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RESEARCH ARTICLE

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Predicted methane production from Italian ryegrass pastures with contrasting chemical composition under sheep grazing in Northern Norway

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Abstract

Background: The Norwegian sheep farming system relies on forages, such as grass silage during winter and grazing cultivated leys and rangeland pastures during summer. Sheep and other ruminants produce enteric methane (CH_4), a greenhouse gas of interest, and there is a need for reliable data on gas emissions from sheep capturing both the indoor feeding period and the grazing season. This study utilized an in vitro gas technique (with standard cow rumen fluid) and modeling approach to estimate CH_4 production and fermentation patterns based on two different qualities of Italian ryegrass (*Lolium multiflorum*) pasture under sheep grazing.

Results: Herbage quality was examined for two 10-day periods, in July and August. Differences in chemical composition of the herbage during these periods had an impact on herbage digestibility and CH_4 production. Total gas production and CH_4 levels were significantly higher for lower quality herbage grazed in July than for higher quality herbage grazed in August ($p < 0.005$). Production of volatile fatty acids in the rumen remained constant between the two periods, but the higher acetate to propionate (A/P) ratio correlated with the higher CH_4 production.

Conclusion: These findings suggest that pasture quality is an important factor to consider when implementing grazing strategies to reduce enteric CH_4 production in sheep.

KEYWORDS
grazing, in vitro, *Lolium multiflorum*, modeling, Norway

INTRODUCTION

Grassland- or rangeland-based sheep farming systems worldwide are a more sustainable option than intensive livestock systems.[1] The availability of land suitable for human-edible crops is limited, but sheep can contribute to food supplies without triggering feed-food competition.[2] In Norway, less than 3.5% of total land area is used for agriculture and around 50% of the agricultural land consists of permanent grasslands and meadows.[3] The sheep industry plays an important role in Norwegian agriculture due to the capacity of sheep to convert biomass from grassland into high-quality protein for human consumption. Ruminants are known to produce enteric methane (CH_4) when digesting their feed, however, there is an urgent need for reliable data on greenhouse gas (GHG) emissions from different types of livestock in their local environment, to provide accurate estimates of how emissions are affected by ruminant diets, during indoor feeding or grazing.

Norway is the largest sheep meat producer in the Nordic region, with 1.16 million sheep slaughtered in 2022.[4] The Norwegian sheep farming system relies heavily on forages, in the form of grass silage during winter and herbage from grazing for 5–6 months on cultivated

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leys during spring and autumn and on rangeland pastures during summer. In Norwegian studies by Lind et al.[5] and Åby et al.[6] enteric CH_4 emissions from sheep fed indoors were measured. Pasture quality and forage intake by grazing animals affect the CH_4 emissions[7] but measurements from grazing sheep in Norway, or other Nordic countries, have not been carried out. There is a probability that the reported emissions from Norwegian sheep only are representative of the indoor season. Farmers need improved information about the annual GHG emissions by accounting for emissions from sheep on pasture to implement good mitigation strategies under arctic conditions with 24-h daylight during the summer.

In vitro studies can predict enteric CH_4 production in ruminants with reliable accuracy,[8,9] and can help to identify promising strategies for later in vivo implementation while reducing experimental costs. It is therefore necessary to carry out direct measurements on pastures to obtain more accurate predictions of overall annual CH_4 emissions from sheep.

This study utilized an in vitro and modeling approach to estimate CH_4 production, digestibility, and fermentation patterns based on two different qualities of Italian ryegrass (*Lolium multiflorum*) pasture grazed by sheep. The hypothesis was that a high-quality pasture (less structural carbohydrate, higher crude protein, better digestibility, more energy/g dry matter [DM]) decreases CH_4 production/kg DM consumed compared to a low-quality pasture.

