Differential rumination, intake, and enteric methane production of dairy cows in a pasture-based automatic milking system

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ABSTRACT

Proper performance monitoring of cows on pasture-based diets is crucial to inform nutritional recommendations that minimize undesirable effects of high ruminant CH₄ emissions into the environment. The prediction of linkages between rumination patterns, methane emissions, and correlated production traits of cows in a pasture-based automatic milking system was tested. A previous 10-d baseline measurement of rumination activity by acoustic methodology of 156 Holstein-Friesian cows was used for frequency analysis of rumination time and identification of 2 treatment groups ($n = 37$ cows/group) represented by cows with consistently high (HR; 75th rumination percentile = 617.55 ± 81.37 min/d) or low (LR; 25th rumination percentile = 356.65 ± 72.67 min/d) rumination. The HR and LR cows were paired by nearest parity, days in milk, body weight (BW), and previous 10-d milk production, and within pairs randomly assigned to 1 of 2 experimental groups managed on a voluntary milking system with diets consisting of at least 75% pasture, plus concentrates. Animal traits, including rumination time, mass flux of CH₄ (QCH_4) and carbon dioxide (QCO_2), milk production, and estimated dry matter intake according to individual QCO_2 fluxes over a 22-d period were analyzed with repeated measure mixed models for a completely randomized design, structural equation modeling, and nonlinear regression. High rumination and methane was seen in older and heavier cows that had greater estimated dry matter intake and milk production. A consistent difference in rumination time and QCH_4 across days was detected between HR and LR, even after adjustment for metabolic BW. Estimated dry matter intake had direct positive effects on rumination and QCH_4 , but no independent direct

effect of rumination on QCH_4 was detected. The LR cows produced more QCH_4 /milk, associated with lower milk, BW, concentrate intake, and greater activity at pasture. A typical dilution of maintenance effect on QCH_4 /milk was detected as a consequence of increasing milk yield and similar significant reduction of QCO_2 /milk. The results raise challenging questions regarding the rumination patterning of grazing dairy cows and alternatives to reduce ruminant methane emissions in grazing dairy cows.

Key words: automatic milking, carbon gas emission, pasture grazing, rumination pattern

INTRODUCTION

The expansion of the dairy industry is increasingly affected by growing international trade, demands for milk products, and pressures to produce more milk on a carbon-constrained economy (Thornton, 2010). Consequently, the added effect of dairy systems on the environment, in particular the issue of rising greenhouse gas emissions, is receiving more attention. According to previous life cycle analysis, enteric CH₄ can represent 50% or more of the total greenhouse gas share in a pasture-based dairy farm, with the rest being nitrous oxide, mostly from urine deposition, and CO₂ associated with on-farm energy use (Zehetmeier et al., 2012; Opio et al., 2013). Thus, reducing CH₄ emissions through practices that maintain or even increase milk production levels is considered to be a major endeavor for the sustainable development of a growing dairy industry worldwide (Pacheco et al., 2014).

Dairy cows managed on pasture-based diets play a pivotal role within the large food provision sector, as these cows convert large amounts of forages inedible to humans into human edible food of greater nutritional value (Pacheco et al., 2014). However, high consumption of fiber commonly seen with pasture-based diets is known to result in higher on-farm CH₄ production and yield of CH₄ per unit of milk (Opio et al., 2013). Therefore, reducing enteric CH₄ is increasingly impor-

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tant in dairy systems where grazed forages remain as predominant diet.

The biochemical pathway of enteric CH₄ synthesis is very well known. High consumption of fiber increases rumen pH and decreases the rate of passage of digesta in the gastrointestinal tract (Allen, 2000). In turn, a shift in ruminal fermentation toward acetate increases the concentration of dissolved hydrogen equivalents for synthesis of CH₄ by rumen methanogens (Moe and Tyrrell, 1979; Knapp et al., 2014). This biochemical pathway could be increasingly complex, and variable due to the inherent, dynamic, and ever-changing diet variability typically seen with grazing-based diets.

Rumination activity (i.e., frequency, intensity, and duration) is also known to increase with the inclusion of high levels of fiber in the diet (Mendes et al., 2013). Rumination plays a 2-fold ingestion-digestion regulation role. First, rumination increases saliva production, which buffers decreases of rumen pH (Mertens, 1997), thereby favoring acetogenic fermentation, which increases milk fat synthesis but also yields more enteric CH₄ (Moe and Tyrrell, 1979). Second, mastication during rumination also reduces feed particle size and increases surface area for rapid fermentation by ruminal microbes (Kennedy, 1985). Thus, rumination has direct effects on digesta turnover, which could exert control on the regulation of DMI (Allen, 2000) and CH₄ production (Pinares-Patiño et al., 2007).

Despite the potential linkages between rumination and CH₄, the quantitative assessment of direct (i.e., independent) and indirect (i.e., dependent) effects of rumination on CH₄ production has received scant attention, particularly in grazing cows. Additionally, a paucity of independent studies have compared diurnal rumination patterns (Gregorini et al., 2012, 2013) or CH₄ emissions (Pinares-Patiño et al., 2007) in grazing dairy cows, but no studies to our knowledge have specifically examined the linkages between 24-h rumination and enteric CH₄ production across several days. Understanding the process of ingestion and digestion, including rumination patterning and CH₄ production, may potentiate the opportunity to implement novel rumination-based feeding practices to reduce enteric CH₄ emissions.

A 2-phase study was conducted to examine the day-to-day variability and functional relationships between 24-h rumination time and CH₄ emissions of lactating Holstein-Friesian cows in a pasture-based automatic milking system. The central hypothesis was the existence of consistent, persistent rumination patterns and CH₄ emissions in animals that exhibit high or low rumination activity. It was also hypothesized that consistently high or low rumination patterns and CH₄

emissions are related to animal traits commonly associated with changes in milk production. Animals showing consistently high rumination levels were also expected to have greater CH₄ emissions and milk production, but would have lower yield of CH₄/milk as a consequence of increased milk production. Thus, the first objective of the study was to test the prediction of temporal consistencies in rumination and CH₄ emission using dairy cows that were previously observed to have high or low rumination levels. The second objective of the study was to compare expected differences in CH₄ emissions and rumination patterns on the basis of performance traits of high versus low ruminating animals. Finally, mechanistic linkages between milk yield, rumination and CH₄ production, and their likely direct and indirect effects on the yield of CH₄/milk were explored by structural equation modeling.

MATERIALS AND METHODS

Study Location and Facility Layout

The study was conducted from May 10, 2014, to June 15, 2014, at the Michigan State University's Pasture Dairy Research Center (PDRC), which is administered and operated by the W.K. Kellogg Biological Station, located in Hickory Corners, Michigan. Protocols for animal handling and care were reviewed, approved, and conducted according to the Michigan State University's Institutional Animal Care and Use Committee, under application number 02/14-024-00.

The PDRC is located on a sandy loamy site. Soils are typical hapludalfs with a sandy loam layer over the first 30 cm followed by variable clay and gravelly sand layers. These soils have a high drainage capacity, relatively rapid infiltration rate, and moderate water-holding capacity. The historical annual precipitation at the PDRC ranges between 760 and 915 mm, the mean annual air temperature 9.5°C (range: -16.7 to 32.2°C), and the frost-free period ranges between 140 to 150 d. The mean air temperature during the study period was 19.4°C, ranging from 12.6 to 25.9°C. A total of 60 mm of precipitation fell over 8 d during the study period.

The PDRC consisted of a grazing platform (east and west sections) of perennial pasture, subdivided into 1-ha paddocks for rotational grazing management of lactating cows. Paddocks were connected via split laneways to a central milking barn with the capacity to milk 2 separate herds. The milking barn layout included 2 freestall pens (58 stalls/pen), each equipped with 1 single-stall automatic milking system (AMS; Astronaut A3, Lely Industries, NV, Maassluis, the Netherlands), and one 2-way drafting gate controlling the exit of cows

to pasture (Grazeway gate, Lely Industries, NV). Additional features of the facility included automatic manure scrapers alongside freestall alleyways. Cow brushes and freestalls covered by double chamber water-mattresses provided additional cow comfort.

Other key features of the PDRC include the use of a split, spring-fall calving system. On a normal year, cows are housed on a partial mixed ration (**PMR**; 65% forage) during winter (usually from December to April), and are rotationally grazed on adjacent pasture the remainder of the year. Pasture utilization is approximately 10 t of DM/ha or between 3 to 3.5 t of DM/cow.

