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[1]

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ABSTRACT: We assessed the interactive effects of gross feed use efficiency (**FUE**, milk yield/kg DMI) background ("high" = **HEFF** vs. "low" = **LEFF**) and graded levels of dietary CP (130, 145, 160, and 175 g/kg DM) on milk production, enteric methane (CH_4) emission, and apparent nitrogen use efficiency (**NUE**, g milk protein nitrogen/g nitrogen intake) with Norwegian Red (**NRF**) dairy cows. Eight early- to mid-lactation cows were used in a 4 × 4 Latin square design experiment (2 efficiency backgrounds, 4 dietary treatments, and 4 periods each lasting 28 d). The diets were designed to be identical in physical nature and energy density, except for the planned changes in CP, which was a contribution of slight changes in other dietary constituents. We hypothesized that HEFF cows would partition more dietary energy and nitrogen into milk components and, as such, partition less energy in the form of methane and excrete less nitrogen in urine and feces compared with their LEFF contemporaries. We observed no interactions between dietary CP level and efficiency background on DMI, other nutrient intake, NUE, CH_4 emission, and its intensity (g CH_4/kg milk). Gradually decreasing dietary CP from 175 to 130 g/kg DM did not affect DMI, milk and energy-corrected milk yield, and milk component yields and daily CH_4 emission. However, decreasing dietary CP increased NUE and reduced urinary nitrogen (**UN**) excretion both in quantitative terms and as proportion of nitrogen intake. The HEFF cows showed improved NUE and decreased CH_4 emission intensity compared with the LEFF cows. In the absence of interaction effects between efficiency background and dietary CP level, our results suggest that CH_4 emission intensity and UN excretions can be reduced by selecting dairy cows with higher FUE and reducing dietary CP level, respectively, independent of one another. Furthermore, UN excretion predictions based on milk urea nitrogen (**MUN**) and cow BW for NRF cows produced very close estimates to recorded values promising an inexpensive and useful tool for estimating UN excretion under the Nordic conditions where ordinary milk analysis comes with MUN estimates.

Key words: dietary crude protein, enteric methane, feed use efficiency, urinary nitrogen

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INTRODUCTION

Recent greenhouse gas (**GHG**) emission from livestock supply chains is estimated at 7.1 Gt CO_2-equivalents per annum accounting for 14.5% of

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all anthropogenic emissions (Gerber et al., 2013b). Although ruminants play an important role in providing high-quality protein essential for human diets, they are an important source of GHG emissions (Opio et al., 2013). Emissions of methane (**CH_4**) and nitrous oxide (**N_2O**) increased globally by nearly 17% from 1990 to 2005, with both gases contributing equally to the increase (Smith et al., 2007). However, such projections are region specific. For example, enteric CH_4 from cattle has shown a downward trend from 1990 to 2014 in the EU-28 countries (EEA, 2016).

Increasing fertilizer and feed prices concerns over food security and increasing regulations to reduce nutrient loss have created pressures to improve agricultural nutrient use efficiency (Powell et al., 2010). In ruminants, the greatest potential in reducing the GHG emissions involves improving animal and herd efficiency. This includes manipulation of dietary composition and feeding techniques to reduce CH_4 generated during enteric fermentation and proper management of manure to reduce CH_4 and N_2O released during storage (Gerber et al., 2013b). Enteric CH_4 emission is proportional to daily DMI (Blaxter and Clapperton, 1965). Selection for low residual feed intake could reduce GHG emissions and improve dietary nitrogen use efficiency (**NUE**) (Basarab et al., 2013). Reports on cattle with contrasting efficiencies have indicated the potential to reduce the environmental impact of meat and milk production (Hegarty et al., 2007; Jones et al., 2011; Connor et al., 2013; Connor, 2015).

We hypothesized that dairy cows with higher gross feed use efficiency (**FUE**, milk yield/kg DMI) would partition more dietary energy and nitrogen into milk components and partition less energy in the form of CH_4 and excrete less nitrogen in urine and feces compared with cows with lower FUE. We also hypothesized that the sensitivity of NUE to increasing levels of dietary CP would differ between these 2 divergent groups.

