Effects of three short-term pasture allocation methods on milk production, methane emission and grazing behaviour by dairy cows

A. Kidane[a,c], E. Prestløkken[a], K. Zaralis[b], and H. Steinshamn[c]*

[a] Norwegian University of Life Sciences, Faculty of Biosciences, Department of Animal and Aquacultural Sciences, Arboretveien 6, 1432 Aas, Norway.

[b] Technological Educational Institution of Western Macedonia, School of Agricultural Technology, Food Technology and Nutrition, Florina, 53100, Greece

[c] ~~NIBIO,~~ Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food Production and Society, Department of Grassland and Livestock, Gunnars veg 6, 6630 Tingvoll, Norway.

*Corresponding author: Alemayehu.sagaye@nmbu.no, NMBU, Department of Animal and Aquacultural Sciences, Arboretveien 6, 1432 Aas, Norway

Abstract

Two short-term grazing experiments were conducted with NRF cows. In Exp 1, 24 cows were randomly assigned to one of the following three pasture allocation methods (**PAM**): weekly pasture allowance (**7RG**), grazing 1/7 of 7RG each day (**1SG**), or grazed as 1SG but had access to grazed part of the paddock within one week (**1FG**). In Exp 2, 7RG was shortened to 5 days (**5RG**). We hypothesized that PAM will affect sward quality, quantity, intake and production differently over a week. Pasture chemical composition changed with advancing grazing days but were not different between treatments. Pasture intake, milk yield, and methane emission were not affected by PAM. In Exp 1, 7RG cows spent less time on grazing, whereas in Exp 2, 1FG cows spent longer on grazing compared to others. Patterns observed in sward quality, and behavioural and physiological adaptations of cows to short-term changes in nutrient supply may explain the observed effects.

Keywords: dairy cow; milk yield; grazing behaviour; methane; pasture

Introduction

Grazed pasture is considered as a low-cost source of nutrients for cows (Wright 2005; Finneran *et al.* 2012). However, in dairy livestock production there is often a requirement for either supplementation with concentrates or implementation of better grazing systems to sustain high yields of the grazing cows. The former comes with an extra cost against the current competing demands for cereal grains and protein ingredients in animal diets, whereas intensive grazing management may require extra resources (Vallentine 2000). Therefore, looking for pasture allocation methods (**PAM**) that could result in an optimal dry matter intake (DMI) with optimal quality to support animal's intrinsic capacity for milk production is vital for a profitable dairy farming.

Previous works comparing different grazing management systems or level of pasture allowances under different conditions resulted in differences on grazing behaviour, DM use efficiency, milk yield in dairy cows, and weight gain and methane (**CH_4**) emission with steers (Virkajärvi *et al.* 2002; DeRamus *et al.* 2003; Abrahamse *et al.* 2008). Such differences could be due to changes in the attributes of the grazed diet (e.g. proportions of morphological fractions, their chemical composition and physical architecture of the grazed sward) on DMI and its quality (Bryant *et al.* 1961; Chacon & Stobbs 1976). For example, in a grazed horizon, from top to bottom, there is a reduction in dietary crude protein with concomitant increment in neutral detergent fiber (Abrahamse *et al.* 2008; Bryant *et al.* 1961) affecting pasture intake and the quality of consumed pasture. With cows on pasture, enteric **CH_4** production is influenced by grazed diet and substrate availability to the rumen microbes. As such, reduced rate of digestion and increased residence time in the rumen (e.g. due to high fiber content) may increase CH_4 production.

Here, we assessed the short-term effects of three different PAM on grazing behaviour, DMI, enteric CH_4 emission, milk yield and its composition with mid-lactation Norwegian Red (NRF) dairy cows. We hypothesized that the quality of grazed forage will deteriorate when cows graze in a horizon with extended grazing days (e.g. weekly rotational grazing) whereas frequent allocation of pasture would optimize forage quality and DMI. It was further hypothesised that grazing behaviour, DMI, and quality of ingested forage would differ between the grazing days as influenced by the PAM resulting also in differences milk yields, milk composition, milk component yields and enteric CH4 emission.

Materials and Methods

Description of Experiments

Two short term grazing experiments were conducted in the year 2014 on early spring pasture (Exp 1; 21 days; 19.05.2014 to 08.06.2014) and on late summer pasture (Exp 2; 19 days; 04.08.2014 to 22.08.2014) with Norwegian Red (NRF) dairy cows. During both experiments, the cows were on pasture except when collected for a.m. milking (between 0630 and 0800 h) and p.m. milking (between 1600 and 1730 h). Time spent on collecting and milking for each group (i.e.; replicate) of four cows was not more than 0.5 h/d due to the proximity of milking shed to the grazed paddocks. The cows had unrestricted access to fresh drinking water all time.

The experiments were carried out at the farm of Animal Production Experimental Centre (Norwegian University of Life Sciences; Norway) following the laws and regulations controlling experiments on live animals under the surveillance of the Norwegian Animal Research Authority.

Experiment 1

Twenty-four mid-lactation (days into milk, DIM ± SD; 124 ± 37) NRF dairy cows with mean bodyweight (BW ± SD) of 572 ± 66 kg were used. Prior to start of Exp 1, the cows grazed for one week on a segment of the same paddock used for the experiment. The experimental herd was composed of 6, 6 and 12 cows from 1st, 2nd and 3rd parity, respectively. These cows were blocked into six groups of four cows per group. Each group was then randomly assigned to one of the three PAM resulting in two groups of cows per treatment. These were: 7 day rotational grazing, 7RG; daily strip-grazing, 1SG; and daily forward-grazing, 1FG. In the 7RG, cows were offered pasture allowance for 7 days on the first day of the grazing week whereas in the 1SG, cows were given a new pasture allowance that was equivalent to 1/7 (estimated DM allowance) of the 7RG each day regulated by forward moving front- and back-electric fences. In the last group (1FG), cows were given daily 1/7 of the equivalent of the 7RG pasture allowance but had, within one week, access to the previously grazed part of the paddock. This meant that the 1FG cows had forward moving front-electric fence for one week. Cows grazed on an early spring pasture that was a primary growth from a 2nd and 3rd year ley dominated by timothy (*Phleum pratense*). In early spring, the experimental fields received 250 kg/ha of artificial fertilizer (N-P-K: 25-2-6). Estimated pasture allowance at entrance (day one of the experimental week) was 25 kg DM/day per cow. This was estimated by cutting herbage mass from 30 spots using a quadrat (50 cm × 50 cm) over 3 days leading into the experimental week. Herbage mass above 60 mm from the ground level was considered. The first week was used as an adaptation period. Grazing was supplemented with a 5 kg/cow per day with a commercial concentrate feed (FORMEL FAVØR 90; produced and supplied by Felleskjøpet Agri SA, Norway). The concentrate feed was fed during milking (a.m. and p.m. milking) in two equal portions. Chemical composition (g/kg DM) of this feed was

