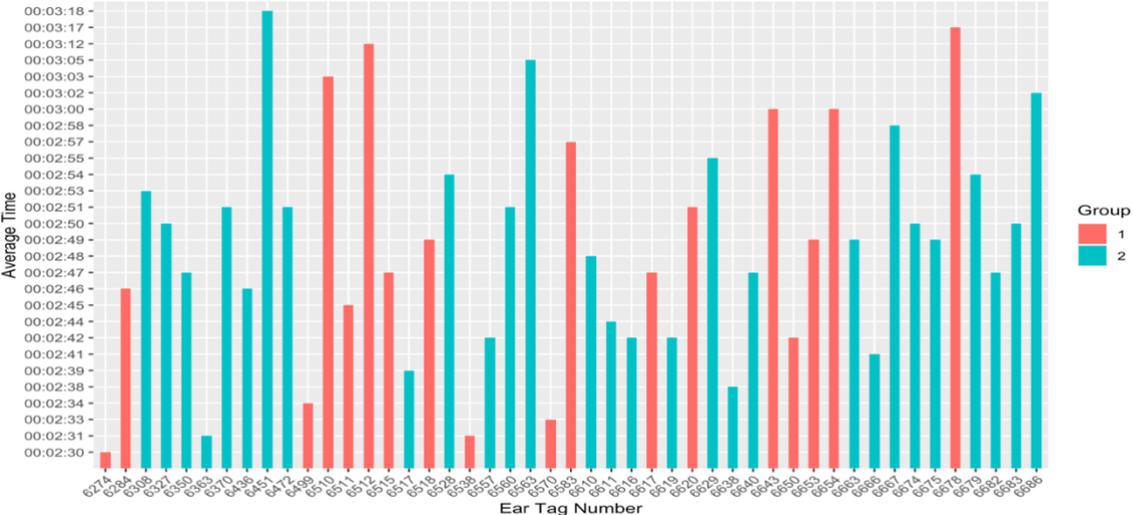


Figure 7. Average time spent by each cow in the Greenfeed unit

Furthermore, the means of methane per day, lactation week, and lactation number for both groups of cows were calculated and plotted in the figures using ‘ggplot’ function in R studio.

3.3 Statistical Model

The following model was used to analyze the differences in methane emission between the two genetic groups (HL and FL):

$$Y_{ijklmn} = \text{Group}_i + \text{Lactation}_j + \text{CalvingMonth-Year}_k + \text{LactationWeek}_l + \text{Group} * \text{Lactation}_m + e_{ijklmn}$$

where Y is records of methane production, Group_i has two levels (i= 1 and 2), Lactation_j is lactation number with three levels (j= 1, 2 and 3), CalvingMonth-Year_k is the month and year of calving, and LactationWeek_l has 26 levels (l= 8-33), Group * Lactation_m has six levels, and e_{ijklmn} is the error term which is randomly associated with nth observation.

The analysis was done using ‘gls’ function from ‘nlme’ package in R studio Version 1.2.5001 (2009-2019). gls stands for "generalized least squares " and calculates least-squares means for factors and their combinations in fitted linear models. Least-squares means (LS means) were calculated for group, lactation, lactationweek, and group*lactation. ANOVA was performed to test the statistical difference between the LS means. The level of significance was set at *P-value* < 0.05.

4. Results

4.1 Descriptive Statistics

The minimum, maximum, mean, and standard deviation of methane are given in Table 3.

Table 3. Minimum, maximum, mean, and standard deviation of methane

	Minimum	Maximum	Mean	Standard Deviation
CH ₄ (g/d)	115	1024	441.3	119.6

The density distribution of methane in the dataset regardless of the group is shown in the density plot (Figure 8A). The Figures 8B and 8C show density distribution of methane in the dataset for group 1 and group 2 respectively. The bell-shaped curve appears to be quite ideal, which illustrates the normal probability distribution of methane. The boxplot shows an insignificant number of outliers, depicting the normal distribution of methane in the data (Figure 9).