MATERIALS AND METHODS

Herbage sampling and a sheep grazing trial were conducted at the Norwegian Institute of Bioeconomy Research (NIBIO) station Tjøtta (65°49′58.3″ N 12°25′46.5″) in Northern Norway during summer 2020. The study plan was reviewed and approved by the Norwegian Food Safety Authority (FOTS 23005). An in vitro experiment on herbage samples was performed at the Swedish Agricultural University (SLU) in Umeå (Sweden), with handling of animals carried out with the permission of Swedish Ethical Committee on Animal Research (represented by the Court of Appeal for Northern Norrland), which approved the experimental protocol (permit no. A 32-16) in line with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

Experimental design

Treatment, pastures, and animals

Herbage sampling was carried out in two 10-day recording periods, period 1 (July 16–25; low-quality pasture) and period 2 (August 11–20; high-quality pasture). In July, the pasture was unfertilized and cut 10 days prior to the first herbage sampling, with a short regrowth period. After period 1, 20 kg/ha of mineral fertilizer (12-4-18 NPK, Felleskjøpet Agri) was applied and the pasture left to grow for 30 days until herbage collection started in period 2.

The pasture was a second-year Italian ryegrass ley dominated by *L. multiflorum* (91%), with 6% smooth meadowgrass (*Poa pratensis*) and 3% other species such as white clover (*Trifolium repens*), meadow fescue (*Festuca pratensis*), and weeds. The experimental area of 2000 m^2 was divided into four parallel blocks of 500 m^2 each. Each block was further split into five (1–5) subplots of 100 (10 × 10 m) m^2 each, resulting in a total of 20 subplots. Herbage samples were collected from each subplot within blocks every second day.

After each sampling of a subplot, the available biomass in each of the four blocks was grazed by a flock of four nonpregnant Norwegian ewes. The 16 ewes were grouped based on initial live weight (66.5 ± 16.8 kg) and age (1.9 ± 1.6 year). Each group of ewes was allocated to the same block and over the 10-day period grazed all five subplots.

Prior to period 1, the ewes were adapted to the pasture for 1 month. Between period 1 and period 2, the ewes grazed a similar pasture nearby. The animals had access to shelter and water during the trial.

Pasture measurements and dry matter calculations

A quadrant (50 × 50 cm) was randomly placed at three positions within each subplot. Compressed herbage height was recorded using a modified plate meter[10] and then herbage mass was mechanically clipped (Bosch Iso cordless grass shears, Robert Bosch GmbH, Germany) at 3 cm above ground level. The herbage was weighed, dried (60°C for 72 h), and weighed to determine DM concentration (%). The dry herbage samples were milled (Retsch SM 2000, Retsch GmbH, Haan, Germany) to pass through a 1-mm screen and analyzed by near-infrared spectroscopy (NIRS; $n = 120$) at the NIBIO laboratory in Særheim.[11]

After herbage sampling, each group of sheep was allowed to graze the subplots for 2 days. Dry matter intake (DMI) by the ewes during that period was estimated using the herbage disappearance method (HDM), based on the difference between herbage mass before and after grazing.[12] One regression per subplot for herbage mass was performed, based on the compressed sward height and DM content before grazing by the sheep. After 2 days of grazing per subplot, post-grazing herbage height was recorded at 100 points and estimated average daily DMI was calculated as the difference between the grass mass before and after grazing, divided by the number of days (2) and number of animals (4) per subplot.

In vitro incubation

The dry herbage samples were pooled within subplots across blocks to one sample per 2-day grazing bout per period, resulting in five samples per period. In vitro incubations were performed to determine in vitro organic matter digestibility (IVOMD) and ruminal fluid digestible organic matter (VOS). Organic matter digestibility (OMD, %) and metabolizable energy (ME, MJ per kg OM) were calculated according to Lindgren.[13]

Two rumen-cannulated lactating Swedish Red cows fed ad libitum on a diet of 600 g/kg grass silage and 400 g/kg concentrate on a DM basis were used as donor animals of rumen inoculum for all incubations. The procedure, sampling, and measurements followed the

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protocol of Fant et al.[14] In short, rumen fluid from the cows was filtered and equal amounts from each cow were blended and buffered to a 1:4 ratio fluid: buffer by volume. Herbage DM substrate (1003 ± 1.8 mg) was weighed into serum bottles, flushed with CO_2, and 60 mL buffered rumen fluid (BRF) was added. All bottles were placed in a water bath and continuously agitated at 39°C for 48 h. The procedures were repeated for two runs with three replicates of each herbage sample in each run.