68.3, 51.3, 227.0, 165.0 and 255.0 ash, crude fat, neutral detergent fiber (NDF), crude protein (CP= N*6.25), and starch, respectively. For cows in the 1SG and 1FG groups, daily fresh pasture offer was made after morning milking.

Experiment 2

Exp 2 followed a similar design as Exp 1. However, the 7RG duration was shortened to 5 day rotations (5RG), and hence the 5 days duration in a rotation was named as an experimental week. The experimental herd was composed of 7, 6 and 11 cows in their 1st, 2nd and 3rd parity, respectively. All cows grazed in the nearby paddocks from early spring to start of the experiment. Daily strip-grazing (1SG) and daily forward-grazing (1FG) were similar as in Exp 1 (i.e., 1/5 of 5RG) and the same allocation procedure of animals into groups and groups to the treatments was followed. In total, 24 late-lactation (DIM ± SD; 201 ± 34) NRF dairy cows (mean BW ± SD; 579 ± 57) grazed on late summer pasture dominated by timothy (*Phleum pratense*).The experimental fields received about 250 and 230 kg/ha of artificial fertilizer (N-P-K: 25-2-6) during early spring and mid-summer, respectively. Estimated pasture allowance during Exp 2 was 24 kg DM/day per cow at start. Similar method of estimation was used as in Exp 1. Grazing was supplemented with 4 kg/cow per day of commercial concentrate feed as described for Exp 1. Similar to Exp1, cows in the 1SG and 1FG groups were offered daily fresh pasture after morning milking.

The grazed paddocks used in Exp 2 were a regrowth after cutting the available grazing field at around 5 weeks ahead of the starting dates for the experiment. The fields were cut in such a way that a paddock planned for 5 days grazing was preceded by a week to adjust for DM yield and stage of maturity at start of grazing week.

Weather data for both experiments

Weather data for weeks leading into and during the experiments is presented in Fig. 1 (Meteorological data for Aas was obtained from: http://www.nmbu.no/fagklim accessed on 10/08/2017).

Measurements and estimations

Sward Height, Sward Sampling and Analysis, and DMI Estimations

Sward height assessment. Sward height (SH) was assessed using falling plate meter (30 cm diameter, applying a standing pressure of 0.203 g/cm^2; produced by Norwegian Institute for Bioeconomy, Grimstad, Norway) to monitor dry matter availability and leftover at the end. This was done from 3 to 4 days before grazing and at the end of each week. However, measurements taken one day before the experimental week (assumed day-0) was used as a decision tool to partition the weekly paddocks into sub-paddocks. The sub-paddocks carrying approximately equal herbage mass were partitioned using movable electric fences.

Sward and concentrate feed samples. In both experiments, sward samples were taken at the beginning of the adaptation week to describe forage quality at start. This was done by taking sward samples from multiple places and making composite of three samples over the whole field before allocation of the field into the grazing groups (replicates). During the weeks that followed, samples were taken at start-, middle- and end-of-grazing week to monitor changes in sward quality over the grazing days. For this, one composite sample per grazing group was taken. For all groups sampling was done on the available area for grazing for the sampling date. This meant that for the 1FG group, sampling at the middle-of-grazing week included old grazed and fresh un-grazed areas. The samples were hand mowed using a sickle at around 60 mm above ground while the cows were in the morning milking session. Samples representing grazed area were taken by walking along a "*W*" transect and cutting a handful of sward after every 10 steps (~3000 g fresh pooled per grazing group). Concentrate feed samples were also taken at regular intervals during each experiment. Both sward and concentrate samples were dried at 60ºC for 48 h and milled through 1.0 mm sieve size using Retsch cutting mill SM 200 (Restech GmbH, Germany) for standard chemical analysis which was later performed in duplicates.

Additional samples of grazed sward were taken for n-alkane composition (odd-chain and C_{32} alkanes) and even-chain alcohols (C_{20}-C_{30}) to estimate individual cow DMI. For this, hand plucked samples (pooled later ~1000 g fresh per grazing group) were taken by walking through a "W" transect in the field during each sampling day. The samples were dried and milled as described above for standard chemical analysis in preparation for analysis.

Sward botanical composition was assessed at start-, middle- and end-of-grazing week of the measurement weeks. For this, about 1000 g fresh sample was taken from the sward samples collected for chemical composition and manually sorted into main botanical components (at species level), plus others (all unidentifiable components) and debris. The proportion of each botanical component was expressed on DM basis after drying the samples at 60ºC for 48 h. Furthermore, these botanical fractions were later bulked by species and analysed for n-alkane and even-chain alcohols in addition to the whole herbage samples as described above.

Sward samples were analysed at Eurofins (Moss, Norway) for ash (550°C for 24 h) and Kjeldahl-N (Kjeltec 2400; Foss, Hillerød, Denmark) using a Cu catalyst. The NDF concentration was measured using heat-stable amylase to remove starch followed by neutral detergent boiling according to ISO standard no 16472 (ISO 16472:2006, 2006).

Values for net energy lactation (NE_{L20}), metabolizable protein (AAT_{20}) and protein balance in the rumen (PBV_{20}) at feed intake of 20 kg DM were estimated according to the Nordic Feed Evaluation System (Volden 2011). The concentrate samples were analysed for dry matter, ash, fat, Kjeldahl N according to EU directive no 152/22009 (Commission, 2009) and for starch content according to AOAC 996.11.