MATERIALS AND METHODS

Animals and Experimental Design

All animal procedures were approved by the national animal research authority of the Norwegian Food Safety Authority (Mattilsynet; FOTS ID: 7844). The experiment was conducted from early-March to early-July 2016 at the metabolism unit (Stoffskifteavdelingen) of the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (Aas, Norway).

The 8 Norwegian Red (**NRF**) dairy cows used in the current experiment were selected from a previous production trial executed in the preceding lactation, with 48 early- to mid-lactation cows (Kidane et al., 2018). In the previous trial, cows with starting BW (mean ± SD) of 566 ± 46.7 kg and initial milk yield of 27.8 ± 5.4 kg/d were used to assess FUE when fed grass/clover silages either low (112 g/kg DM) or optimal (142 g/kg DM) in CP, supplemented with a fixed level of a commercial concentrate diet, Formel Favør 90 (Felleskjøpet Agri SA, Lillestøm, Norway). From this trial, 2 contrasting efficiency groups of cows were selected (low FUE cows = **LEFF** vs. high FUE cows = **HEFF**; 5 cows in each group) at a comparable BW and level of DMI. The selected LEFF cows had lower milk yield and milk component yield than the HEFF cows for a unit DMI. This has resulted in differences in NUE and residual feed intake between the 2 groups. Thus, the LEFF cows had lower NUE and higher residual feed intake than their HEFF counterparts. The cows were rumen cannulated before the next calving, and 8 selected cows (4 LEFF and 4 HEFF) were used in the present experiment.

The cows in each group were assigned to the experimental diets using a 4 × 4 Latin square design (i.e., 4 diets over 4 periods each lasting 28 d). The cows were housed in tie-stalls with rubber mat floors topped with sawdust beddings. All data were collected at individual cow level as described later.

Feeds and Feeding

Feeds. The cows were fed a total mixed ration (**TMR**) with graded levels of dietary CP (Table 1). The rations were prepared as TMR to minimize the selective consumption of individual feed components (Coppock et al., 1981) and, hence, enforce planned daily intake of nutrients. All cows were fed these TMR diets ad libitum (assuming 10% refusal rate). This was achieved by weighing the refusal every day at 0630 h immediately before new feed was offered and adjusting DM on offer to 110% of the DMI of the previous day. Any suspicious DMI-based large refusal rate from a particular day was overridden in the estimation of daily DM offer. A minimum of 5-kg fresh feed (~10% of daily allowance) was added as an adjustment to previous-day intake if an

Table 1. Ingredient inclusion rate, chemical composition, and energy value of the total mixed rations (TMR) fed at 4 levels dietary CP concentrations

	Dietary Treatments			
	130	145	160	175
Ingredients in TMR				
Grass silage	500.0	500.0	492.5	492.5
Concentrate[1]	425.0	425.0	425.0	415.0
Barley pellet	65.0	37.5	17.5	0.0
Protein supplement[2]	10.0	37.5	65.0	92.5
Chemical composition of TMR (analyzed/estimated)				
DM content, g/kg fresh	411.0	411.0	415.0	415.0
OM	939.1	939.0	938.5	938.0
Ash	60.9	61.0	61.5	62.0
CP	118.2	134.0	149.1	166.7
Starch	227.3	224.1	221.8	211.2
aNDFom[3]	399.2	392.1	391.8	391.1
pdNDF[4], g/kg NDF	797.8	803.4	800.2	809.8
iNDF[5]	80	77.1	78.2	74.4
ADF	241.2	238.1	239.1	239.3
Crude fat	24.8	24.0	24.9	22.8
FPF[6]	15.0	15.0	14.5	14.8
RestCHO[7]	159.0	153.0	139.0	134.0
NE_l[8], MJ/kg DM	6.7	6.6	6.6	6.6

Values are in g/kg DM, unless otherwise stated.

[1]Commercial compound feed composed (g/kg DM basis) of oats (351), barley (201), rye (171), SoyPass (78), sugarcane molasses (65), rapeseed cake (41), maize gluten meal (30), wheat bran (20), whole oil seeds (*Brassica* spp., 17), oat bran (6.0), and some minerals and vitamin premixes (20).