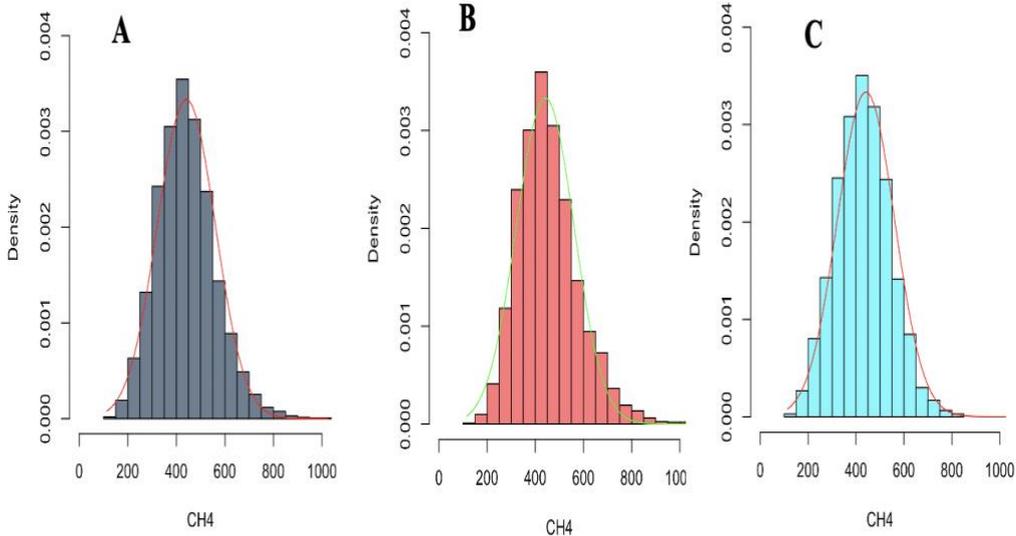


Figure 8. Density distribution of methane, A) Regardless of group, B) For group 1, C) For group 2

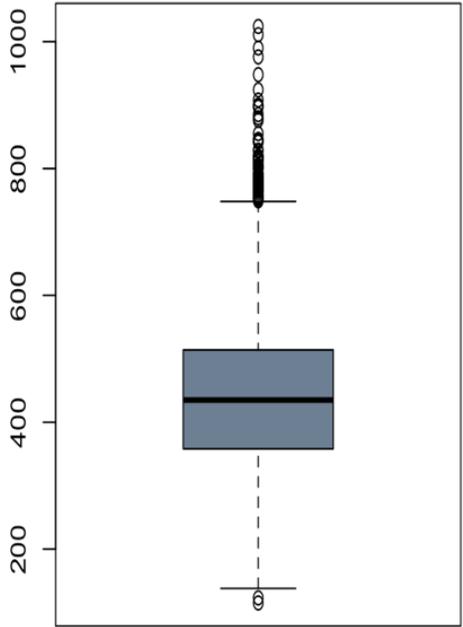


Figure 9. Boxplot of methane

The average CH₄ per day for both groups of cows across the sampling days is given in Figure 10. It can be clearly seen that Group 1 shows higher average values than Group 2.