Estimation of dry matter intake and its digestibility. Dry matter intake was estimated for the last two experimental weeks using dosed C_{32} n-alkane as an external marker and odd-chain alkanes and even-chain alcohols of dietary origin as internal markers. For this, cows were dosed with a 640 mg/d of C_{32} n-alkane impregnated into paper bungs in two equal portions during a.m. and p.m. milking. The marker dosing started 7 days ahead of the start of faecal sampling to harmonize variation in faecal n-alkane concentrations (Mayes *et al.* 1986a). Faecal samples were collected for a series of 5 days twice daily (i.e. during a.m. and p.m. milking). About 500 g of fresh faecal sample was taken from each cow through rectal palpitation. These samples were frozen at collection and stored until completion of the experiment. Later, the samples were thawed and dried using air forced oven at 60ºC for 48 h and milled through 1.0 mm sieve size. Lastly, the samples were pooled by cow and by experimental week on equal weight basis.

The n-alkane and even-chain alcohols contents of the grazed sward, its botanical components, concentrate feed, and faecal samples were analysed as described in Mayes *et al.* (1986a). Pasture DMI was estimated (one estimate per week, per cow) with adjustments made for concentrate intake as described in (Mayes *et al.* 1986b; Dove & Mayes 2005) with weighting for alcohol concentrations in diets and faeces. Total diet dry matter digestibility was estimated based on total intake and faecal output estimates with the dosed C_{32} n-alkane and its concentration in faeces as described by

Dove and Mayes (2005) with faecal recovery correction factors for alkanes based on cattle studies carried out elsewhere (Mayes, personal communication; Dillon et al. 2002).

Body Weight, Milking, Milk Sampling and Analysis

Cow body weight was measured at start and end of each experimental week, in an enclosure designed for handling and weighing, after a.m. milking. Cows were milked twice daily in a parlour using milking machines. Milk samples were taken at the start of adaptation week (day 0; a.m. milking) and at 12 sampling points during the following two weeks of each experiment. The samples were collected in bottles containing Bronopol tablets (2-Bromo-2-nitropane-1,3 diol, Broad Spectrum Microtabs® II) as preservative and stored chilled (4ºC) until analysis on milk protein, fat, lactose and urea using infrared milk analyser (MilkoScan 6000; Foss Electric, Hillerød, Denmark).

Energy-corrected milk (ECM) yield was calculated for individual cow based on mean milk fat, protein and lactose composition, and fresh milk yield according to Sjaunja *et al.* (1991).

Grazing Behaviour

During both experiments, four cows from each treatment were fitted with RumiWatch Noseband Sensors (NBS, FW-Version 1.16) developed by ITIN+HOCH (ITIN+HOCH GmbH, Fütterungstechnik, Switzerland). The NBS recorded cow jaw movements. These jaw movements were matched to eating, ruminating, drinking and other activities by the NBS. These data were collected continuously from the middle of the adaptation week to the end of each experiment. Prior to analysis, data were converted to a comma separated values (CSV) and split into hourly summaries using the RumiWatch Converter software (V0.7.3.2; Itin+Hoch GmbH, Liestal, Switzerland) for each day of recording and for individual cows. A recent report on validation of the system is described in Zehner *et al.* (2017).

Enteric Methane Measurement

Enteric methane (**CH_4**) production was estimated using sulphur hexafluoride (SF_6) as a marker (Johnsen *et al.* 1994) for 8 days during Exp 1, and 7 days during Exp 2. Two cows from each replicate (n = 4; total of 12 cows) were used for this purpose during both experiments. Even though, the plan was to measure on 4 days of each experimental week during both experiments, one sampling day was missed for all cows due to technical reasons contributed by a very wet weather condition during Exp 2. Samples were collected on days 1, 3, 5, and 7 of each experimental week during Exp 1. However, during Exp 2, samples were collected on days 1, 2, 3 and 5 of experimental week 1, and days 2, 4 and 5 of experimental week 2. For Exp 2, it later appeared during sample analysis that the marker was not detected for some cows at random. Therefore, CH_4 estimates were averaged per cow per week for Exp 2.

The sampling technique involved placing a permeation tube containing ultra-pure SF_6 into the rumen several days before sampling as described by McGinn *et al.* (2006). Steel permeation tubes filled with SF_6 gas (mean ± SD = 2583.9 ± 80.9 mg) and predetermined release rate (mean ± SD; 4.38 ± 0.80 mg/d; r^2=0.999) (Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Saskatchewan, Canada) were used.

For CH_4 sampling, cows were mounted with a depressurized gas collection canisters and a halter system as described in McGinn *et al.* (2006) for 24 h gas sample collection. This method involves sampling breathed and background air from around nasal proximity through a tubing into an evacuated canister mounted to the neck of the cows. The flow into the canister was regulated for 24 h using an in-line capillary tubing (McGinn *et al.* 2006). Furthermore, each sampling day, two sets of canisters and halters were placed in the grazing area at about grazing-cow-head position to correct for background air in the sampled gas.

At the end of each experiment, the daily gas samples were analysed in triplicates per cow using gas chromatography (GC, Model 7890A Agilent, Santa Clara, CA, US) equipped with flame ionization detector for CH_4 and an electron capture detector for SF_6 analysis. Daily enteric CH_4 emission was calculated according to McGinn *et al.* (2006):

$$Q_{CH4} = \frac{C_{CH4} - C_{CH4b}}{C_{SF6} - C_{SF6b}} Q_{SF6} \frac{MW_{CH4}}{MW_{SF6}}$$

Where: Q_{CH_4} - daily enteric CH_4 emission (g/day)

Q_{SF_6} - predetermined marker release rate (g/day)

C_{CH_4} and C_{SF_6} - *the* CH_4 and SF_6 *mixing ratios in the canisters* (µ*mol/mol)*

$C_{CH_4^b}$ and $C_{SF_6^b}$ - *the background* CH_4 and SF_6*, respectively, measured with air samples collected from the grazed field*

MW_{CH4} / MW_{SF6} *- molecular weight ratio used to account for the differences in the density of the gases*