[2]Protein supplement composed of 44.1% barley, 41.4% DEMP (yeast-based microbial crude protein supplied by Alltech; Alltechnology Ireland Limited), and 14.5% urea, on DM basis, and produced by the Center for Feed Technology (Fôrtek, Norwegian University of Life Sciences, Norway).

[3]NDF corrected for ash.

[4]Potentially degradable NDF.

[5]Indigestible NDF.

[6]Sum of fermentation products in feeds (NorFor, 2011) contributed from the silage portion.

[7]Residual carbohydrates corrected for low-molecular-weight fractions (urea and NH_3-N) as in the Nordic feed evaluation system (NorFor, 2011).

[8]Calculated NE_l based on the proportion of ingredients and their energy values in the TMR.

individual cow was reported to have negligible refuse. The cows had individual feed troughs and free access to gauged waterlines to monitor daily water intake.

Feeding, feed sampling, and analysis. All cows were fed at 0630, 1400, and 1830 h with daily DM offer partitioned into 3 meals at the ratio of 50:30:20 in the respective order. Area around the feed trough was maintained clean for each cow and tossed feed, whenever existed, was put back on regular intervals during a day. During each feed delivery time of the day, feed availability for individual cow was monitored in the troughs to make sure that the partitioning of daily DM on offer into the 3 portions functioned properly and also if the ad libitum feeding was achieved.

Representative grab feed and refuse samples were taken on Mondays and Thursdays of each week. Duplicate samples were used for immediate DM analysis to follow up consistency in TMR preparation and to estimate DMI. Additional duplicate samples were taken for chemical analysis and kept frozen at −20 °C until the end of the experiment. The latter were freeze-dried and milled using cutting mill (Retsch SM 200, Retsch GmbH, Germany) at different sieve sizes, as described later, for the various analyses intended. Separate silage samples were also taken from a batch of silage bales intended as part of the TMR for fermentation products.

Feed (TMR) samples for starch and in sacco 288 h indigestible NDF (**iNDF**) determination were milled through 0.5- and 1.5-mm sieve sizes, respectively, whereas samples for other analysis were milled through 1.0-mm sieve size. These samples were analyzed for DM content (103 °C overnight), ash using ISO 5984 method (550 °C for a minimum of 4 h), and Kjeldahl-N using Method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) with Kjeltec 2400/2460 Auto Sampler System (Foss Analytical, Hilleroed, Denmark) and estimated CP = N × 6.25. Total starch content of the TMR diet was analyzed using AACCI Method 76-13.01 (Megazyme amyloglucosidase/α-amylase method). The NDF was determined with an ANKOM^{220} fiber analyzer (ANKOM Technology, Fairport, NY) according to Mertens (2002) using sodium sulfite and α-amylase and corrected for ash and hereafter expressed as aNDFom. The iNDF was determined after 288 h in sacco incubation following the Nordic feed evaluation system (NorFor, 2011). The ADF was determined according to Method 973.18 (AOAC, 2000) with the modification that the samples were not washed with acetone and were corrected for ash. Silage fermentation products (**FPF**) and ammonia-N in fresh silage samples were analyzed by Eurofins (Eurofins Food & Feed Testing Norway AS, Moss, Norway) as described in Dønnem et al. (2011).

Milking, Milk Sampling, and Analysis

Cows were milked twice a day (AM = between 0630 and 0730 h; PM = between 1830 and 1930 h) in the tie stalls. Milk yield was recorded on all

days. Milk samples were taken on days 1, 8, 11, 15, and 22 (separate AM and PM samples, 10 samplings per cow per period) in bottles containing Bronopol tablets (2-bromo-2-nitropane-1,3 diol, Broad Spectrum Microtabs II) as preservative, stored chilled (4 °C) until analyzed for milk protein, fat, lactose, and urea using infrared milk analyzer (MilkoScan 6000; Foss Analytical, Hilleroed, Denmark). Energy-corrected milk (**ECM**) yield was calculated for individual cow based on mean milk chemical composition and milk yield according to Sjaunja et al. (1991).