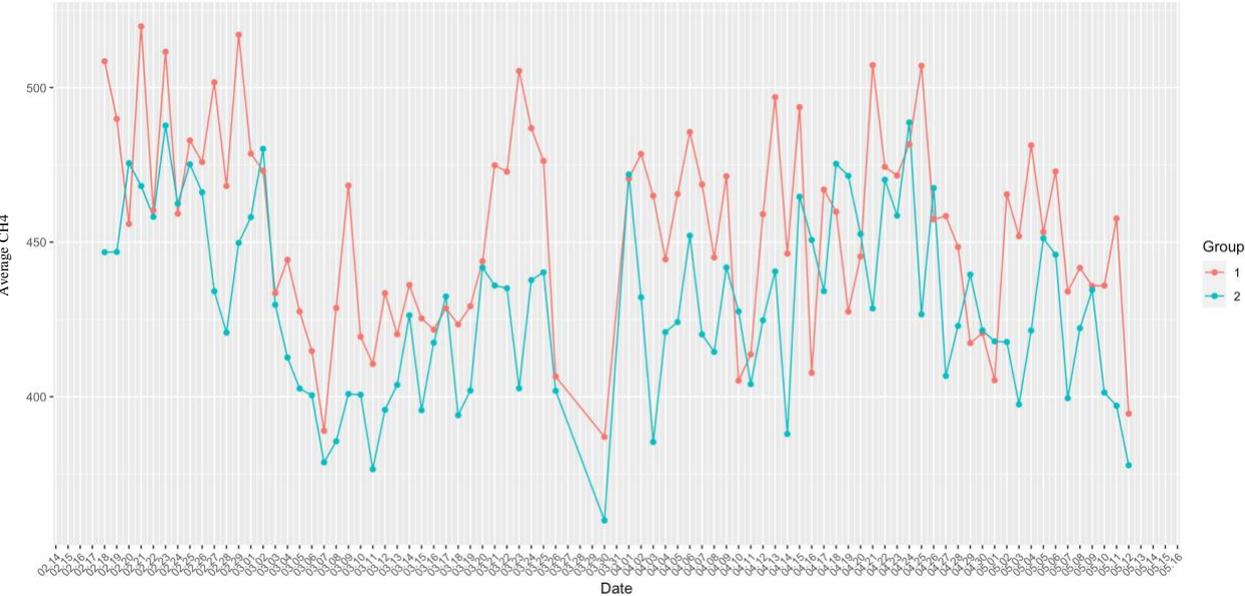


Figure 10. Average CH₄ per day for group 1 and group 2

The average methane production per lactation week is also higher for Group 1 in comparison to Group 2 (Figure 11), except at lactation week 9 where Group 2 has considerably greater value.



Figure 11. Average CH₄ per lactation week for group 1 and group 2

The average CH₄ per lactation number shows unexpected pattern for Group 1 (Figure 12). Group 2 presents an increase in average methane production from 1st lactation to 3rd lactation. Whereas, average methane is highest in 2nd lactation and lowest in 3rd lactation for group 2. This may be because of least number of observations for group 2 in lactation 3.

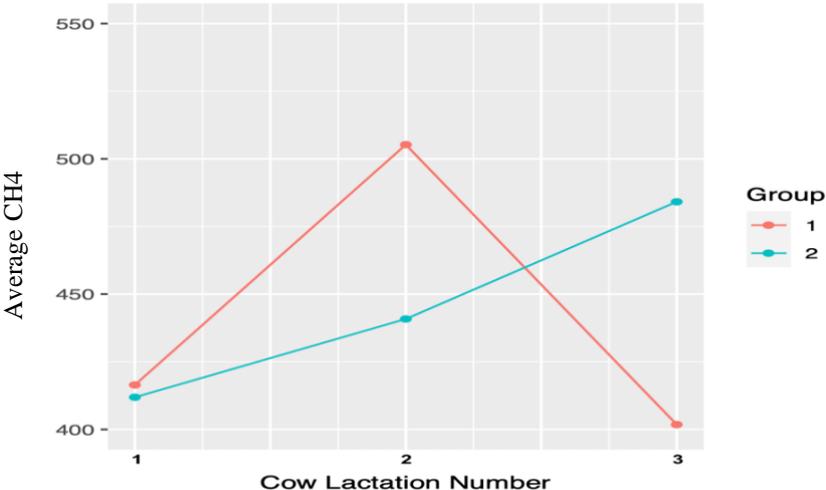


Figure 12. Average CH₄ per lactation number for group 1 and group 2

4.2 Factors Affecting Methane Production

The analysis of methane production showed that the effect of genetic group on methane emission was not significant (p-value: 0.802993), whereas lactation number, calving month-year, lactation week and group * lactation number all had a significant effect (p-value <0.005). LS means for genetic group are given in Table 4, the difference in methane production between two groups was significant (p-value: 0.0358). Table 5 presents the LS mean for methane production in each lactation. Regardless of the group, cows in lactation 2 produced the highest methane (461g/d), and those in lactation 1 emitted the least amount (400g/d). The LS mean was smaller for lactation 3 than lactation 2. There was a significant difference in methane emissions between lactation 1 and 2, and lactation 1 and 3 (p-value < 0.0001), however the difference was insignificant between lactation 2 and 3 (p-value: 0.4146).

Table 4. Least squares mean (LS-mean) for methane production (g/d) with standard errors for group 1 and 2

Group	Least squares means	Standard Error
1 (HL)	444	4.67
2 (FL)	430	5.70

Table 5. Least squares mean (LS-mean) for methane production (g/d) with standard errors for lactation number 1, 2 and 3

Lactation number	Least squares means	Standard Error
1	400	5.43
2	461	6.03
3	450	6.68

Table 6 shows least squares means for lactation week 8 to 33. Lactation week 28 had lowest methane production (416 g/d), while methane production in lactation week 11 was greatest (489 g/d). Means followed by a common letter are not significantly different according to the Tukey-test. The pairwise differences showed that the differences in LS means were significant between lactation weeks 11-13, 11-19, 11-20, 11-21, 11-22, 11-23, 11-24, 11-25, 11-26, 11-27, 11-28, 11-29, 11-30, and 12-28 (p-value <0.05).