The present study started at the onset of the grazing season of 2014 (May 1) and lasted a total of 47 d (Table 1). The first 15 d served for the dietary adjustment of cows onto a pasture-based feeding system comprising approximately 25% concentrates and 75% grazed pasture. Thereafter, a 10 d pre-experimental period was used for baseline determination of the rumination activity of cows, followed by a 22-d experimental period for comparison of high and low ruminating animals (Table 1).

Animals

The lactating herd consisted of 156 (148 ± 8 on any given study day) Holstein-Friesian cows (80% US Holsteins plus 20% New Zealand Friesians), grazed and milked in 2 separate herds (65 ± 10 cows/group in any given study day). The lactation profile and characteristics of cows was as follows: parity (2.15 ± 1.08), milk production (kg/d; 29.39 ± 11.61), DIM (174.20 ± 162.24), milking frequency (milking/d; 1.89 ± 0.76), BW (kg; 609.91 ± 96.98), concentrate intake (kg/d; 4.86 ± 1.96), and rumination time (min/d; 489.07 ± 121.15).

Upon completion of the pre-experimental phase, 2 rumination groups ($n = 37$ cows/group) represented by cows with consistently high rumination (**HR**; 75th rumination percentile) and low rumination (**LR**; 25th rumination percentile) were selected according to the previous 10-d rumination time. The HR and LR groups included 14% and 16% of NZ Friesian cows, respectively. The rest of the cows in the HR and LR groups were US Holsteins. Cows within each rumination group were paired by nearest parity, DIM, BW, and previous 10-d production and within pairs reassigned to 1 of the 2 experimental herds according to a completely randomized design. Our objective was to achieve even distribution of similar HR and LR cows between the 2 herds. Cows remained in the assigned herd until completion of the study.

Feeding Protocols, Grazing Management, and Herbage Characteristics

Before commencing with the study, cows received ad libitum forage-based PMR (65% forage as haylage and corn silage) plus a supplement pellet during milking in the AMS at a rate of 1 kg of feed for every 8 kg of milk, up to a maximum of 7 kg/d. The average DM, CP, NDF, and NEL of the pellet was 89.40%, 19.30%, 9.94%, and 2.05 Mcal/kg, respectively. At study onset, cows were transitioned onto a pasture-based diet where the same pellet concentrate was offered at a rate of 1 kg of feed for every 6 kg of milk, with the same maximum of 7 kg/d. This level of supplementation of concentrate remained over the entire study.

Cows had 24 h access to pasture from the milking barn. Grazing management consisted of the rotational grazing of the 2 herds on a separate platform of 16 ha of pasture containing 72% perennial ryegrass (*Lolium perenne*) and 28% white clover (*Trifolium repens*). Each day, herds received 2 breaks of fresh pasture on alternating locations of the farm (north and south); this 2-way grazing system (i.e., A-B grazing schedule) was decided for improved traffic of cows on a voluntary milking system (Lyons et al., 2013). Pasture allocations were made available from 1000 to 2200 h and from 2200 to 1000 h, respectively. Indirect estimation of pregrazing and postgrazing herbage mass (to ground level) was conducted from measurements of sward height collected with a rising plate meter and a predicting equation for spring ryegrass-white clover pasture developed on site ($HM = 125 \times SH$, $R^2 = 0.96$, where HM = herbage mass in kilograms of DM/ha; SH = sward height in centimeters). Herbage mass was determined using a total 30 sward height readings collected alongside pasture allocations. The prescribed pasture allowance (from ground level) was 30 kg of DM/cow per day, equally divided into 2 allocations of 15 kg of DM/cow. Average pregrazing herbage mass was $3,200 \pm 155$ kg of DM/ha, and the average postgrazing herbage mass following grazing was $1,707 \pm 63$ kg of DM/ha. The resulting average grazing intensity of $1,493 \pm 130$ kg of DM/ha (or about 47% of available pasture) was equivalent to a pasture disappearance of $1,040 \pm 59$ kg of DM/herd per day, or 16.0 ± 0.9 kg of DM/cow per day. Perennial ryegrass plants were in a phenological stage V3 to E2 (3 collared leaves to second node palpable or visible; Moore et al., 1991). The phenological stage of white clover plants was early (<15 cm) to mid (<30 cm) vegetative (Kalu and Fick, 1981). The average CP (4010 CN combustion system, Costech Analytical Technologies Inc., Valencia, CA), NDF and ADF (200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY),

Table 1. Characteristics of the study

Phase (duration)	Animals	Objective	Measurements and database	Management characteristics
Adaptation (15 d)	156 milking cows	Conditioning cows to grazing-based diets consisting of 75% and 25% concentrate	NA ¹	<ul style="list-style-type: none"> • Voluntary milking system • Milking access based on lactation days and milk yield • Two-way grazing system • Two allocations of perennial ryegrass and white clover pasture per day (AM and PM) • Daily herbage allowance of 30 kg of DM/cow evenly split between two daily pasture allocations • Pellet concentrate fed during milking at 1 kg of feed per every 6 kg of milk; maximum 7 kg
Pre-experimental (10 d)	156 milking cows	Baseline determination of herd rumination time and detection of low ruminating (LR; 25th percentile) and high ruminating (HR; 75th percentile) animals	<ul style="list-style-type: none"> • Parity and days in lactation • Body weight • Milk production • Rumination time 	
Experimental (22 d)	74 cows, including 37 HR and 37 LR cows	Monitoring and assessment of rumination time and methane and carbon dioxide emissions and yield of LR and HR animals	<ul style="list-style-type: none"> • Parity and days in lactation • Body weight and BW change • Milk production • Rumination time • Methane and carbon dioxide fluxes • Intake and conversion • Activity 	

¹NA = not applicable.

and in vitro DM digestibility (48 h incubation; Daisy II, Ankom Technology Corp.) of pasture ($n = 16$) over the 22-d experimental period was $23.5 \pm 2.6\%$, $45.3 \pm 4.1\%$, $27.1 \pm 1.9\%$, and $77.1 \pm 4.8\%$, respectively.

Animal Measurements

Milk Performance and BW. The AMS identified each cow visit by an electronic identification neck collar (QWES-HR Tag, SCR Engineers Ltd., Netanya, Israel) and collected daily information on BW, milk production, milkings/day, milking refusals/day, milking failures/day, total visits/day, and amount of concentrates distributed during milking. Data of the AMS operation and records of individual cows were retrieved through the use of the AMS software (T4C software; Lely Industries N.V., Maassluis, the Netherlands). Body weight change was determined as the slope of the regression of daily BW measurements over the 22-d experimental period.

Rumination Time and Animal Activity. Estimation of daily rumination time (min/d) and activity index of cows were collected by electronic loggers (QWES-HR Tag, SCR Engineers Ltd.) enclosed in the neck identification collars. A description of the equipment is provided in the manufacturer's patent (Bar-Shalom, 2003), and the accuracy of the device as a monitoring tool for rumination time (Schirrmann et al., 2009) and activity, including number of steps and walking activity of grazing cows, was previously tested, validated, and reported (Elischer et al., 2013). Briefly, the collar incorporates a 1-dimensional accelerometer and onboard microprocessor, which integrates motion data on a 2-min resolution and provides a weighed activity index in 12×2 h periods per day. In addition, a built-in microphone and acoustic microprocessor are used to filter digitalized sounds of regurgitation and mastication during rumination. Acoustic data are filtered on a 2-min resolution, and also summarized into 12×2 h periods per day. All processed data are stored in the tag for up to 22 h, before the first 2-h period is overwritten. Finally, all activity and rumination data are transferred to the main database by stationary infrared readers located in the AMS. The AMS software provided a 24-h average rumination time and activity index, as well as segmented information of rumination time and weighed activity in 12×2 h periods per day.