In vitro predicted methane production

Gas production was monitored using a Gas Production Recorder (GPR-2, Version 1.0 2015, Wageningen, UR), with readings every 12 min adjusted to normal air pressure (101.3 kPa). Measurement of CH_4 production in vitro was performed following Ramin and Huhtanen.[15] In short, gas samples (0.2 mL) were withdrawn from each bottle at 2, 4, 8, 24, and 48 h during the incubation period and analyzed using a Varian Star 3400 CX gas chromatograph. Mean blank gas production within each run was subtracted from the sample gas production. Predicted CH_4 production was calculated, and model simulations used a mean retention time of 50 h, corresponding to the maintenance level of feed intake in sheep as described by Ramin and Huhtanen.[15]

Volatile fatty acids

Liquid samples were extracted from the bottles after 48 h of incubation, with 0.6 mL liquid residue preserved at −20°C. Volatile fatty acid (VFA) concentration was determined by ultra-performance liquid chromatography (UPLC; Waters Acquity), following the method of Puhakka et al.[16] Total VFA concentration (mmol/L) was calculated by mean blank VFA concentration. Total VFA production (mmol) was derived by multiplying the concentration difference (sample – blank) by the sample volume (60 mL). Molar proportion of individual VFA was related to total VFA.

Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2021). Data on total gas and CH_4 production parameters, total VFA production, and molar proportions of VFA were subjected to analysis by a mixed effect model, with sampling period (July and August) as a fixed effect and bottle, run and days as random effects. The chemical composition of herbages was analyzed as the mean of the three samples from each subplot using the above model without run and bottles. Outliers, defined as 1.5 times the interquartile range (IQR = Q3 – Q1) greater than the third quartile (Q3), or 1.5 times the interquartile range less than the first quartile (Q1), were removed from the statistical analysis. Linear regressions between predicted CH_4 and chemical composition (mean per 2 days subplots) were performed to look for relationships. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

The estimated DMI of the sheep was higher ($p < 0.005$) in August (2.4 kg DM/animal/day) than in July (1.5 kg DM/animal/day). The chemical composition of herbage sampled in the two periods differed significantly for all parameters except potentially digestible NDF (pdNDF; $p = 0.07$) water-soluble carbohydrates (WSC; $p = 0.23$; Table 1). The herbages from August had significantly higher ME content ($p < 0.005$) than those from July. Percentage NIRS digestibility and in vitro OM digestibility were similar within period (67% and 68%, respectively, for July; 77% and 76%, respectively, for August). Crude protein (CP) concentration was significantly higher for August herbages (+32%) compared with July herbages. The sward height before and after grazing was greater in August (27.5 and 8.8 cm, respectively) than in July (13.5 and 5.8 cm, respectively), but the herbage mass offered to the animals was similar ($p > 0.05$; data not shown) due to the lower DM concentration of the grass in August (15%) compared to July (20%).

Estimated asymptotic in vitro gas production did not differ between the herbages from the two periods ($p = 0.208$; Table 2). Total in vitro gas production (+8%) and fermentation rate (+19%) in July were significantly higher ($p < 0.005$) than in August. Asymptotic CH_4 production (+10%) and predicted in vivo CH_4 production (+11%;

TABLE 1 Chemical composition (analyzed by NIRS) and in vitro incubation parameters of Italian ryegrass herbage sampled in Norway during two periods (July and August).