Table 6. Least squares mean (LS-mean) for methane production (g/d) with standard errors for lactation weeks 8 to 33

Lactation Week	Least squares means	Standard Error
8	461 ^{abc}	34.18
9	456 ^{abc}	32.69
10	450 ^{abc}	17.59
11	489 ^c	12.37
12	470 ^{bc}	12.06
13	434 ^{ab}	9.93
14	446 ^{abc}	8.87
15	452 ^{abc}	8.72
16	440 ^{abc}	8.96
17	443 ^{abc}	7.75
18	442 ^{abc}	7.79
19	422 ^{ab}	7.88
20	422 ^{ab}	7.48
21	436 ^{ab}	6.51
22	436 ^{ab}	6.54
23	422 ^{ab}	7.08

24	421 ^{ab}	7.27
25	422 ^{ab}	8.12
26	421 ^{ab}	8.61
27	425 ^{ab}	9.20
28	416 ^a	8.65
29	430 ^{ab}	9.48
30	433 ^{abc}	10.82
31	420 ^{ab}	10.04
32	422 ^{ab}	10.06
33	430 ^{ab}	21.12

The LS means for methane production per week were plotted across the lactation weeks (Figure 13). The lactation curve for methane emission showed an irregular pattern from week 8 to 33. The difference of means is not statistically significant between those lactation weeks where standard error bars overlap each other.

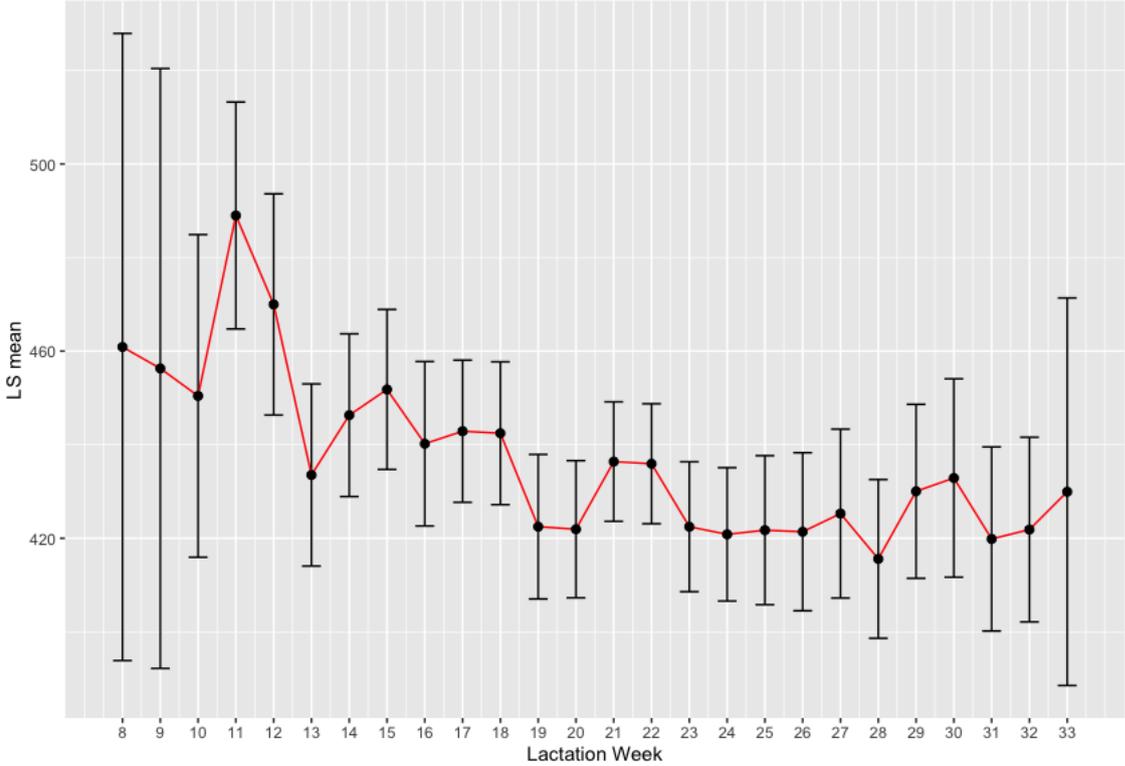


Figure 13. Lactation curve for methane emission from week 8 to 33

Table 7 shows the effect of the interaction between lactation number and group on methane production in NRF cows. LS mean for group 1 was highest in 2nd lactation and lowest in 1st lactation. Group 2 followed the same trend for 1st and 2nd lactation. In lactation 3, group 2 had the highest LS mean, whereas group 1 showed smaller LS mean in 3rd lactation than that of 2nd lactation. There was a significant difference between all combinations of groups and lactation number ($p < 0.0005$), except group 1 and 2 in lactation number 1.