Rumen Fluid Samples for Volatile Fatty Acids and Ammonia Nitrogen Analysis

Samples for VFA and ammonia nitrogen (NH_3-N) analysis were collected at 9 time points over 24-h cycle starting on day 17 during each period. These time points (i.e., 0400, 0600, 0800, 1000, 1200, 1500, 1800, 1900, and 2100 h) created a lag period ranging from 0.5 to 11.5 h between feeding (meals) and sampling. The samples (10 mL) were preserved with 0.5 mL of 98% formic acid and stored at 4 °C until analysis. The rumen fluid VFA were analyzed by gas chromatography (TRACE 1300 Gas Chromatograph equipped with Stabilwax-DA column 30 m, 0.25 mm i.d., 0.25 µm; Thermo Fischer Scientific S.p.A., Milan, Italy), whereas the rumen fluid NH_3-N was analyzed using Method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) with a modification that block digestion was not carried out.

Total Feces and Urine Collection, and Analysis

During the third week of each period, total feces and urine were collected over 72 h for digestibility (A. Kidane et al., unpublished data) and nitrogen balance estimates. Daily feces were collected, weighed, mixed thoroughly, and subsampled (10% of daily yield). These samples were kept frozen at −20 °C until the 72-h collection was completed. At completion, the samples were thawed and thoroughly mixed until uniform consistency. Then after, 2 duplicate samples (500 g each) were prepared. One set of the duplicate samples was oven dried at 103 °C for DM analysis, and the second set was further frozen in preparation for lyophilization. The latter samples were prepared and analyzed for DM and Kjeldahl-N content as described for the TMR samples.

Urine samples were collected using rubber tube strapped over the vulva by using a harness and glue to avoid urine loss and contamination with feces. Daily urine was collected in a 30-L plastic container containing 1.5 L of 10% (vol/vol) H_2SO_4 to preserve the urine. At completion of each day collection, total volume and pH of the collection were recorded; duplicate samples were taken and kept frozen at −20 °C until analysis. The samples were later analyzed for Kjeldahl-N using Method 2001.11 (AOAC, 2002) to estimate total urinary nitrogen (**UN**) excretion.

Enteric Methane Measurement

Enteric CH_4 production was estimated using sulfur hexafluoride (SF_6) as a marker (Johnson et al., 1994). Brass permeation tubes filled with SF_6 gas (mean ± SD = 2338 ± 148.9 mg) and predetermined mean (± SD) release rate of 4.614 (± 0.228; r^2 = 0.999) mg/d were prepared by Agriculture and Agri-Food Canada (Semiarid Prairie Agricultural Research Centre, Saskatchewan, Canada). On days 25, 26, 27, and 28, cows were mounted with a depressurized CH_4 collection yokes and a halter system as described in McGinn et al. (2006) for 24-h gas sample collection. Furthermore, on the sampling days, 2 yoke and halter sets were placed in the barn on 2 corners at about a cow-head position to account for the background concentration of the marker and CH_4. At the end of the experiment, the gas samples (in triplicates per day) were analyzed using gas chromatography (GC, Model 7890A Agilent, Santa Clara, CA) 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):

$$Q_{CH_4} = \frac{C_{CH_4} - C_{CH_4^b}}{C_{SF_6} - C_{SF_6^b}} Q_{SF_6} \frac{MW_{CH_4}}{MW_{SF_6}}$$

where Q_{CH_4} is daily enteric methane emission (g/d); Q_{SF_6} is predetermined marker release rate (g/d); C_{CH_4} and C_{SF_6} are CH_4 and SF_6 mixing ratios in the yokes (µmol/mol); $C_{CH_4^b}$ and $C_{SF_6^b}$ are background CH_4 and SF_6 levels in air samples from the barn; and MW_{CH_4} / MW_{SF_6} is molecular weight ratio used to account for the differences in the density of the gases.