	Period		SEM		*p*-value
Parameter	July	August	July	August	Periods
NIRS herbage chemical composition ($n = 40$), g/kg DM					
DM	200	150	4.8	4.2	0.003
OM	932	919	1.1	1.1	<0.005
NDF	566	501	11.0	10.9	<0.005
iNDF	245	148	4.6	4.5	<0.005
pdNDF	353	329	9.2	9.1	0.069
ADF	313	280	6.0	5.9	<0.005
CP	102	150	4.8	4.8	<0.005
WSC	208	181	16.4	16.2	0.23
Digestibility, %	67	77	1.4	1.4	<0.005
In vitro chemical composition ($n = 10$)					
IVOMD, %	68	76	0.6	0.5	<0.005
ME, MJ/kg DM	10.5	11.9	0.10	0.08	<0.005

Abbreviations: ADF, acid-detergent fiber; CP, crude protein; DM, dry matter; iNDF, indigestible neutral detergent fiber; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber; OM, organic matter; pdNDF, potentially digestible NDF; SEM, standard error of the mean; WSC, water-soluble carbohydrate.

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TABLE 2 Effects of harvesting herbage in July or August on predicted in vivo total gas and methane (CH_4) production.

Parameter	Period		SEM		p-value
	July	August	July	August	Periods
Total gas, mL/g DM					
Asymptotic gas	277	269	4.4	4.2	0.208
Predicted in vivo gas	245	228	2.6	2.6	<0.005
Rate, L/h	0.074	0.061	0.0011	0.0010	<0.005
CH_4, mL/g DM					
Asymptotic CH_4	45.0	40.7	4.78	4.78	<0.005
Predicted in vivo CH_4	36.9	33.0	0.84	0.81	<0.005
Rate, L/h	0.052	0.056	1.08	1.09	<0.005

Abbreviations: CH_4, methane; DM, dry matter; SEM, standard error of the mean.

TABLE 3 Effects of harvesting herbage in July and August on in vitro total VFA production, VFA molar proportions, and VFA molar ratio at 48 h of incubation of buffered rumen fluid.

Parameter	Period		SEM		p-value
	July	August	July	August	Period
Total VFA production, mmol/g DM	4.26	4.36	0.364	0.363	0.458
VFA molar proportions, mmol/mol:					
Acetate	651	645	4.0	4.1	0.064
Propionate	246	249	8.8	8.8	0.055
Butyrate	104	106	5.8	5.8	0.273
A/P, mol/mol	2.65	2.59	0.019	0.019	0.029

Abbreviations: A, acetate; DM, dry matter; P, propionate; SEM, standard error of the mean; VFA, volatile fatty acid.

mL/g DM) were higher for the herbage from July compared with the August herbages ($p < 0.005$). The mean response of CH_4 production to increased iNDF concentration in the herbage was 0.22 g CH_4/kg of DM per 1 g/kg of DM in iNDF. The WSC:CP ratio in July (2.03) was twice that in August (1.02). The mean response in CH_4 production to CP was −0.52 g CH_4/kg of DM per 1 g/kg of DM in CP, with the highest adjusted $R^2 = 0.54$ of all regressions tested (data not shown).

Total VFA and molar proportion of butyrate were similar ($p = 0.458$ and 0.273, respectively) between periods (Table 3). There was a trend for July herbage for higher acetate molar proportion in the BRF ($p = 0.066$) and a lower propionate molar proportion ($p = 0.055$) compared with August herbage. A significant difference in A/P ratio ($p = 0.029$) was identified, with August having a lower ratio in BRF than July.

DISCUSSION

Performance of in vivo studies is costly and labor demanding compared to the use of in vitro studies to screen the effect of different diets on CH_4 production. Fant and Ramin[9] found a high correlation between CH_4 production between predicted in vivo and observed in vivo study. However, as pointed out by Yáñez-Ruiz et al.[17] testing diets in vitro do not guarantee a similar result when tested in vivo. The present study was the first to use an in vitro approach to estimate CH_4 production and fermentation patterns under grazing sheep in Nordic conditions. The results contribute to develop best mitigation practices under arctic conditions with 24-h daylight during the grazing season.