Fluxes of Methane and Carbon Dioxide. A gas capture and quantification system (C-Lock Inc., Rapid City, SD) retrofitted into the AMS stalls was used for spot measurement of enteric methane (QCH_4 ; g/d) and expired carbon dioxide (QCO_2 ; g/d) fluxes during milking, which lasted between 6 to 14 min. Data ac-

quisition included ambient pressure, temperature, humidity, CH_4 and CO_2 concentrations [by nondispersive infrared (NDIR) sensors; maximum span: $CO_2 = 0$ to 4%, $CH_4 = 0$ to 2%; Advanced Gasmittter, Sensors Inc., Saline, MI], detection of muzzle position relative to the air sampling inlet (0 or 1), airflow rate, and radio frequency identification (RFID) specific to each cow. All variables were logged at 1-s intervals. The system was recently validated by Hammond et al. (2013) against equivalent gas measurements conducted on respiration chambers, or by the use of the sulfur-hexafluoride (SF_6) tracer technique. A more complete description and operation of the equipment at the PDRC and calculation of gas fluxes is provided in Huhtanen et al. (2015).

All 74 cows were sampled multiple times during the 22-d experimental period, with each of the sample points distributed over the diurnal feeding cycle of cows, according to their own voluntary milking schedule. This large sample size and use of distributed sampling over time provided highly repeatable measurements of QCH_4 [$Rep = \delta_{cow}^2 / (\delta_{cow}^2 + \delta_{residual}^2) = 0.79$] and QCO_2 ($Rep = 0.84$). Thus, protocols minimized most sampling variability or bias that could be associated with voluntary changes in feeding rate, timing, or both, of feeding relative to the time of gas sampling (Hegarty, 2013). Double point calibration of NDIR sensors (Advanced Gasmittter, Sensors Inc., Saline, MI) and gas recovery testing were conducted during the study. The low standard gas mixture was 500 ppm CH_4 and 1,500 ppm CO_2 and the high concentration gas was 1,500 ppm CH_4 and 10,000 ppm CO_2 (Matheson Tri-Gas Inc., Basking Ridge, NJ). The balance gas was nitrogen. Gas recovery testing was performed by controlled release of CO_2 at 30 cm horizontal distance from the AMS manifold. This protocol mimicked qualifying animal nostril position during sampling periods. Portable cylinders prefilled with CO_2 (KEE Action Sports, LLC, Sewell, NJ) were used. Release trials lasted 3 min and were repeated 9 times. The mean recovery of CO_2 was 99.7% with a standard error of the mean of 0.3%.

Gas flux data were calculated using the open-circuit gas quantification principle (McLean and Tobin, 1987). This principle combined the continuous measurement of active airflow rate and concentration of emitted gases captured by the system's intake manifold (70 L), as follows:

$$Q_{C(i)} = C_{p(i)} \times (\text{Conc}_{(i)} - B\text{Conc}_{(i)}) \times Q_{\text{air}(i)},$$

where C_p = the measured capture rate of gas at any time i , Conc = the concentration of captured gas (ppm), $B\text{Conc}$ = the background concentration of gas (ppm), and Q_{air} = the volumetric air flow rate (L/min)

passing through the system's manifold, automatically determined on a dry gas basis at 1 atm, upstream of the intake manifold.

All data were processed automatically, near real time. The algorithm also included automatic inspection and filtering by muzzle position to remove bias of estimations associated with the capturing of diluted gas (Huhtanen et al., 2015). The acquisition of at least 3 min of filtered gas data (i.e., head position <30 cm from the system's air inlet) was required to qualify a sampling period as valid. Sampling periods that did not meet this criterion were not included in the analysis. Finally, daily fluxes of QCH_4 and QCO_2 by individual animal were calculated as the moving average of all flux measurements collected within a 3-d period. This calculation included between 5 and 13 gas flux samples.

Estimated Intake. The direct determination of individual DMI by common methodologies (i.e., external markers) requires obtrusive sampling (i.e., marker dosing and fecal collection), and can create interferences with grazing behavior and traffic of cows in a voluntary milking system. Consequently, unobtrusive, measurement of QCO_2 was used as an internal biomarker for estimation of individual DMI according to Casper and Mertens (2010). This model is based on very strong relationships between DMI and known CO_2 emissions adjusted for milk yield [QCO_2 , g/d = $[821.3 + (126.0 \times DMI) - (1.18 \times \text{milk})]/0.27$; $n = 1,252$; $R^2 = 0.86$, $SER = 217.7$, $P < 0.01$], and covers ample range of diet variability, milk production (5.1 to 56.6 kg/d, average of 23.0 kg/d), and CO_2 emissions (3,552 to 17,222 g/d, average of 10,444 g/d), as shown for cows of the present study. The model was previously tested and validated for incorporation into the Cornell Net Carbohydrate and Protein System v 6.1 (CNCPS v6.1; Fox et al., 2004) for comparison of DMI and CO_2 emissions from different feedstuffs and feeding systems, including high-forage diets (Higgs et al., 2013; Russomanno et al., 2013).

Pasture intake was estimated as the difference between the DMI estimated from CO_2 data and the known consumption of concentrates measured by the AMS. Finally, feeding efficiency and rumination intensity were calculated as follows. Feeding efficiency was the quotient between estimated DMI and the activity recorded on individual cows. Rumination intensity was the quotient between rumination time and the estimated DMI of individual cows.

Calculations and Statistical Analysis

All statistical analyses were conducted with SAS version 9.2 (SAS Institute Inc., Cary, NC). All rumina-

tion data from the original 156 cows were previously inspected for reliability and outliers using descriptive statistics produced with the UNIVARIATE procedure. Two cows with incomplete 2-h rumination periods were removed from the data set as accuracy of 24-h estimation could be affected by the use of incomplete 2-h measurement periods. Outliers were defined as any cow exhibiting mean rumination values above or below 1.5 times the interquartile range ($Q3-Q1$) determined for the herd population. Cows identified as outliers ($n = 11$) were removed from the data set and excluded from the classification of HR and LR groups. In addition, 2 sets of QCH_4 and QCO_2 data did not correspond to animals within the study due to RFID failure by the gas system; data were removed from the data set.

Inspection of pre-experimental rumination data (-10 to 0 d) for rumination time (min/d) and sorting of HR and LR cows were conducted with the FREQUENCY procedure. The criteria of classification evaluated the frequency distribution of rumination time for all cows in the herd ($n = 156$) together with the detection of the 75th and 25th percentile for classification of subsets of cows into HR and LR groups, respectively. This detection criteria produced 2 symmetric groups of experimental cows ($n = 37/\text{group}$).

All data (1 to 22 d) of the resulting 74 experimental cows were analyzed by least squares ANOVA for a completely randomized design, using maximum likelihood for repeated measure mixed models with the MIXED procedure. The model assumption for normal distribution of residuals was met; however, differences ($P < 0.05$) in variance between rumination groups were detected according to the Brown and Forsythe's test. Therefore, weighted least square analyses were used to account for variance differences between groups. Parity, DIM, milk production, milking frequency, milking refusals, AMS visits, DMI, concentrate intake, pasture intake, estimated feed conversion (milk/DMI), QCH_4 , QCO_2 , QCH_4/QCO_2 ratio, yield of QCH_4/milk , yield of QCO_2/milk , rumination time, rumination intensity (rumination/DMI), activity, average BW, and adjusted response variables for metabolic BW ($\text{kg}^{0.75}$) were analyzed by the following mixed model:

$$Y_{ijkl} = \mu + R_i + C_{j(i)} + D_k + R_i D_k + e_{ijkl}$$

where Y_{ijkl} = the dependent response variable, μ = the overall mean; R_i = rumination group i (HR or LR), $C_{j(i)}$ = the random effect of cow j nested within group i , D_k = the repeated effect of day k (0 to 22), $R_i D_k$ = the interaction between rumination group and day, and e_{ijkl} = the residual error term. The term $C_{j(i)}$ was defined as SUBJECT; therefore, models evaluated changes on

response variables over time within each animal. Selection for best covariance among unstructured, compound symmetry, and autoregressive order 1 structure were conducted by the lowest Bayesian information criterion (Littell et al., 2006). All models used the Kenward and Roger (1997) method for determination of degrees of freedom for the test fixed effects.

Repeatability analysis of QCH_4 , QCO_2 , and rumination was conducted with the MIXED procedure of SAS. Following Huhtanen et al. (2015), the source of variance associated with animal and residual (i.e., variance due to instrument, measurement scale, environment, etc.) was used to model the repeatability of traits, as follows:

$$\text{repeatability} = \delta^2_{\text{cow}} / (\delta^2_{\text{cow}} + \delta^2_{\text{residual}}),$$

where δ^2 is variance.