Estimation of Urinary Nitrogen Excretion Based on Milk Urea Nitrogen

Total daily UN excretion was calculated based on measured urine volume and analyzed nitrogen content of the urine samples. Two predictive models are developed for estimating UN excretion by using

simple regression of the observed daily UN excretion on milk urea nitrogen (**MUN**) and cow BW in a similar fashion to what was developed for other breeds elsewhere (Jonker et al., 1998; Kohn et al., 2002). We further checked the predictive values of the existing UN prediction models developed for different breeds (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Kohn et al., 2002) with our measured values.

Statistical Analysis

Data collected over the experimental days were analyzed as repeated measurements ANOVA with SAS Mixed Models (2002 to 2012, SAS for Windows 9.4, SAS Institute Inc.; Cary, NC) using AR(1) covariance structure and a cow within efficiency group as a subject. Daily DM and its component intakes (NDF, CP, OM, and starch) were adjusted for refuse DM content and chemical composition before statistical analysis.

The effect of level of CP and efficiency background on feed and nutrient intake, milk and its component yields, and enteric CH_4 emission were assessed using the following model:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \theta_k + C_{l(j)} + D_{m(k)} + (\alpha\beta)_{ij} + \varepsilon_{ijklm}$$

where Y_{ijklm} is response variable, μ is the overall mean, α is the effect level of dietary CP protein, β is the effect of efficiency background (block), θ is the effect of period, C is the random effect of cow with in block, D is the effect of day of measurement within a period, $\alpha\beta$ is the interaction effect of level of CP and efficiency background, and ε_{ijklm} is residual error term.

Rumen fluid VFA and NH_3-N concentrations were measured at frequent time intervals, and rumen pH was monitored continuously at 10-min interval over 24-h period. Therefore, the effect of level of CP and efficiency background on rumen fermentation parameters was tested taking account of meal (AM, PM, or evening feeding) and time relative to these meals using the following model:

$$\begin{aligned} Y_{ijklmn} = {} & \mu + \alpha_i + \beta_j + \theta_k + C_{l(j)} + F_m + TRF_{n(m)} \\ & + (\alpha\beta)_{ij} + (\beta TRF)_{jn(m)} + (\alpha TRF)_{in(m)} \\ & + (\alpha\beta TRF)_{ijn(m)} + \varepsilon_{ijklmn} \end{aligned}$$

where Y_{ijklmn} is response variable, μ is the overall mean for a response variable, α is the effect level of dietary CP, β is the effect of efficiency background (block), θ is the effect of period, C is the random effect of cow with in block, F is the fixed effect of meal (AM, PM, or evening feeding), TRF is the effect of time relative to meal (feeding) in minutes, $\alpha\beta$ is the interaction effect of level of CP and efficiency background, βTRF is the interaction effect of efficiency background and time relative to feeding, αTRF is the interaction effect of level of dietary CP and time relative to feeding, $\alpha\beta TRF$ is the 3-way interaction effect of level of dietary CP with efficiency background and time relative to meals, and ε_{ijklmn} is residual error term.

Sum of squares for dietary CP levels were partitioned into orthogonal contrasts to assess linear and quadratic responses of the tested parameters to the graded levels of dietary CP. Statistical significance is declared at $P < 0.05$.

RESULTS

Feed Intake

Data on mean daily DM and nutrient intakes are presented in Table 2. Mean daily DMI, nutrient (NDF, starch, CP), and free drinking water intakes were not affected by the efficiency background ($P > 0.1$). Similarly, except for the CP intake which linearly increased ($P < 0.001$) with increasing dietary CP level as planned, all other parameters were not affected by the dietary treatments. The interaction effects of dietary CP level and efficiency background were not significant for all intake parameters described.

When expressed in relation to metabolic BW (**$BW^{0.75}$**), intake of the above parameters maintained similar trend and hence was not affected by either the efficiency background, dietary CP level, or their interaction effects ($P > 0.1$). However, CP intake (g/kg $BW^{0.75}$) significantly ($P = 0.014$) increased with increasing dietary CP level in a linear pattern ($P < 0.001$). Mean (± SE) dietary fiber intake (i.e., g aNDFom/kg BW) was similar between the efficiency backgrounds (11.7 ± 0.60) and between dietary CP levels.