Herbage

Herbages harvested in the two periods differed for all chemical and nutritional parameters except concentrations of WSC. Therefore, the agronomic management regime applied (i.e., no application of chemical fertilizer and 10 days of regrowth in July vs. 20 kg/ha chemical fertilizer and 30 days of regrowth in August) successfully differentiated herbage quality between the two periods. The change of agronomic regime, increased herbage digestibility and CP concentration, maintained WSC concentration and decreased the concentrations of structural carbohydrates (NDF, iNDF, and ADF) in August compared with July. The difference in chemical composition between the two periods was greater for the ratio of nonstructural carbohydrate to iNDF. These differences might have been amplified by dry weather in the weeks preceding the July sampling.

Herbage ME content can be used to assess herbage quality.[18] The higher ME content of the August herbage (11.9 MJ/kg DM)

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compared to that from July (10.5 MJ/kg DM) indicated an overall better herbage quality offered in August. Herbage quality in the two periods was comparable to that of some forages described in the Nordic feeding table (Norfor),[19] with August herbage corresponding to high-digestibility meadow (Norfor code: 006-0502) and July herbage to low-digestibility meadow (Norfor code: 006-0504). Due to the management regime applied, the results differed from those reported by Rivero et al.[20] who found that a longer regrowth period increased the concentration of WSC and decreased the concentrations of CP and NDF.

Methane

In a study by Åby et al.[6] they recorded a DMI of 1.73 kg DM/animal/day for Norwegian White sheep while Lind et al. (pers com) found an average of 2.14 kg DM/animal/day. We did not establish a regression for the post-herbage mass, which likely resulted in overestimation of DMI due to higher stem bulk density and lower leaf bulk density, and thus DM density, in the lower half of the herbage. This effect might have been greater for August due to higher post-herbage height after grazing.

According to Rinne et al.[21] increased herbage digestibility enhances rumen fermentation and thus increases CH_4 production in ruminants. However, higher digestibility is associated with higher feed intake and passage rate, lower fiber content and as a result, a lower CH_4 production per kg DMI.[22] In the present study, the pasture in August had higher digestibility than in July but a lower digestible NDF content, which may explain the lower CH_4 production estimated in August. The higher CP content in August herbage due to application of nitrogen fertilizer may also play a role. It is suggested by Jentsch et al.[23] that fermentation of CP produces less CH_4 than fermentation of carbohydrates. Lower CH_4 production can therefore be attributable to replacement of carbohydrates by CP in the diet.[24] This trend was confirmed by the negative slope of the regression between CP on CH_4 (−0.52 g CH_4/kg of DM per 1 g/kg of DM in CP). It was even more apparent on comparing the WSC:CP ratio in the herbages, which was twice as great in July as in August. In vitro gas and CH_4 emissions found by Sun et al.[25] for incubated ryegrass with DM, NDF, and ADF concentrations comparable to ours, were lower than those found in this study (35–36 mL CH_4/g DM and 40–41 mL CH_4/g DM, respectively). Their CH_4/total gas ratio was comparable (14.6%–14.9%) to that in the present study (14.8%–15.4%). The mean values obtained for seven different perennial ryegrass species incubated by Purcell et al.[26] with lower content of NDF, higher CP, and similar WSC concentrations as in the present study, were in a similar range (33.9–35.1 mL CH_4/g DM). The literature shows that our results are comparable when using the in vitro approach for predicted CH_4 production from ryegrass.

On converting our results expressed as mL CH_4/g DM into g CH_4/kg DM, we obtained values of 26.6 and 23.6 g CH_4/kg DM for July and August, respectively. Åby et al.[6] found average CH_4 emissions of 16.1–25.2 g CH_4/kg DM for two Norwegian sheep breeds and two silage qualities (early and late). On using the National Research Institute for Agriculture, Food and the Environment (INRAE) formula[27] instead of our DMI estimate to calculate daily CH_4, we obtained values of 1.6 and 1.7 kg DMI for July and August herbage, respectively. Daily CH_4 production by our ewes was then 41.6 and 40.1 g/animal and day in the two periods. These results are comparable to findings by Åby et al.[6] of 40.2 g CH_4/sheep and day at daily DMI of 1.73 kg DM.