Body weight change was analyzed with the following mixed model:

$$Y_{ijkl} = \mu + R_i + C_{j(i)} + e_{ijkl}$$

where Y_{ijkl} = the dependent response variable, μ = the overall mean, R_i = rumination group i (HR or LR), $C_{j(i)}$ = the random effect of cow j , and e_{ijkl} = the residual error term.

The relationships between estimated DMI, milk production, rumination, QCH_4 , and output yield of QCH_4 /milk were examined by structural equation modeling, using the CALIS procedure of SAS (Hartmann, 1992). This multifactorial analysis allows partitioning of multiple regression coefficients into direct effects (standardized partial regression coefficients) of explanatory variables on dependent variables, and indirect effects of independent variables through the correlation with other independent variables. The standardized regression coefficients (r) estimated by the model assessed the strength, direction, and dependency of relationships between the independent explanatory variable, dependent variables, and the observed variable of consideration (Pugesek et al., 2003). A chi-squared test was used to verify goodness of fit by the model. Additional nonlinear regression analysis was used to model likely relationships between milk production and yield of QCH_4 /milk and QCO_2 /milk, across all experimental cows. The following exponential decay model was fitted using the NLIN procedure of SAS:

$$Y_i = a - b * (1 - \text{Exp}^{-c * X_i}),$$

where Y_i = QCH_4 /milk or QCO_2 /milk at milk production i , X_i = milk production i , a = the gas yield at zero milk production, b = the maximum gas yield, and c

= the rate constant decay of gas yield with increasing milk production.

The significance level for all analyses was set at $P < 0.05$. All results were reported as least squares means $\pm$ SEM, when appropriate.

RESULTS

Rumination Patterns

Low to moderate repeatability was detected for rumination time (repeatability = 0.26) of experimental cows included in the high- and low-rumination groups (Figure 1). A significant rumination group ($P < 0.001$), day ($P < 0.001$), and interaction between rumination group and day effect ($P = 0.001$) was observed on rumination time (Table 2, Figure 2A). Similarly, when rumination time was adjusted for metabolic BW, rumination group ($P < 0.001$), day ($P < 0.001$), and the interaction of rumination group by day ($P = 0.004$) remained significant (Figure 2B). Consistent differences in rumination over time were observed between HR and LR groups. Rumination time was 42% greater for cows in the HR group (Table 2). The rumination time by HR cows showed increased day-to-day variation particularly around d 2 to 3, 11 to 12, 16 to 17, and 19 to 20 (Figure 2A).

Methane Production

High repeatability of QCH_4 (repeatability = 0.79) and QCO_2 (repeatability = 0.84) were detected. Methane fluxes were not affected ($P = 0.74$) by an interaction between rumination group and days, but significant differences between rumination groups ($P = 0.001$) and days ($P < 0.001$) were observed; the HR cows consistently emitted 18% more CH_4 throughout the study (Figure 3A). Similarly, when QCH_4 fluxes were adjusted for metabolic BW, rumination group ($P = 0.052$) and day ($P < 0.001$) effects remained significant (Figure 3B), and no interaction between rumination groups and days ($P = 0.89$) was detected. The LR cows had a greater ($P < 0.001$) QCH_4 /rumination time ratio than the HR cows (1.00 ± 0.06 vs. 0.68 ± 0.03) despite their lower QCH_4 and rumination time (Table 2).

Milk Production and Traits of High and Low Ruminating Cows

The production traits of the HR and LR cows are shown in Table 2. The HR cows had lower DIM and greater parity and BW compared with LR cows, but their BW change was not different from that measured on LR cows. The milk production and milking frequency of cows varied according to a rumination

Table 2. Characteristics and production variables (least squares mean $\pm$ SEM) of high (HR) and low (LR) rumination cows in a pasture-based automatic milking system

Variable ²	Rumination group		P-value ²		
	HR	LR	G	D	G $\times$ D
Animals					
DIM	144 $\pm$ 5	183 $\pm$ 5	<0.001	NA	NA
Parity	2.45 $\pm$ 0.03	1.48 $\pm$ 0.03	<0.001	NA	NA
BW (kg)	633.16 $\pm$ 13.32	586.72 $\pm$ 18.12	0.043	<0.001	<0.001
BW change (kg/d)	-0.136 $\pm$ 0.14	-0.514 $\pm$ 0.23	0.161	NA	NA
Milk production and AMS use					
Milk (kg)	36.89 $\pm$ 1.80	22.87 $\pm$ 1.53	<0.001	<0.001	<0.001
Milkings/d	2.13 $\pm$ 0.09	1.64 $\pm$ 0.08	<0.001	<0.001	0.015
Refusals/d	0.20 $\pm$ 0.04	0.13 $\pm$ 0.03	0.181	0.004	0.001
AMS visits/d	2.34 $\pm$ 0.11	1.78 $\pm$ 0.10	<0.001	<0.001	0.022
Estimated DMI					
Pasture (kg)	17.09 $\pm$ 0.66	15.31 $\pm$ 0.66	0.061	<0.001	0.372
Concentrate (kg)	5.82 $\pm$ 0.25	3.84 $\pm$ 0.27	<0.001	<0.001	0.203
Total intake (kg)	22.91 $\pm$ 0.68	19.15 $\pm$ 0.71	<0.001	<0.001	0.563
Total intake (% BW)	3.65 $\pm$ 0.12	3.39 $\pm$ 0.12	0.137	<0.001	0.202
Feed conversion (kg/kg)	1.61 $\pm$ 0.08	1.19 $\pm$ 0.06	<0.001	<0.001	0.452
Gas flux					
QCH_4 (g/d)	413.33 $\pm$ 13.81	338.69 $\pm$ 17.46	<0.001	<0.001	0.741
QCO_2 (g/d)	13,428.13 $\pm$ 315.54	11,760.21 $\pm$ 390.53	<0.001	<0.001	0.944
QCH_4/QCO_2 ratio	0.031 $\pm$ 0.001	0.029 $\pm$ 0.001	0.087	<0.001	0.381
QCH_4 /milk (g/kg)	12.25 $\pm$ 0.78	16.39 $\pm$ 1.09	0.003	<0.001	0.870
QCO_2 /milk (g/kg)	398.91 $\pm$ 21.11	577.26 $\pm$ 32.61	<0.001	<0.001	0.081
Activity and rumination					
Activity index	574.51 $\pm$ 17.47	719.16 $\pm$ 31.81	<0.001	<0.001	0.049
Rumination (min/d)	618.22 $\pm$ 7.05	357.38 $\pm$ 5.89	<0.001	<0.001	0.002
Rumination intensity (min/kg)	28.19 $\pm$ 0.99	19.95 $\pm$ 0.97	<0.001	<0.001	0.068
Feeding efficiency (g/activity)	42.12 $\pm$ 1.86	30.40 $\pm$ 2.36	<0.001	<0.001	0.052

¹Feed conversion = kilograms of milk per kilogram of DMI; activity index = daily activity recorded by accelerometer technology; rumination intensity = rumination time per kilogram of DMI; feeding efficiency = grams of DMI per unit of animal activity.

²Probability value of ANOVA for rumination group (G), day (D), and interaction between rumination group and day (G $\times$ D); NA = not applicable; QCH_4 = flux of methane; QCO_2 = flux of carbon dioxide.

Figure 1. Frequency distribution of cows as a function of rumination time (min/d). Grey bars indicate the 25th rumination percentile of cows classified as low ruminating animals. White bars indicate the 75th rumination percentile of cows classified as high ruminating animals.

Figure 2. Temporal trend of rumination time (A) and rumination time adjusted for metabolic BW (B) by cows classified as high (HR) and low (LR) rumination animals. Vertical bars denote the standard error of means ($n = 37$).

group by study day interaction, but the HR cows always produced more milk and milked more frequently in the AMS compared with LR cows. The total DMI also differed across study days and between HR and LR cows, but the difference between rumination groups disappeared when it was expressed on % BW basis. The intake of concentrates and gross feed conversion also varied across study days and were greater for HR cows. A tendency for greater pasture intake was also noted for HR cows.

As shown in Table 2, the QCH_4 and QCO_2 were greater for HR cows than LR cows and regardless of the observed variability in gas production detected across study days. The QCH_4/QCO_2 ratio remained near 3% despite the slight tendency for lower QCH_4/QCO_2 ratio noted for LR cows. The yield of QCH_4 and QCO_2 per unit of milk output also varied across study days and was lower for HR than LR cows. A study day by rumination group interaction was observed on the activity and rumination of cows, with the LR cows always displaying greater activity and lower rumination than HR cows. The rumination intensity and feeding

efficiency varied by study day and were greater for HR than LR cows.