Statistics

Statistical analyses were carried out using repeated measures ANOVA in SAS PROC MIXED (SAS Institute Inc.2002-2012) as multiple measurements per animal over days cannot be regarded as independent units of observations (Littell *et al*. 1998; Abrahamse *et al*. 2008). Therefore, the analysis was performed with day as the repeated factor where within-cow variation was modelled using autoregressive (AR1) covariance structure. Whenever existed and contributed significantly to the model, day 0 (pre-experimental) values were used as covariates. For most of the data, whenever data structure allowed, the following basic model was fitted as a repeated measure:

$$Y_{ijklmn} = \mu + T_i + R_j + C_k + W_l + D_m + (D*T)_n + PreMY + e_{ijklmn}$$

Where: Y_{ijklmn}= the response variable; μ = overall mean; T_i = the fixed effect of PAM (i =1-3); R_j = the random effect of replicate (j = 1-2); C_k= *the random effect of cow within a replicate (*k =1-4; except for grazing behaviour and methane measurement where k =1-2) *;* W_l = the fixed effect of experimental week (l =1-2); D_m = the fixed effect of day in an experimental week (m = 1-7 for Exp 1; and m = 1-5 for Exp 2); $(D*T)_n$ = the fixed effect of the interaction between day in an experimental week and PAM; *PreMY = the fixed effect of a covariate (e.g. day 0 milk yield);* e_{ijklmn} *= the residual error term.* For behavioural data, the model further included time of the day, and its interaction effects with PAM and day of the week. However, for DMI data, since only one DMI estimate per cow per week was available, the statistical analysis was carried out by omitting day and covariate effects from the model.

Statistical significance was declared at $P \leq 0.05$. Shorthand presentations were used in tables with full P-values for tendencies ($0.05 < P \leq 0.1$).

Results

Sward Height, Sward Chemical and Botanical Composition

Data on pre- and post-grazing SH are presented in Table 1. Mean pre-grazing SH of 36.6 cm for the two measurements weeks of Exp 1 reduced to around 16.0 cm in the 1SG group after 7 days of grazing. Exp 2 started with a well regulated pre-grazing SH (15.4 cm) which was diminished to 9.6 cm after 5 days of grazing.

Data on sward botanical composition was merged for the measurement weeks and changes observed over the grazing days relative to pre-grazing values in the measurement weeks are presented in Table 1. Timothy was the dominant grass species (> 60%) on DM basis in both experiments while the remaining 40% of the herbage was composed of Meadow fescue (*Festuca pratensis Huds.*), Perennial ryegrass (*Lolium perenne L.*), mixed species of white (*Trifolium repens* L.) and red (*Trifolium pratense* L.) clover and other species at variable proportions. The proportion of the main botanical components diminished with increasing share of debris (especially in Exp 2) with advancing grazing days in the field. The proportion of clover in the grazed sward was relatively low (<5% of herbage mass on DM basis).

Mean chemical composition of the grazed sward, is provided in Table 2 and changes in sward chemical composition brought about by the different PAM over the grazing days of week are illustrated in Fig. 2 and Fig. 3.

Sward chemical composition was not affected by the different PAM with the exception of the CP content ($P = 0.081$) and estimated net energy for lactation ($NE_{L20;}$ $P = 0.068$). These parameters tended to be lower in the 5RG group during Exp 2. However within each treatment there was a significant change in chemical composition of the swards over grazing days ($P < 0.05$) for most of the parameters except for ash content (Exp 1) and estimated organic matter digestibility (Exp 2). Here, the CP content decreased ($P < 0.001$) while the NDF content increased (effect of day in a week; $P < 0.001$; Fig. 2 and Fig.3; Panel “A”) over the grazing days. The interaction effect between PAM and days of grazing were not significant ($P > 0.1$) for the analysed sward parameters. Furthermore, the estimated NE_{L20} and AAT_{20} of the grazed sward declined significantly with grazing days in a week ($P < 0.001$). The effect was consistent in both experiments and the pattern was uniform for all treatments without any treatment, and treatment by grazing day interaction effects (Fig. 2 and Fig. 3 and panels “C” and “D”).

In addition, changes were observed in sward chemical composition of the pre-graze samples of the three weeks from both experiments. As a result, there was a drop in CP and NE_{L20} contents and an abrupt increment in NDF content during Exp 1. For Exp 2, the observed differences especially in CP were the opposite. Here, the CP content of the pre-graze pasture showed an in increment from adaptation week to the last week of the experiment (Fig. 3a).

Dry Matter Intake

Pasture and total DMI of cows are presented in Table 3. During Exp 1, estimated herbage intake of cows was not affected by the PAM ($P > 0.1$). Mean daily pasture DMI was around 12.0 kg making the total DMI to 16.5 kg/cow. During Exp 1, estimated mean pasture DMI intake for measurement week 2 (10.7± 0.80) was lower than that of measurement week 1 (13.4 ± 0.82) ($P = 0.001$). Estimated diet (grazed pasture + concentrate feed) digestibility was not different between the three PAM ($P > 0.1$). However, measurement week influenced estimated diet digestibility (% ± SE) where week 1 had higher DM digestibility (78.9 ± 0.34) than week 2 (75.1 ± 0.36).

During Exp 2, pasture DMI was not influenced by the PAM or week of measurement. But, there was a tendency for interaction of measurement week by the PAM ($P = 0.08$) for DMI. As a result, cows in the 5RG tended to have higher estimated pasture DMI than the other two treatments during week 1 but not in week 2. Estimated diet digestibility was different between the three PAM ($P = 0.018$). However, the observed interaction effect ($P < 0.016$) of PAM and week of measurement indicated that this difference existed only during measurement week 1 whereby the 5RG treatment resulted in higher diet digestibility than the other two treatments.

Grazing Behaviour

Data on grazing behaviour and related activities are presented in Table 4, whereas grazing and rumination patterns over the 24 h cycle are shown in Fig 4.

Cows exhibited shorter but intensive grazing patterns during Exp 2 with mean day-length of 15.45 h. During both experiments, cows had almost similar grazing patterns as indicated by peaks just before and after a.m. milking, before p.m. milking, and just before sunset.