Table 7. Least squares mean (LS-mean) for methane production (g/d) with standard errors for combination of group 1 and 2 with lactation number 1, 2 and 3

Group	Lactation number	Least squares means	Standard Error
1 (HL)	1	399	5.96
1 (HL)	2	500	6.52
1 (HL)	3	432	13.78
2 (FL)	1	400	5.75
2 (FL)	2	423	7.36
2 (FL)	3	468	7.14

Table 8 presents least square means and standard error for CalvingMonthYear. Cows calving in autumn months (September to November) produced more methane than those in winter months (December and January). There was a statistically significant difference between autumn months and winter months (p -value < 0.05), however within months difference was insignificant (p -value > 0.05).

Table 8. Least square means and standard error for CalvingMonthYear

CalvingMonthYear	Least square means	Standard Error
2019-09	419	5.16
2019-10	424	4.84
2019-11	427	4.92
2019-12	328	23.63
2020-01	397	34.19

5. Discussion

This is the first study to compare methane emissions between two genetic groups of Norwegian Red cows. There are large genetic differences in milk yield and mastitis resistance between both groups (HL and FL). The average breeding value for milk yield is 21 index points higher for HL than FL. Conversely, FL has an average breeding value for mastitis 18 index points higher than HL, and for somatic cell count, the average is 9 index points higher for FL than HL (Heringstad, 2018). In the current study, it was hypothesized that genetic selection either for milk yield or mastitis resistance have affected the methane production in Norwegian Red cows.

In the current study, a large variation was observed in the number of observations per cow (Figure 4) which can be understood by the fact that some cows had more access to Greenfeed unit during the sampling period. Hence, the average time spent by each cow in the unit also varied (Figure 7). Similarly, the fluctuation in number of observations per day could also be either due to visits of more cows on some days or technical limitations (Figure 5). Unequal number of visits per cow and different number of cows per lactation week led to the huge variation in the number of observations per lactation week (Figure 6).

The mean methane emission of 441g/day in the current experiment was less than the study done by (Kidane, Øverland, et al., 2018) who measured 482.3 g/d and 465.7 g/d in high feed-efficiency cows and low feed-efficiency cows respectively using sulphur hexafluoride (SF₆) tracer technique. However, in another experiment (Kidane, Prestløkken, et al., 2018) reported lower average values in a herd of Norwegian red cows, possibly because of short experimental duration. (Waghorn et al., 2016) also reported lower mean values of methane in dairy cows, measured with GreenFeed system. The range of 115–497g/day can be explained by the uneven number of measurements per day (Table 3).

Despite the higher number of cows and observations of group 2 (FL), group 1 (HL) showed higher average methane production per day and per lactation week (Figure 10, 11). The possible reason behind that is high milk producer animals release more methane, supported by (Garnsworthy et al., 2012a) who determined a positive linear relationship between methane emission index and daily average milk yield. Nevertheless, the large fluctuation can be attributed to the varying number of observations. The average methane production increased

from lactation number 1 to 2 for group 1, which is in accordance with the finding from (Kandel et al., 2017). Surprisingly, there was a sudden drop in methane emission from lactation number 2 to 3. The possible reason can be very small number of observations in 3rd lactation compared to 1st and 2nd (Table 1).

The effect of genetic group on methane emission was not significant, however, the difference in methane production between two groups was significant. It can be inferred that selection for milk yield or mastitis resistance does have an effect on methane production, which supports our hypothesis. Previous researches also proved that selection for milk yield indirectly reduces the methane emission in high producer animals (Bell et al., 2010; Bell et al., 2016; Pryce & Bell, 2017; Richardson et al., 2021; Yan et al., 2010). (Yan et al., 2010) reported decreased rate of methane emission after selection for milk productivity traits. selection for milk fat and protein alongwith providing low-forage diets to high-yielding cows in a farm offers 12% decline in methane emissions per kilogram (Bell et al., 2010).