Relationships Between Rumination and Methane Production

Several relationships between independent, dependent, and observed variables were found (Figure 4). Estimated intake had a positive direct effect ($P < 0.001$) on rumination time, QCH_4 , and milk production, with additional indirect effects ($P < 0.001$) on milk production explained by positive changes in rumination time (Figure 4). Despite these observations, rumination time had no direct effect ($P > 0.05$) on QCH_4 or yield of QCH_4 /milk; however, rumination time did have a negative indirect effect ($P < 0.001$) on the yield of QCH_4 /milk as a result of positive effects on milk production (Figure 4). In turn, yield of QCH_4 /milk directly increased ($P < 0.001$) by increases in QCH_4 and concurrent decreases ($P < 0.001$) in milk yield (Figure 4). A negative exponential relationship ($P < 0.001$)

Figure 3. Temporal trend of methane (QCH_4) fluxes (A) and methane fluxes adjusted for metabolic BW (B) in cows classified as high (HR) and low (LR) rumination animals. Vertical bars denote the standard error of means ($n = 37$).

Figure 4. Structural equation modeling depicting relationships (direct and indirect) between estimated DMI, rumination time, methane flux (QCH_4), milk production, and yield of QCH_4 /milk of dairy cows in a pasture-based automatic milking system. The significant (solid arrows) and nonsignificant (dashed arrows) paths between independent (solid white rectangle), dependent (gray rectangles), and observed output (dashed white rectangle) variables are shown together with their corresponding standardized regression coefficient and level of significance (** $P < 0.0001$; NS = $P > 0.05$; $n = 74$).

was found between milk production and QCH_4 /milk, or QCO_2 /milk (Figure 5).

DISCUSSION

The present study used novel technologies and methods to investigate the day to day variability in 24-h rumination time of grazing dairy cows and potential associations with feed intake and CH₄ emissions. In addition, many results generated from this study have been previously unexplored. Results therefore provide new insights on alternative techniques for grazing research, as well as on factors that can affect the rumination time and CH₄ emissions in grazing cattle.

Rumination Patterns

Low to moderate trait repeatability suggests that a high proportion of the variability in rumination time was due to factors that were not related to the cow, for example variations due to instrument or environmental effects. However, cows that were previously classified as HR or LR ruminating animals maintained consistent rumination time throughout the study (Figure 2A). The HR cows ruminated 618 min/d, which equated to a 42% increase over the 357 min/d by LR cows. A similar high difference of rumination time among cows was shown in previous studies (Beauchemin, 1991;

Dado and Allen, 1994), although to our knowledge, the extent and causes of this variation as well as their implications on rumination patterning over time have not been documented, particularly in grazing cows.

Figure 5. Relationship between yield of methane (QCH_4 /milk; circles) and carbon dioxide (QCO_2 /milk; squares) per unit of milk and the milk production of dairy cows on a pasture-based automatic milking system. Values for cows classified as high (HR; solid symbols; $n = 37$) or low (LR; open symbols; $n = 37$) ruminating animals are shown with corresponding exponential functions showing reductions of QCH_4 /milk [solid line; $Y = 43 \pm 6 - 36 \pm 5 \times (1 - \exp^{-0.06 \pm 0.01 \times \text{milk}})$; $R^2 = 0.69$] and QCO_2 /milk [dashed line; $Y = 1,474 \pm 140 - 1,252 \pm 114 \times (1 - \exp^{-0.06 \pm 0.01 \times \text{milk}})$; $R^2 = 0.84$] as a function of milk production, across all animals.

In the present study, inherent behaviors that dairy cows typically express on a pasture-based system (with voluntary milking), including the occurrence of distinct grazing behaviors, may have contributed to the observed differences in rumination patterns. Ruminants, including dairy cows, can display a high degree of variability in grazing patterns, usually driven by behavioral adjustments that individual cows make to cope with changes in forage intake (Hancock, 1954; Hodgson, 1990). Grazing behavior can affect the daily rumination time and rumination patterning of grazing cows (Gregorini et al., 2012), as it was shown previously that feed-restricted animals can compromise rumination time to sustain intake (Greenwood and Demment, 1988). Likewise, the selectivity for nutritious plant parts (Hardison et al., 1954) or species (Rutter et al., 2004) also differs among dairy cows. Consequently, increased selectivity has the potential to alter rumination if cows select and digest diets with contrasting fiber level, particularly effective NDF (Beauchemin, 1991; Mertens, 1997). Perennial ryegrass and white clover (as in this study) have a different distribution within grazed horizons, with prostrate clover plants usually containing lower NDF than taller perennial ryegrass plants (Enriquez-Hidalgo et al., 2014). Thus, it is possible that diet choice and selectivity in response to plant species composition and spatial patterns may have influenced the rumination time if cows achieved diets differing in ryegrass and clover content (Rutter et al., 2004), but diet selection was not measured in the present study.

Grazing cows also behave according to a complex herd organization, displaying an entire suite of correlated behaviors (i.e., behavioral syndromes) according to their social status, age, and hierarchical position (Phillips and Rind, 2002). The HR cows, on average had higher parity, which is consistent with the observation of greater rumination in older, often more dominant animals, than younger subordinate counterparts (Gregorini et al., 2013). The HR cows also had greater milk production, concentrate intake, and estimated intake, in total. These cows also displayed lower grazing effort according to a lower activity (Elischer et al., 2013), which in turn may have provided additional time to milk in the AMS more frequently or to program increases in rumination time as a consequence of reduced grazing activity, as was shown previously (Kennedy et al., 2011). Differences in rumination time appear to be a consequence of complex associations between feed intake, modifications of grazing activity, and milk production, which in the present study were possibly driven by positive effects of feed intake on both rumination time and milk production (Figure 4). Thus, higher intake with reduced activity at pasture (as a consequence of greater concentrate intake and degree of satiety) is sug-

gested as a possible driver for higher rumination in HR cows. This observation agrees with Kononoff and Heinrichs (2003), who reported increased rumination time with increases in DMI in dairy cows fed forage-based diets. The positive effect of feed intake on rumination time has been extensively reported in older literature (Welch and Smith, 1969). Conversely, less rumination time by LR cows could be driven by increases of grazing activity at pasture, as these cows milked in the AMS less frequently while consuming less concentrate and displaying a greater degree of activity (Table 2). This response could be related to an attempt to compensate reductions of instantaneous intake rate associated with increased depletion of pasture allocations over time (as LR cows may have remained longer time at pasture, which is consistent with greater measured activity), and lower concentrate consumption (Welch and Smith, 1969). In addition, the lower rumination time and estimated intake by LR cows could be related to lower energy requirements associated with their lower milk production and body size, or to a subordinate position within the herd, as these cows also had smaller body size and parity (Table 2).

The consistent differences in rumination time after adjustment for metabolic BW (Figure 2B) suggests that other biological or physiological differences that were not allometrically scaled to BW may have affected rumination. These may include differences in digestive capacity (Dado and Allen, 1994) or retention of digesta in the rumen and its rate of passage (Ørskov et al., 1988). The influence of these factors on the rumination of dairy cows at pasture was previously suggested in the study of Gregorini et al. (2013), but to our knowledge a paucity of studies have investigated the effect of digestive and physiological drivers on rumination. It is likely that a complex arrangement of both interacting abiotic factors (i.e., distance to pasture or water) and biotic factors (i.e., pasture quality and quantity), which can affect both physiological and behavioral mechanisms, regulate the rumination time of cows at pasture; however, this hypothesis requires further development and investigation.

The HR cows also showed increased rumination variability on particular study days (Figure 2A). This day effect was previously shown to account for up to 62% of the total variation in rumination time (Hancock, 1954). This day to day variation could be related to the high milk production of HR cows as dairy cows with high requirements usually show greater degree of response to changes in temperature (West, 2003), diet composition (Beauchemin, 1991), or modifications of feed intake (Maulfair et al., 2010). In the present study, greater day to day variability in rumination time by HR cows were correlated with day to day differences

in estimated DMI ($r = 0.49$) and animal activity ($r = -0.45$), but following a post hoc inspection of weather data, no evidence was detected to support that these daily variations in rumination time, DMI, or activity were related to changes in daily air temperature, precipitation, wind speed and direction, atmospheric pressure, or solar radiation (not shown).