During Exp 1, cows on 1SG and 1FG groups spent more time (min/h) on grazing compared to 7RG ($P < 0.05$). However, the expected interaction effect of grazing day by PAM on time spent on grazing – that cows in the 7RG group would spend more time on grazing towards the end of grazing week to compensate for differences in pasture physical structure and quality - was not observed ($P > 0.1$). The treatment by time of the day effect on eating/grazing was significant ($P < 0.001$) (Table 4 and Fig. 4a) as indicated clearly by early start of grazing from 7RG compared to the other PAM.

During Exp 2, cows on 1FG spent more time on grazing compared to 1SG. Time spent on rumination decreased from 5RG to 1FG, but the hypothesized interaction effect of treatment by day of grazing on either eating or rumination was not observed ($P > 0.1$).

Enteric Methane Emission

Daily enteric CH_4 production (yield; g/d), and intensity (g CH_4/kg ECM) is provided in Table 5. The different pasture allocation methods did not affect enteric CH_4 yield and its intensity during both experiments ($P > 0.1$). However, the significant interaction effect of PAM by measurement day during Exp 1 ($P < 0.05$) indicated that cows in the 7RG group had the lowest CH_4 production on day 1 of the measurement week 2.

Overall, during Exp 1, mean (±SE) daily CH_4 production was 287.5 ± 8.68 g/day per cow with mean intensity of 10.5±0.41 g CH_4/kg ECM. For Exp 2, the values were 292.4 ± 5.04 g/day per cow and 13.6 ± 1.49 8 g CH_4/kg ECM in the respective order. The PAM by week interaction effect for daily CH_4 during Exp 2 indicated cows in the 7RG group produced higher CH_4 in measurement week 1 than 2, whereas cows in the 1SG produced less CH4 in measurement week 1 than 2.

Animal Performance

Milk yield and chemical composition are summarized in Table 6 and mean ECM yield over the grazing days are presented in Fig.5. During Exp 1, milk and ECM yield were not affected by the different PAM ($P > 0.1$) or by day of grazing in a week ($P > 0.1$). However, significant PAM by grazing day interaction effect ($P < 0.05$) was observed for milk yield, milk lactose, and milk protein and milk urea contents in the absence of the main effect of PAM.

During Exp 2, again the effects of PAM on milk yield and chemical composition were not significant ($P > 0.1$). However, the effects of grazing days on milk yield and ECM were significant ($P < 0.001$) with significant interaction effects of grazing days by PAM for milk yield ($P < 0.01$).

Cow BW change over the experimental days was not affected by PAM during both experiments (Table 6). However, cows in all groups tended to lose BW relative to starting BW over the experimental days during Exp 1 (measurement day effect, $P = 0.058$). During Exp 2, cows in 1SG and 1FG maintained BW whilst those in 5RG on average lost BW (linear estimate ± SEM; 343 ± 295 g/d).

Discussion

Sward Characteristics

Maintaining grazed swards to a low post-grazing SH is a strategy for improving grass utilization (Ganche et al 2015). Low post-grazing SH usually increases leaf proportion, and as such, improves herbage quality (Peyraud and Delagarde, 2013). The observed mean post-grazing SH from our experiments was much higher than what is reported with long season grazing conditions in other parts of Europe (Ganche et. al., 2015; Dale et al., 2008). However, high pre-grazing SH, fast growth of herbage with heavy DM accumulation on the days that followed, and a lax grazing intensity might have contributed to such a higher post-grazing SH. In addition, we observed excessive trampling and lodging of the grazed sward over the grazing week, especially during Exp1. As a result, accurate representation of post-grazing SH as an indicator of the degree of pasture utilization was not possible. During Exp 2, the observed mean post-grazing SH in all PAM was not as extreme as in Exp 1 but again closer to 10 cm which could be considered high. McGilloway et al., (1999) argue that cows cannot be ‘forced’ to utilize herbage to the same extent as they do in current systems of rotational grazing (between 6 and 8 cm residual SH) to maximize intake. Nevertheless, the observed post-grazing SH implied large residual biomass in the grazed field which under practical farming conditions could be grazed by a follow-up group of non-lactating animals.

For sward botanical composition, the level of clover in the experimental pastures was much lower than what would be expected from a grass/clover mixed stand. However, similar low levels were reported for grassland managed under conventional production systems here in Norway (Adler et. al., 2013).The proportion of debris (dead organic matter) increased over the grazing days in both experiments. These could justify some of the changes in chemical composition, particularly the increasing NDF content (Thomson 1983; Hodgson 1985) with the concomitant decline in CP content of the grazed sward.

In all PAM, sward quality in terms of CP, metabolizable protein supply and NE_{L20} declined with advancing grazing days following a similar pattern. Thus, contrary to our expectations, there was a lack of a significant effect of PAM, and its interaction with days of grazing on pasture quality. The observed changes in chemical composition appeared to be mainly due to the rapid plant phenological development well known for spring growth of timothy (Heide *et al.* 1985) and changes in sward structure. In addition, the expected selective grazing behaviour and removal of the top horizons of the sward by grazing animals may have contributed to this. Grazing alone could have resulted in more of the structural components of the sward (Bryant *et al.* 1961; Delagarde *et al.* 2000). However, the rapid maturity of the pasture appeared to have stronger effects than the effects of grazing as suggested by changes observed in each of the three weekly pre-grazing sward chemical compositions.

The increasing CP content of the grazed sward during the two measurement weeks of Exp 2, in contrast to what was observed in Exp 1, is likely to be due to the differences in stage of maturity of the regrowth as modulated by different cutting dates and the inherent differences in the paddocks allocated for the experiment.

Dry Matter Intake from Grazed Pasture

Pasture DMI during Exp 1 was relatively comparable between treatments. A generous DM allowance (25 kg DM/day estimated at 60 mm above ground level) and abrupt DM accumulation in the days that followed had resulted in a lax grazing intensity. Even for the 1SG group where cows were restricted to roughly 1/7th of the area for the 7RG group - theoretically without access to 6/7th of the allowance to 7RG at a given day - the estimated DMI was not different from the others. This is suggestive of the lax nature of pasture DM available for grazing at the time. Furthermore, we estimated pasture DMI, retrospectively, based on energy balance (data not presented). This was based on requirements for the achieved level of production (i.e., milk production, maintenance, pregnancy, and bodyweight changes) and estimated herbage energy values. The estimate of intake was higher than we observed with n-alkane method. Considering the amount of herbage available for selective grazing and the expected better quality of the consumed diet (Ayantunde et al 1999), such inflation in DMI estimate is plausible. This is because the digestibility and, hence, energy contents of the sward samples were estimated on samples cut above 60 mm from the ground which would be inferior in quality to the selectively consumed sward. Animal performance was dependent on the latter. Therefore, retrospectively estimating DMI based on samples cut above 60 mm from the ground level should be higher than expected.