Additionally, the predicted in vitro CH_4 production in our experiment is similar to those measured in vivo on sheep fed ryegrass in New Zealand.[25,28] Warner et al.[29] found that increased digestibility reduced CH_4 production per unit of digestible DM. A study cited by Hristov et al.[30] found that changes in chemical composition of feed accounted for 20% of the variation in CH_4 emissions from sheep fed fresh ryegrass of different compositions, while feed intake accounted for 80% of the variation when using the respiration chamber technique.

These findings align with our own, suggesting that variations in CH_4 emissions were relatively little influenced (11%) by the chemical composition of the ryegrass herbages, which aligns with da Cunha et al.[31] who found that the sward structure (sward height and herbage mass) is more important for explaining the CH_4 emissions than the chemical content of a pasture.

Volatile fatty acids

VFAs were extracted after 48 h of incubation. Hetta et al.[32] found an increase of acetate, propionate, and butyrate over time in in vitro recordings (7 measures over 96 h) but the A/P ratio remained unchanged in the BRF.

Several studies have found that decreasing forage digestibility and increasing fiber content influence total rumen VFA and molar proportions of VFA, with a greater acetate and lower propionate proportion.[26,33–35] Total VFA in BRFs in the present study was not influenced, but a tendency of acetate and propionate molar proportions was found, resulting in a lower A/P ratio in the August herbages. Lower A/P ratio means that a greater number of VFAs act as a net sink for hydrogen, reducing their capacity to form CH_4. Rivero et al.[20] found similar results in experiments on autumn and spring standard ryegrass cultivar pasture with a A/P ratio of 3.08 and 2.67, respectively, but the CH_4 output after 24 h of incubation (33.4 and 34.1 mL/g DM) was slightly lower than in our study. Purcell et al.[26] highlighted the lack of differences in rumen in vitro fermentation and the relative proportions of the major VFAs in BRF when the differences in composition (WSC and NDF) are small. We were able to accentuate these trends through agronomic management, which ensured that differences in chemical composition between harvesting periods were significant.

In this experiment, we used adult ewes. However, under the Norwegian sheep farming system, ewes and their lambs are gathered from the mountain summer pastures during August, and lambs not ready

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for slaughter (<40 kg live weight) are separated from their mothers and finished on Italian ryegrass. The pasture in August may cause higher feed intake of the animals resulting in higher absolute CH_4 emissions. However, the weight gain of lambs on these pastures is similar to that of lambs fed a grass silage and concentrate diet[36] and the CH_4 yield is likely to be lower with increasing pasture quality. Using pasture for fattening as a mitigating option must be considered regarding application of fertilizer and use of fuel against use of a diet consisting of grain-based concentrate and grass silage. The protein sources in the concentrate are in most cases imported protein, and the production of grass silage also needs fertilizer and fuel for machinery. Calculation of the emission intensity, g CH_4/kg meat, is out of the scope of this experiment but is important to include in future research. To recommend farm practices for more sustainable production, not only GHG emissions from livestock but also the environment, yield, quality, and profitability of the entire system must be considered.

CONCLUSIONS

Observed differences in chemical composition of ryegrass herbage during the season led to differences in in vitro CH_4 production in sheep grazing. Herbages from July and August differed in qualities, leading to differences in predicted total gas and CH_4 production. Although the VFA production remained constant during the rumen in vitro incubations, the molar proportions of individual VFA and A/P ratio showed differences that could explain the observed differences in predicted CH_4 production. The overall conclusion is that high-quality herbage may reduce CH_4 intensity in grazing sheep, so improving pasture quality is a tool to mitigate CH_4 emissions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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