Estimation of Feed Intake

Although a measure of DMI is important to inform nutritional recommendations, our capacity to routinely measure the individual DMI of grazing dairy cows is highly limited (Hills et al., 2015). In the past, different techniques based on agronomic methods (i.e., clipping of pasture before and after grazing) or external markers (i.e., n-alkenes, chromium oxide, etc.) have been proposed to estimate DMI of free ranging animals, all with varying results, limitations for use in large groups of grazing animals (Mayes and Dove, 2000), or both. In the present study, an alternative technique for estimation of DMI at the individual animal level was proposed. First, highly repeatable quantification of QCO_2 fluxes was conducted with a noninvasive sampling technique (Huhtanen et al., 2015). Then, empiric estimation of DMI was conducted by interpolation, using a previously published linear regression of QCO_2 on DMI and milk production (Casper and Mertens, 2010). Interestingly, the average between the individual pasture DMI of HR and LR cows (16.2 ± 0.2 kg of DM; Table 2) determined by the proposed CO_2 technique was no different to the herd average DMI that was calculated by difference between the pregrazing and postgrazing herbage mass (16.0 ± 0.9 kg of DM). This finding is relevant in 2 ways. First, it supports the use of CO_2 fluxes as an internal biomarker for accurate estimation of individual DMI (both in total and of pasture) of grazing cows. Second, the proficiency to routinely use QCO_2 measurements for estimation of DMI at the individual animal level could open new opportunities for novel grazing research as well as for developing protocols to monitor, evaluate, and manage feed intake in commercial farms (Hills et al., 2015). Importantly, the present study indicates that repeatable and individual measurements of QCO_2 can produce estimates of pasture DMI that are consistent with field measurements of pasture disappearance.

Methane Production

High repeatability of carbon gas fluxes suggests that most of the observed variability in animal gas production was related to differences among animals and not to the instrument or sampling technique. As expected,

differences in QCH_4 between HR and LR cows were also observed (Figure 3A), with HR cows producing more QCH_4 than LR cows even after adjustment for metabolic BW (Figure 3B). This greater CH_4 emission was also coincident with greater estimations of DMI in HR cows (Table 2), because intake level had a strong positive effect on CH_4 output (Figure 4). Feed intake is regarded as the single most important predictor of CH_4 production in dairy cows (Dijkstra et al., 2013). Increased DMI results in more total CH_4 production because more feed fermented in the rumen yields more hydrogen (H^+) equivalents for methanogenesis (Moe and Tyrrell, 1979; Bannink et al., 2006) by archaea microorganisms (Ellis et al., 2008). However, the formation of CH_4 is not constant. As DMI increases above the maintenance intake level a fractional reduction occurs in the yield of CH_4 per unit of feed intake or milk production (Johnson and Johnson, 1995; Shibata and Terada, 2010; Knapp et al., 2014), as noted in this study (Figure 5). Increased DMI is associated with lower rumen retention time, lower diet digestibility (Tyrrell and Moe, 1975) particularly NDF (Huhtanen et al., 2009), and reduced rumen pH, and shift in fermentation of carbohydrates (and some protein) toward propionate, which is a pathway that competes with the formation of CH_4 (Ellis et al., 2008). In the present study, HR also increased milk production because increased intake had positive direct effects on QCH_4 and milk yield (Figure 4). Likewise, HR cows consumed more concentrate (both in total and as a proportion of diets; 20% and 25% for LR and HR, respectively), which may have further reduced the yield of QCH_4 /milk. Increasing the intake and energy density of pasture-based diets with concentrates increases milk yield, but also can lower rumen pH, shifting the molar proportion of fatty acids toward propionate; thereby resulting in proportionally less CH_4 (Ellis et al., 2008; Knapp et al., 2014).

In the present study, the average pasture DMI of HR and LR cows (16.2 ± 0.7 kg of DM; Table 2) estimated from CO_2 production (Casper and Mertens, 2010) was similar to the herd average DMI calculated by difference between pregrazing and postgrazing herbage mass (16.0 ± 0.9 kg of DM). Consequently, the results of the present study support the use of CO_2 flux as an internal biomarker for accurate estimation of individual DMI (both in total and of pasture) of grazing cows. In addition, this demonstrated proficiency to measure DMI by a noninvasive methodology and with reasonable accuracy can increase study cows consumed (estimated pasture + concentrate) between $3.2\times$ to $3.8\times$ the maintenance intake level while producing a decreasing amount CH_4 /milk, particularly in HR cows consuming greater proportion of concentrates. Thus, the lower reduction of QCH_4 /milk in LR compared with HR could

be related to a high proportion of fiber (i.e., forage) over starch (i.e., concentrate) in their diet. Consequently, a further decrease of QCH_4 /milk through higher intake of pasture (i.e., pasture level >75%) was more difficult in LR cows, because the extent to which rumen fill limits feed intake generally increases with the level of fiber in diet (Allen, 2000). Thus, a high proportion of fiber, as commonly seen in pasture-based diets, will favor the production of CH_4 and would reduce the capacity to dilute QCH_4 /milk due to increased limitations on feed consumption.

The strong relationship between estimated feed intake and CH_4 production (Figure 4) is also consistent with older reports. Moe and Tyrrell (1979) found that at feeding levels less than $1.5\times$ the maintenance intake level, a high correlation existed between intake and CH_4 production ($r = 0.66$); however, at intake levels greater than $3.5\times$ the level of maintenance, the correlation decreased ($r = 0.53$). It appears that at feeding levels greater than $3.5\times$ the maintenance intake level (similar to this study), the effect of increased intake on CH_4 production is more variable (Shibata et al., 1993). Greater CH_4 emission variability is thought to be related to a greater inherited variability among cows (Vlaming et al., 2008) and associated differences in their potential for milk production (Dijkstra et al., 2013), diet selection (Knapp et al., 2014), rumen fermentation (Ellis et al., 2008), physiological differences in the regulation of feed intake and its effect on CH_4 production (Pinares-Patiño et al., 2007), or a combination of these.

Differences in grazing behavior and correlated differences of grazing efficiency may have also affected CH_4 production. For instance, the LR cows showed greater overall activity (Table 2), a metric which is a strong predictor of total number of steps ($r = 0.75$) and indicator of the overall walking activity of cows in a pasture-based system (Elischer et al., 2013). In addition, the LR cows had lower visits to the AMS (Table 2), indicating that most of the increased activity by LR cows was likely related to changes in grazing activity. Therefore, despite increasing their grazing activity at pasture, the LR cows had lower estimated DMI (in total and of pasture) because their overall feeding efficiency (feed intake/overall activity) was lower (Table 2). Conversely, more total CH_4 output in HR was associated with greater estimated DMI, both overall and per unit of activity, which may have indicated greater intake per unit of walking effort or greater grazing efficiency (Table 2). The potential effect of changes in grazing efficiencies on CH_4 output and correlated changes on grazing and rumination patterning represents a gap of knowledge according to the current literature and more studies on the subject are certainly needed.

Relationships Between Rumination Time, Methane Production, and Milk Yield

The mechanistic relationships (both direct and indirect) between rumination, QCH_4 , and milk production have not been previously investigated or modeled, particularly in grazing dairy cows. Results from this study indicated no independent direct effect of rumination time on QCH_4 (Figure 4), but interestingly LR cows produced 32% more QCH_4 per minute of rumination (0.68 vs. 1.00 g/min) despite the lower QCH_4 and rumination time (Table 2). The actual mechanisms through which cows can achieve different QCH_4 /rumination ratio are unclear, but it could be possible that different QCH_4 /rumination ratios are the result of changes in DMI and associated modifications in grazing behavior. First, a more favorable rumen condition for CH_4 formation in LR cows may have occurred as consequence of lower concentrate intake. In turn, lower concentrate intake by LR cows was associated with lower estimation of DMI (Table 2), which may have reduced rumination time as it was shown that the level of intake also had a direct effect on rumination (Figure 4). Lower concentrate intake may have also resulted in lower fluctuations of ruminal pH, which would have created more favorable rumen conditions for acetate production, thereby increasing CH_4 production and QCH_4 /rumination ratios (Lana et al., 1998). Second, greater QCH_4 /rumination ratio in LR cows was also associated with increases, rather than decreases, in activity at pasture. As discussed previously, LR cows may have compromised rumination time in the attempt to maintain satisfactory intake (Greenwood and Demment, 1988), which in grazing dairy cows can occur either through greater intake per bite, if pasture depletion is low (Laca et al., 1994), or increased grazing time and activity (Hodgson, 1990), as shown for LR cows. Lower concentrate intake may have further increased the motivation (i.e., low satiety) or need (i.e., increased pasture depletion over time) of LR cows to increase grazing activity at pasture, which may have challenged the opportunity for rumination.