During Exp 2, the estimated pasture DMI was similar between grazing groups but the level of intake appeared unlikely in relation to the stage of lactation and observed animal performance. Here, contrary to Exp 1, the DMI estimate based on energy balance was lower than DMI estimate with the marker method suggesting that the latter might have been inflated. This is because intake from pasture alone amounted to about 135 g/kg BW$^{0.75}$, and total intake (pasture plus concentrate feed) was about 163 g/kg BW$^{0.75}$. This estimate is much higher than what is suggested by Van Vuuren and Van den Pol-van Dasselaar (2006) (i.e., 110 to 120 g DMI/kg BW$^{0.75}$) for cows fed pasture alone.

However, the methods used for estimation did not result in differences in DMI estimates between the PAM. Overall, the observed effects of grazing treatments on pasture chemical composition and DMI did not support our hypothesis. Therefore, the expected effects of grazing treatments on milk yield and its chemical composition would be marginal.

Grazing Behaviour

The hypothesized effects of grazing day by PAM on cows grazing behaviour was not observed during both experiments. During both experiments, cows exhibited similar grazing patterns as indicated by the peaks. These peaks were marked as “before morning milking” (most probably disrupted by gathering for milking), “after morning milking” (probably a continuation of the morning grazing), “afternoon grazing”, and “evening grazing” culminated by darkness. During Exp 1, the 7RG group commenced grazing earlier and culminated morning grazing earlier than the other two groups. This, pattern was absent during Exp 2, under which both pasture and daylight conditions differed from Exp 1. This may highlight the importance of behavioural changes of cows, over a short term, as adaptations to changes in grazing conditions (Gibb 2006; Chilibroste *et al.* 2012).

The grazing pattern observed in Exp 1 suggested that the 7RG cows were not anticipating fresh pasture allocation probably learnt from the adaptation week. They started early morning grazing every day ahead of the other two groups. It could also be that the other two groups expected their daily fresh offer (Jamieson & Hodgson 1979) and had to wait until this was made. With housed dairy cows fed on total mixed ration, increased feed alley attendance (i.e., similar pattern of eating activity) was observed when fresh feed is offered (DeVries *et al.* 2003). Peyraud *et al.* (1996)

suggested cows may abandon grazing as the sward structure may represent physical limitation to prehend the grass. However, this might not seem to be the case in Exp 1 as herbage allowance was not restrictive. However, under relatively pasture limiting conditions, as observed in Exp 2, it could be argued that the stubble structure could have posed a physical limitation (Peyraud *et al.* 1996).

The shorter rumination time for the 1SG group compared to others during Exp 1, against observed longer time spent on grazing suggested that DM intake rates were lower for the group (Stobbs 1970). This was also supported by the numerically lower estimated DMI for the groups and corroborates the multifaceted nature of factors influencing feed intake by grazing animals. For example, number of bites per unit of time and the average size of each bite mass (Fuerst-Waltl *et al.* 1997) affect herbage DMI as influenced by available herbage mass and sward surface height (Gibb 2006). As a result, under restrictive sward mass and height conditions, dairy cows might attempt to maintain intake by increasing grazing time.

In general, time spent on grazing during Exp 1 was shorter than that observed during Exp 2. This would reflect the higher amount of DM available during Exp 1 which would have allowed higher intake rate. Similar outcomes were reported with previous studies (Phillips & Leaver 1986). The declining forage availability and relatively restrictive day length available for grazing as observed in Exp 2, necessitated greater intensity of grazing activity (Realini *et al.* 1999; Gekara *et al.* 2005). Furthermore, animals would spend more time on grazing activity because they obtain less mass per bite (Arnold & Dudzinski 1978; Chilibroste *et al.* 2012). However, lower bites per day and reduced grazing time were reported in rotational grazing systems (Pulido and Leaver, 2003) where cows anticipated movement to a fresh allocation of herbage in situations where low herbage allowance and low sward heights created difficulties in prehension.

Methane Production

Dry matter intake is the main determinant of enteric methane production. Lack of difference in both daily enteric methane production and its intensity (g CH4/kg ECM) would reflect the achieved level of DMI. The observed values were close to recent reported values from the same herd (Storlien *et al.* 2017) or from elsewhere with other breeds (Robertson & Waghorn 2002; Muñoz *et al.* 2015; Muñoz *et al.* 2016) under grazing conditions, and dairy cows fed silages of different sources and proportions (van Gastelen *et al.* 2015). It was also much lower than what we have recently observed (Kidane *et al.* 2018) for NRF cows from the same herd fed total mixed ration diets at similar stage of lactation. Recent review of enteric methane from dairy cattle production by Knapp *et al.* (2014) presented comparable results based on mean values from 11 published works comprising of 35 dietary treatments.

The observed interaction effect of PAM and day on daily CH_4 production in Exp1 was seen only during measurement week 2. During Exp 2, this effect was not tested for reasons described earlier. The lack of effects of PAM on enteric methane emission could be due to the level of achieved DMI and observed changes in pasture quality.

Animal Performance

Milk production on pasture is influenced by herbage intake and the nutritive value of the herbage. Pasture fed cows are often challenged in achieving high milk yields due to intake limitation from pasture alone. As a result, DMI from grazed pasture alone could suffice for milk production up to 28 kg/d with requirement for additional supplementation for high producing cows (Van Vuuren & Van den Pol-van Dasselaar 2006; Van den Pol-van Dasselaar *et al.* 2009).

Our effort to moderate achieved DMI and its quality on milk yield and milk quality using the three pasture allocation methods was not successful. This was contrary to other reports where frequent allocation of herbage improved intake and milk production (Abrahamse *et al.* 2007; Abrahamse *et al.* 2008). Indeed, McFeely et al., (1975) and Chenais et al., (1995) reported lack of difference between grazing groups on milk yield and composition using a relatively longer grazing intervals than what we implemented here. It may be the case that residence time in a paddock might not be the main determinant of animal performance at similar stocking rate and management (Hoden *et al.* 1991; Dalley *et al.* 2001).