No previous research proved the actual decrease in emission rate by selecting cows for mastitis resistance. However, (Kandel et al., 2018) calculated positive genetic correlation (0.22) and (Zetouni et al., 2018) computed very low correlation (0.02) between udder health and methane production. There is higher probability of uncertainty in the experiment by (Zetouni et al., 2018) because of very few number of observations. Regarding the current study, since both groups of the cows were a product of selection for higher milk production (Heringstad et al., 2007), the non-significant effect of the genetic group can be understood. Further research with large number of observations may provide more knowledge on the associations between methane emissions and milk production and health traits.

The results showed that the effect of lactation number on the methane emission was significant. Cows in 2nd parity had increased methane production than those in 1st lactation. (Vanlierde et al., 2015) concluded a rising pattern from 1st to 3rd lactation. Similarly, (Garnsworthy et al., 2012b) reported an increasing trend, however there was reduction in methane in 3rd lactation than 2nd in current experiment. This could be due to less number of observations in 3rd lactation compared to 2nd lactation (633 vs 1453).

Our results suggested an effect of lactation week on methane emission, as shown by other studies (De Haas et al., 2011; Garnsworthy et al., 2012b; Munger & Kreuzer, 2006). The methane curve showed an irregular pattern from lactation week 8 to 33 (Figure 13). It was hypothesized that the curve for methane emission should follow the normal lactation curve, i.e. an increase in methane emission until the peak milk yield and a slight decrease afterwards. Peak milk yield is obtained around week 7 and starts decreasing gradually (Ferris et al., 1985), whereas our experiment started at lactation week 8, the methane curve should ideally show a steady decreasing pattern. Our results were not exactly in accordance with the previous researches, where (Munger & Kreuzer, 2006) found constant methane emissions throughout the lactation period and (Hattan et al., 2001; Sutter & Beever, 2000) proposed an increase in methane emission as the lactation continues.

Although Lactation week showed a significant effect on methane production, many of the pairwise comparisons were not significantly different. The pairwise differences showed non-significant differences between the lactation weeks except for week 11, because of the high standard errors (Table 6). Unequal number of observations per lactation week could also be a reason in this case. (High, 2011) mentioned that presence of unequal sample sizes, unequal variances, random effects, or repeated measures in an analytic study can provide biased results, while analysing the differences in the means from the standard error bar graphs. Further study with more number of observations over the whole lactation is needed to rule out these experimental limitations.

Calving season showed a significant effect on methane production in the current study, similar to the findings by (Vanlierde et al., 2016). Cows calving in autumn months (September to November) produced more methane than those in winter months (December and January). There can be many factors behind this variation such as milk yield, feed intake, diet composition and herd management etc. For instance, (Vanlierde et al., 2016) proposed differences in daily methane emissions between cows calving in autumn and spring, because of seasonal variation in feed intake. Since the information about feed intake, milk yield and other herd characteristics is not available in our case, it is not possible to mention a solid reason and further investigation is needed to find out all possible reasons. There was not much research done on estimating the direct effect of calving season on methane emission, however, some researchers added it as fixed effect in the model (Munger & Kreuzer, 2006; Van Engelen et al., 2015).

The results showed that the effect of the interaction between lactation number and group on methane production in cows was significant. Group 1 (HL) showed decreased amount of methane in 3rd lactation compared to 2nd lactation, attributed to few observations. Group 2 (FL) followed the increasing trend from 1st to 3rd lactation. There was a significant difference between all combinations of group and lactation number, except group 1 and 2 in lactation number 1. The higher number of observations for group 2 in 1st lactation may explain this non-significant difference. No previous research was carried out for investigating effect of the interaction between parity number and genetic group on methane production.

To our understanding, the deviation in the results from the expectation is mainly because of small and uneven number of observations. This can be ruled out by increasing the number of cows and recordings per cows and distributing evenly among lactations and lactation weeks. This study did not include the feed intake and milk yield of cows, addition of these variables can elaborate the unambiguity. Moreover, measurement over full lactation can give a better understanding.

6. Conclusion

This study concluded that despite of the non-significant effect of genetic group, high milk yield group (HL) and mastitis resistance group (FL) showed significant differences in their methane emissions. High milk yielding group released higher quantity of methane than the mastitis resistant group. Thus, supporting the hypothesis that selection for mastitis resistance or milk yield affects the methane production in Norwegian Red cows. Moreover, Parity, lactation stage and calving season showed significant effects on methane production. The current study provides the initial understanding and further experiments are required for building a solid conclusion.

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