Reducing Methane Through Increased Milk Yield

This study showed that rumination time was positively related to milk production, and increased milk production was associated with reduced yield of QCH_4 /milk (Figure 4). However, no evidence was present to support the hypothesis that rumination time has independent, direct effects on QCH_4 or observed changes in QCH_4 /milk (Figure 4). Consequently, most of the change in milk production, QCH_4 , and resulting yield of QCH_4 /milk was more likely a differential response

to direct and indirect effects of feed intake on milk and CH_4 production (Figure 4).

The study also confirmed a typical reduction of $Q\text{CH}_4/\text{milk}$ and $Q\text{CO}_2/\text{milk}$ with increased milk yield (Figure 5). This could be attributed to a dilution of fixed requirements for maintenance, and to a lower extent to an improved efficiency for milk production (Dijkstra et al., 2013; Knapp et al., 2014). First, it was noted that increasing milk yield was associated with exponential decreases in $Q\text{CO}_2/\text{milk}$ (Figure 5). Increased milk yield increased the amount of ME retained as milk over the amount of CO_2 expired as heat (Moe et al., 1972; Ferrell and Oltjen, 2008). This effect was not constant, but decreased proportionally as maintenance costs were increasingly diluted by greater milk yield (Figure 5). Second, a similar exponential decay of $Q\text{CH}_4/\text{milk}$ with increased milk yield was observed (Figure 5). The $Q\text{CH}_4/Q\text{CO}_2$ ratio only had a tendency to decrease at lower milk yield, remaining near 3% (Figure 5). Cows producing less milk, such as those in LR, had lower estimated intake and $Q\text{CH}_4$, and proportionally greater loss of energy as $Q\text{CO}_2$, which could be consequence of lower milk production, greater BW decrease (at least numerically), and increased walking activity (Table 2). Consequently, most of the decreases in $Q\text{CH}_4/\text{milk}$ with increasing milk production were proportional to the increasing dilution of maintenance costs rather than direct consequence of increased digestive (i.e., lower total $Q\text{CH}_4$) or metabolic (i.e., lower total $Q\text{CO}_2$) efficiencies affecting the net energy for milk production (Dijkstra et al., 2013).

In the present study, greater estimation of gross feed conversion by HR cows was related to a greater estimation of feed intake and milk production (Table 2), with similar net reductions of $Q\text{CH}_4/\text{milk}$ as published in previous reports (see Knapp et al., 2014). Again, differences in feed conversion may have resulted from greater dilution of maintenance costs because $Q\text{CH}_4/Q\text{CO}_2$ was similar between HR and LR cows (Table 2). In addition, HR cows were not mobilizing body reserves any more than LR cows (Table 2), which confirms that higher gross feed conversion was mostly a response of the dilution of maintenance costs. In addition, lower feed conversion by LR cows could be related to additional increases of grazing energy expenditure (Brosh, 2007) associated with increased activity (Table 2), but this correlated effect was not quantified in the present study.

The most commonly reported means to reduce CH_4 emissions is to reduce the amount of CH_4 per unit of product (Pacheco et al., 2014), and previous reviews (Boadi et al., 2004; Shibata and Terada, 2010; Waghorn and Hegarty, 2011; Dijkstra et al., 2013; Knapp et al., 2014) have discussed potential mitigation alternatives

through improvements to animal production and on-farm efficiencies. The present study confirms ample opportunity for reduction of enteric CH_4 emissions per unit of product through practices that increase milk yield and dilute maintenance costs. The monitoring and manipulation of rumination patterns alone appears to have limited applicability to reduce enteric CH_4 emissions in grazing cattle, but further manipulative studies are need to confirm this observation.

CONCLUSIONS

The results from this study raise complex and challenging questions on the relationships between rumination patterns, intake, enteric CH_4 emissions, and milk production. The results show that grazing dairy cows display consistent rumination patterns and greater rumination was noticed in heavier cows that had lower days in lactation, greater parity, and greater intake, milk production, total $Q\text{CH}_4$, and reduction of $Q\text{CH}_4/\text{milk}$. However, no empirical evidence supports that these responses were the consequence of independent direct effects of rumination on $Q\text{CH}_4$ and $Q\text{CH}_4/\text{milk}$. This study therefore raises the hypothesis that coupled differences of grazing behavior and physiological differences among cows could be mechanistically geared toward a different regulation of DMI with implications for digestion rates, rumination patterns, and CH_4 emissions. Further studies, under experimentally controlled conditions, could provide additional insights into rumination patterning and potential associations with grazing behavior and enteric CH_4 emissions in grazing dairy cows. The dilution of maintenance costs appears to explain most of the variation in enteric CH_4 emissions per unit of product in grazing dairy cows. Finally, the use of $Q\text{CO}_2$ as an internal biomarker for determination of individual pasture DMI produced reliable results that were comparable with direct measurements of pasture disappearance.