The effects observed under our conditions suggested only fluctuations of daily DMI on milk yield as could be seen from the oscillation in milk yields. The latter was manifested in the grazing day x PAM interaction effects. Such daily fluctuations are often the main challenges in optimizing rations for grazing dairy cows (Van Vuuren & Van den Pol-van Dasselaar 2006; Van den Pol-van Dasselaar *et al.* 2009). Here, these fluctuations occurred in a non-particular manner between the different PAM over the measurement days of each week. As such, the observed effects in the absence of main effects of grazing treatments suggested that the achieved level of nutrient intake under the different PAM, even though fluctuated between days, might not have been different. Moreover, the perceived behavioural adaptations of cows to adjust DMI and its quality under different PAM in the absence of time restriction for grazing (Pérez-Ramírez *et al.* 2008), could also provide some buffer to maintain milk yield and composition.

Conclusions

The lack effects of the different PAM on enteric methane emission, milk yield and milk composition could be due to lack of the anticipated differences between the treatments in sward qualities over each week. As a result, the achieved level of nutrient intake might not have been different. Secondly, the resilience of dairy cows to adapt to changing nutritional conditions under such a short experimental periods may accommodate some fluctuations in DM and nutrient intake. Furthermore, behavioural adaptations of cows to adjust feed intake under different PAM could also provide some physiological plasticity to maintain milk yield and composition.

Acknowledgements: This work was funded by the Norwegian Agricultural Agreement Research Fund (Project number 225266; The Research Council of Norway), Norsk senter for økologisk landbruk (NORSØK), County Governors in Møre og Romsdal (FMLA), Møre and Romsdal County Authority, TINE SA and Oikos – Organic Norway. We would like to thank The James Hutton Institute for supplying n-alkane bungs and later analysis of faeces, herbage and feed samples for n-alkane and long chain alcohol contents.

The authors do not have any conflict of interest.

References

Abrahamse P.A., Dijkstra J. & Tamminga S. (2007) Frequent reallocation of strip grazing cows improves productivity. *Journal of Dairy Science* **90**, 636-.

Abrahamse P.A., Dijkstra J., Vlaeminck B. & Tamminga S. (2008) Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. *Journal of Dairy Science* **91**, 2033-45.

Arnold G.W. & Dudzinski M.L. (1978) *Ethology of free-ranging domestic animals. Developments in Animal and Veterinary Sciences, 2.* Elsevier Scientific Publishing Company, Amsterdam.

Bryant H.T., Blaser R.E., Hammes R.C. & Hardison W.A. (1961) Method for Increased Milk Production with Rotational Grazing1. *Journal of Dairy Science* **44**, 1733-41.

Chacon E. & Stobbs T. (1976) Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. *Australian Journal of Agricultural Research* **27**, 709-27.

Chilibroste P., Mattiauda D.A., Bentancur O., Soca P. & Meikle A. (2012) Effect of herbage allowance on grazing behavior and productive performance of early lactation primiparous Holstein cows. *Animal Feed Science and Technology* **173**, 201-9.

Dalley D.E., Roche J.R., Moate P.J. & Grainger C. (2001) More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. *Australian Journal of Experimental Agriculture* **41**, 593-9.

Delagarde R., Peyraud J.L., Delaby L. & Faverdin P. (2000) Vertical distribution of biomass, chemical composition and pepsin - cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. *Animal Feed Science and Technology* **84**, 49-68.

DeRamus H.A., Clement T.C., Giampola D.D. & Dickison P.C. (2003) Methane emissions of beef cattle on forages: efficiency of grazing management systems. *Journal of Environmental Quality* **32**, 269-77.

DeVries T.J., von Keyserlingk M.A.G. & Beauchemin K.A. (2003) Short Communication: Diurnal Feeding Pattern of Lactating Dairy Cows. *Journal of Dairy Science* **86**, 4079-82.

Dillon P., Crosse S., O’Brien B. & Mayes R.W. (2002) The effect of forage type and level of concentrate supplementation on the performance of spring-calving dairy cows in early lactation. *Grass and Forage Science* **57**, 212-23.

Dove H. & Mayes R.W. (2005) Using n-alkanes and other plant wax components to estimate intake, digestibility and diet composition of grazing/browsing sheep and goats. *Small Ruminant Research* **59**, 123-39.

Finneran E., Crosson P., O'Kiely P., Shalloo L., Forristal P.D. & Wallace M. (2012) Economic modelling of an integrated grazed and conserved perennial ryegrass forage production system. *Grass and Forage Science* **67**, 162-76.

Fuerst-Waltl B., Appleby M.c., Solkner J. & Oldham J.D. (1997) Grazing behaviour of dairy cattle in relation to genetic selection for milk production. *Die Bodenkultur* **48**, 199-209.

Gekara O.J., Prigge E.C., Bryan W.B., Nestor E.L. & Seidel G. (2005) Influence of sward height, daily timing of concentrate supplementation, and restricted time for grazing on forage utilization by lactating beef cows. *J Anim Sci* **83**, 1435-44.

Gibb M. (2006) Grassland management with emphasis on grazing behaviour. In: *Fresh Herbage for Dairy Cattle* (eds. by Elgersma A, Dijkstra J & Tamminga S), pp. 141-57. Springer, Printed in the Netherlands.

Heide O.M., Hay R.K.M. & Baugerod H. (1985) Specific Daylength Effects on Leaf Growth and Dry-Matter Production in High-Latitude Grasses. *Annals of Botany* **55**, 579-86.

Hoden A., Peyraud J.L., Muller A., Delaby L., Faverdin P., Peccatte J.R. & Fargetton M. (1991) Simplified rotational grazing management of dairy cows: effects of rates of stocking and concentrate. *The Journal of Agricultural Science* **116**, 417-28.

Hodgson J. (1985) The control of herbage intake in the grazing ruminant. *Proceedings of the Nutrition Society* **44**, 339-46.