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REFERENCES

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren, and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *J. Theor. Biol.* 238:36–51.
- Bar-Shalom, A. 2003. Method and system for monitoring physiological conditions of, and/or suitability of animal feed for ruminant animals. U.S. patent 2003/0205208 A1.
- Beauchemin, K. A. 1991. Ingestion and mastication of feed by dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* 7:439–463.
- Boadi, D., C. Benchaar, J. Chiquette, and D. Massé. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Can. J. Anim. Sci.* 84:319–335.
- Brosh, A. 2007. Heart rate measurements as an index of energy expenditure and energy balance in ruminants: A review. *J. Anim. Sci.* 85:1213–1227.
- Casper, D. P., and D. R. Mertens. 2010. Carbon dioxide, a greenhouse gas, is sequestered by dairy cattle. *J. Dairy Sci.* 93(E-Suppl. 1):843–844.
- Dado, R. G., and M. S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variable for lactating dairy cows. *J. Dairy Sci.* 77:132–144.
- Dijkstra, J., J. France, J. L. Ellis, A. B. Strathe, E. Kebreab, and A. Bannink. 2013. Production efficiency of ruminants: Feed, nitrogen and methane. Pages 10–25 in *Sustainable Animal Agriculture*. E. Kebreab, ed. CAB International, Wallingford, UK.
- Elischer, M. F., M. E. Arceo, E. L. Karcher, and J. M. Siegford. 2013. Validating the accuracy of activity and rumination monitor data from dairy cows housed in a pasture-based automatic milking system. *J. Dairy Sci.* 96:6412–6422.
- Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modeling of methane production in cattle. *J. Agric. Sci.* 146:213–233.
- Enriquez-Hidalgo, D., D. Hennessy, T. Gilliland, M. Egan, J. F. Mee, and E. Lewis. 2014. Effect of rotationally grazing perennial ryegrass white clover or perennial ryegrass only swards on dairy cows feeding behaviour, rumen characteristics and sward depletion patterns. *Livest. Sci.* 169:48–62.
- Ferrell, C. L., and J. W. Oltjen. 2008. ASAS Centennial Paper: Net energy systems for beef cattle – Concepts, application, and future models. *J. Anim. Sci.* 86:2779–2794.
- Fox, D. G., L. O. Tedeschi, T. P. Tytlutski, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Technol.* 112:29–78.
- Greenwood, G. B., and M. W. Demment. 1988. The effect of fasting on short-term cattle grazing behaviour. *Grass Forage Sci.* 43:377–386.
- Gregorini, P., B. DelaRue, K. McLeod, C. E. F. Clark, C. B. Glassey, and J. Jago. 2012. Rumination behavior of grazing dairy cows in response to restricted time at pasture. *Livest. Sci.* 146:95–98.
- Gregorini, P., B. DelaRue, M. Poruau, C. Glassey, and J. Jago. 2013. A note on rumination behavior of dairy cows under intensive grazing systems. *Livest. Sci.* 158:151–156.
- Hammond, K., D. J. Humphries, L. A. Crompton, P. Kirton, C. Green, and C. K. Reynolds. 2013. Methane compared emissions from growing dairy heifers estimated using an automated head chamber to respiration chambers or SF6 techniques. *Adv. Anim. Biosci.* 4:447.
- Hancock, J. 1954. Studies of grazing behaviour in relation to grassland management 1. Variations in grazing habits of dairy cattle. *J. Agric. Sci.* 44:420–433.
- Hardison, W. A., J. T. Reid, C. M. Martin, and P. G. Woolfolk. 1954. Degree of herbage selection by grazing cattle. *J. Dairy Sci.* 31:89–102.
- Hartmann, W. M. 1992. The CALIS Procedure: Extended User's Guide. SAS Institute Inc., Cary, NC.
- Hegarty, R. S. 2013. Applicability of short-term emission measurements for on-farm quantification of enteric methane. *Animal* 7:401–408.
- Higgs, R. J., K. L. Russomanno, T. F. Christoph, and M. E. Van Amburgh. 2013. Predicting methane and carbon dioxide emissions using the CNCPS. *J. Dairy Sci.* 96(E-Suppl. 1):598.
- Hills, J. L., W. J. Wales, F. R. Dunshea, S. C. Garcia, and J. R. Roche. 2015. Invited review: An evaluation of the likely effects of individualized feeding of concentrate supplements to pasture-based dairy cows. *J. Dairy Sci.* 98:1363–1401.
- Hodgson, J. 1990. *Grazing Management: Science Into Practice*. Longman Scientific and Technical, Harlow, UK.
- Huhtanen, P., E. H. Cabezas-Garcia, S. Utsumi, and S. Zimmerman. 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. *J. Dairy Sci.* 98:3394–3409.
- Huhtanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. *J. Dairy Sci.* 92:5031–5042.
- Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.* 73:2483–2492.
- Kalu, B., and G. Fick. 1981. Quantifying morphological stage of development of alfalfa for studies of herbage quality. *Crop Sci.* 21:267–271.
- Kennedy, E., J. Curran, B. Mayes, M. McEvoy, J. P. Murphy, and M. O'Donovan. 2011. Restricting dairy cow access time to pasture in early lactation: The effects on milk production, grazing behaviour and dry matter intake. *Animal* 5:1805–1813.
- Kennedy, P. M. 1985. Effect of rumination in reduction of particle size of digesta by cattle. *Aust. J. Agric. Res.* 36:819–828.
- Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983–997.
- Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci.* 97:3231–3261.
- Kononoff, P. J., and J. Heinrichs. 2003. The effect of corn particle size and cottonseed hulls on cows in early lactation. *J. Dairy Sci.* 86:2438–2451.
- Laca, E. A., R. A. Distel, T. C. Griggs, and M. W. Demment. 1994. Effects of canopy structure on patch depression by grazers. *Ecology* 75:706–716.
- Lana, R. P., J. B. Russell, and M. E. Van Amburgh. 1998. The role of pH in regulating ruminal methane and ammonia production. *J. Anim. Sci.* 76:2190–2196.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. *SAS System for Mixed Models*, 2nd ed. SAS Institute Inc., Cary, NC.
- Lyons, N. A., K. L. Kerrisk, and S. C. Garcia. 2013. Comparison of 2 systems of pasture allocation on milking intervals and total daily milk yield of dairy cows in a pasture-based automatic milking system. *J. Dairy Sci.* 96:4494–4504.
- Maulfair, D. D., G. I. Zanton, M. Fustini, and A. J. Heinrichs. 2010. Effect of feed sorting and chewing behaviour, production, and rumen fermentation in lactating dairy cows. *J. Dairy Sci.* 93:4791–4803.
- Mayes, R. W., and H. Dove. 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. *Nutr. Res. Rev.* 13:107–138.
- McLean, J. A., and G. Tobin. 1987. *Animal and Human Calorimetry*. Cambridge University Press, New York, NY.

- Mendes, A. M. D., D. K. D. Silva, M. D. Ferreira, A. S. Veras, G. D. G. Ferreira, R. B. Vigoderis, H. G. S. Arcanjo, J. M. Silva, J. S. de Almeida, A. C. A. Fotius, and G. D. Farias. 2013. Ingestive behaviour of pastured crossbred dairy cows offered different supplement types. *Trop. Anim. Health Prod.* 45:231–237.
- Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy Sci.* 80:1463–1481.
- Moe, P. W., W. P. Flatt, and H. F. Tyrrell. 1972. Net energy value of feeds for lactation. *J. Dairy Sci.* 55:945–958.
- Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. *J. Dairy Sci.* 62:1583–1586.
- Moore, K. J., L. E. Moser, K. P. Vogel, S. S. Waller, B. E. Johnson, and J. F. Pedersen. 1991. Describing and quantifying growth stages of perennial forage grasses. *Agron. J.* 83:1073–1077.
- Opio, C. P., A. Gerber, A. Mottet, G. Falcucci, M. Tempio, T. MacLeod, B. Vellinga, B. Henderson, and H. Steinfeld. 2013. Greenhouse gas emissions from ruminant supply chains—A global life cycle assessment. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Ørskov, E. R., C. Fraser, V. C. Mason, and S. O. Mann. 1988. A study on consistency of differences between cows in rumen outflow rate of fibrous particles and other substrates and consequences for digestibility and intake of roughages. *Anim. Prod. Sci.* 47:45–51.
- Pacheco, D., G. Waghorn, and P. H. Janssen. 2014. Decreasing methane emissions from grazing forages: A fit with productive and financial realities? *Anim. Prod. Sci.* 54:1141–1154.
- Phillips, C. J. C., and M. I. Rind. 2002. The effects of social dominance on the production and behaviour of grazing dairy cows offered forage supplements. *J. Dairy Sci.* 85:51–59.
- Pinares-Patiño, C. S., G. C. Waghorn, A. Machm, I. Ier, B. Vlaming, G. Molano, A. Cavanagh, and H. Clark. 2007. Methane emissions and digestive physiology of non-lactating dairy cows fed pasture forage. *Can. J. Anim. Sci.* 87:601–613.
- Pugesek, B. H., A. Tomer, and A. Von Eye. 2003. *Structural Equation Modeling: Applications in Ecological and Evolutionary Biology*. Cambridge University Press, Cambridge, UK.
- Russomanno, K. L., T. F. Christoph, R. J. Higgs, and M. E. Van Amburgh. 2013. Utilization of byproducts from human food production as feedstuffs for dairy cattle and relationship to greenhouse gas emissions and environmental efficiency. *J. Dairy Sci.* 96(E-Suppl. 1):35.
- Rutter, S. M., R. J. Orr, N. H. Yarrow, and R. A. Champion. 2004. Dietary preference of dairy cows grazing ryegrass and white clover. *J. Dairy Sci.* 87:1317–1324.
- Schirmann, K., M. A. G. von Keyserlingk, D. Weary, D. Veira, and W. Heuvelink. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. *J. Dairy Sci.* 92:6052–6055.
- Shibata, M., and F. Terada. 2010. Factors affecting methane production and mitigation in ruminants. *Anim. Sci. J.* 81:2–10.
- Shibata, M., F. Terada, M. Kurihara, T. Nishida, and K. Iwasaki. 1993. Estimation of methane production in ruminants. *Anim. Sci. Technol.* 64:790–796.
- Thornton, P. K. 2010. Livestock production: Recent trends, future prospects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365:2853–2867.
- Tyrrell, H. F., and P. W. Moe. 1975. Production efficiency in the high producing cow: Effect of intake on digestive efficiency. *J. Dairy Sci.* 58:1151–1163.
- Vlaming, J. B., N. Lopez-Villalobos, I. M. Brookes, S. O. Hoskin, and H. Clark. 2008. Within- and between animal variance in methane emissions in non-lactating dairy cows. *Aust. J. Exp. Agric.* 48:124–127.
- Waghorn, G. C., and R. S. Hegarty. 2011. Lowering ruminant methane emissions through improved feed conversion efficiency. *Anim. Feed Sci. Technol.* 166–167:291–301.
- Welch, J. G., and A. M. Smith. 1969. Effect of varying amounts of forage intake on rumination. *J. Anim. Sci.* 28:827–830.
- West, J. W. 2003. Effects of heat stress on production in dairy cattle. *J. Dairy Sci.* 86:2131–2144.
- Zehetmeier, M., J. Baudracco, H. Hoffman, and A. Heibenhauer. 2012. Does increasing milk yield per cow reduce greenhouse gas emissions? A systems approach. *Animal* 6:154–166.