Jamieson W.S. & Hodgson J. (1979) Effect of Daily Herbage Allowance and Sward Characteristics Upon the Ingestive Behavior and Herbage Intake of Calves under Strip-Grazing Management. *Grass and Forage Science* **34**, 261-71.

Johnsen K., Huyler M., Westberg H., Lamb B. & Zimmerman P. (1994) Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. *Environmental Science & Technology* **28**, 359-62.

Kidane A., Overland M., Mydland L.T. & Prestlokken E. (2018) Interaction between feed use efficiency and level of dietary crude protein on enteric methane emission and apparent nitrogen use efficiency with Norwegian Red dairy cows. *J Anim Sci* **96**, 3967-82.

Knapp J.R., Laur G.L., Vadas P.A., Weiss W.P. & Tricarico J.M. (2014) Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science* **97**, 3231-61.

Littell R.C., Henry P.R. & Ammerman C.B. (1998) Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* **76**, 1216-31.

Mayes R.W., Lamb C.S. & Colgrove P.M. (1986a) The Use of Dosed and Herbage N-Alkanes as Markers for the Determination of Herbage Intake. *Journal of Agricultural Science* **107**, 161-70.

Mayes R.W., Wright I.A., Lamb C.S. & Mcbean A. (1986b) The Use of Long-Chain N-Alkanes as Markers for Estimating Intake and Digestibility of Herbage in Cattle. *Animal Production* **42**, 457-.

McGinn S.M., Beauchemin K.A., Iwaasa A.D. & McAllister T.A. (2006) Assessment of the sulfur hexafluoride (SF6) tracer technique for measuring enteric methane emissions from cattle. *Journal of Environmental Quality* **35**, 1686-91.

Muñoz C., Hube S., Morales J.M., Yan T. & Ungerfeld E.M. (2015) Effects of concentrate supplementation on enteric methane emissions and milk production of grazing dairy cows. *Livestock Science* **175**, 37-46.

Muñoz C., Letelier P.A., Ungerfeld E.M., Morales J.M., Hube S. & Perez-Prieto L.A. (2016) Effects of pregrazing herbage mass in late spring on enteric methane emissions, dry matter intake, and milk production of dairy cows. *Journal of Dairy Science* **99**, 7945-55.

Pérez-Ramírez E., Delagarde R. & Delaby L. (2008) Herbage intake and behavioural adaptation of grazing dairy cows by restricting time at pasture under two feeding regimes. *Animal* **2**, 1384-92.

Peyraud J.L., Comeron E.A., Wade M.H. & Lemaire G. (1996) The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. *Annales De Zootechnie* **45**, 201-17.

Phillips C.J.C. & Leaver J.D. (1986) The Effect of Forage Supplementation on the Behavior of Grazing Dairy-Cows. *Applied Animal Behaviour Science* **16**, 233-47.

Realini C.E., Hodgson J., Morris S.T. & Purchas R.W. (1999) Effect of sward surface height on herbage intake and performance of finishing beef cattle. *New Zealand Journal of Agricultural Research* **42**, 155-64.

Robertson L.J. & Waghorn G.C. (2002) Dairy industry perspectives on methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. *Proceedings of the New Zealand Society of Animal Production* **62**, 213-8.

Sjaunja L.O., Baevre L., Junkarinen L., Pedersen J. & Setälä J. (1991) A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in Performance Recording of Animals: State of the Art, 1990: Proceedings of the 27th Biennial Session of the International Committee for Animal Recording (ICAR). (eds. P. Gaillon and Y.Chabert), Paris, France, 2-6 July 1990. Wageningen Academic Publishers, Wageningen, the Netherlands.

Stobbs T.H. (1970) Automatic measurement of grazing time by dairy cows on tropical grass and legume pastures. *Tropical Grasslands* **4**, 237-44.

Storlien T.M., Prestlokken E., Beauchemin K.A., McAllister T.A., Iwaasa A. & Harstad O.M. (2017) Supplementation with crushed rapeseed causes reduction of methane emissions from lactating dairy cows on pasture. *Animal Production Science* **57**, 81-9.

Thomson N.A. (1983) Factors Influencing the Accuracy of Herbage Mass Determinations with a Capacitance Meter. *New Zealand Journal of Experimental Agriculture* **11**, 171-6.

Vallentine J. (2000) *Grazing management*. Academic Press London, UK.

Van den Pol-van Dasselaar A., Vellinga T.V., Johansen A. & Kennedy E. (2009) To graze or not to graze, that's the question In: *(eds. Hopkins, A. et al.) Biodiversity and animal feed– Future challenges for grassland production . The 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, June 9-12, 2008* (eds. by Hopkins A, Gustafsson T, Bertilsson J, Dalin G, Nilsdotter-Linde N & Spörndly E), pp. 706-16. Grassland Science in Europe, Uppsala, Sweden.

van Gastelen S., Antunes-Fernandes E.C., Hettinga K.A., Klop G., Alferink S.J., Hendriks W.H. & Dijkstra J. (2015) Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets. *J Dairy Sci* **98**, 1915-27.

Van Vuuren A.M. & Van den Pol-van Dasselaar A. (2006) *Grazing systems and feed supplementation*. Wageningen UR Frontis Series Volume 18, Springer, Dordrecht, the Netherlands.

Virkajärvi P., Sairanen A., Nousiainen J.I. & Khalili H. (2002) Effect of herbage allowance on pasture utilization, regrowth and milk yield of dairy cows in early, mid and late season. *Animal Feed Science and Technology* **97**, 23-40.

Volden H. (2011) Feed calculations in NorFor. In: *NorFor- The Nordic feed evaluation system* (ed. by Volden H), pp. 55-8. Wageningen Academic Publishers, The Netherlands.

Wright I.A. (2005) Future prospects for meat and milk from grass-based systems. In: *Grasslands: Developments Opportunities Perspectives* (eds. by Reynolds SG & Frame J), pp. 161-79. Science Publishers, Inc. in Association with FAO, Enfield, New-Hampshire, USA.

Zehner N., Umstätter C., Niederhauser J.J. & Schick M. (2017) System specification and validation of a noseband pressure sensor for measurement of ruminating and eating behavior in stable-fed cows. *Computers and Electronics in Agriculture* **136**, 31-41.