Milk Yield, Its Chemical Composition, and Component Yields

Milk yield, its chemical composition, and component yields are presented in Table 3. Mean (± SE) milk yield was greater ($P = 0.019$) for cows from the HEFF (23.3 ± 0.65 kg/d) than from the LEFF (20.4 ± 0.62 kg/d) group. Similarly, ECM was significantly greater ($P = 0.0045$) for cows from the HEFF (24.0 ± 0.61 kg/d) than cows from the LEFF

Table 2. Mean daily DM and nutrient intake and intake per unit metabolic BW ($BW^{0.75}$) of 2 groups of dairy cows (HEFF vs. LEFF) fed a total mixed ration with CP levels of 130, 145, 160, and 175 g/kg DM

	Eff.[1]			Dietary CP level					Effects (*P*-value)			Contrast for CP	
Parameters	HEFF	LEFF	SE	130	145	160	175	SE	Eff.	CP	CP × Eff.	Linear	Quadratic
DM and nutrient intake, kg/d													
DMI	19.8	19.0	*0.58*	18.9	19.9	19.6	19.1	*1.08*	*0.24*	*0.50*	*0.19*	*0.88*	*0.16*
OM	18.5	17.8	0.55	17.7	18.7	18.4	17.9	*1.02*	*0.23*	*0.49*	*0.20*	*0.87*	*0.16*
aNDFom[2]	7.87	7.65	0.15	7.65	7.91	7.84	7.63	*0.29*	*0.20*	*0.48*	*0.089*	*0.88*	*0.14*
CP	2.81	2.69	0.09	2.22[a]	2.67[b]	2.92[c]	3.18[d]	*0.17*	*0.25*	*<0.001*	*0.27*	*<0.001*	*0.33*
Starch	4.19	3.92	0.15	3.94	4.20	4.09	3.99	*0.27*	*0.11*	*0.53*	*0.10*	*0.90*	*0.20*
Water[3]	70.4	74.1	3.47	65.6	75.0	73.1	75.3	*6.49*	*0.29*	*0.20*	*0.47*	*0.082*	*0.30*
Intake per unit $BW^{0.75}$, g/kg													
DM	151.4	145.0	8.69	144.3	153.1	149.5	145.9	*14.79*	*0.50*	*0.81*	*0.58*	*0.99*	*0.40*
OM	142.0	136.0	8.17	135.3	143.6	140.2	136.9	*13.90*	*0.49*	*0.81*	*0.58*	*0.99*	*0.40*
aNDFom	60.3	58.5	2.42	58.4	60.8	59.8	58.5	*4.36*	*0.51*	*0.82*	*0.53*	*0.90*	*0.40*
CP	21.6	20.3	1.84	17.2[a]	20.5[ab]	22.1[ab]	24.2[c]	2.94	*0.52*	*0.014*	*0.74*	*<0.001*	*0.66*

Means in a row with different superscripts for the dietary CP levels are significantly different at $P < 0.05$.

[1]Eff. is gross feed efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.

[2]NDF corrected for ash.

[3]Measured free drinking water intake.

Table 3. Milk and energy-corrected milk yield, chemical composition, and component yields of 2 groups of dairy cows (HEFF vs. LEFF) fed a total mixed ration with CP levels of 130, 145, 160, and 175 g/kg DM

	Eff.[1]			Dietary CP level					Effects (*P*-value)			Contrast for CP	
Parameters	HEFF	LEFF	SE	130	145	160	175	SE	Eff.	CP	CP × Eff.	Linear	Quadratic
Yield, kg/d													
Milk	23.3	20.4	*0.60*	21.3	22.1	22.4	21.7	*0.78*	*0.019*	*0.86*	*0.97*	*0.80*	*0.40*
ECM[2]	24.0	20.3	*0.56*	21.3	22.4	22.7	22.2	*0.74*	*0.005*	*0.72*	*0.82*	*0.49*	*0.34*
Chemical composition, %													
Fat	4.13	4.00	*0.206*	3.94[a]	4.10[ab]	4.04[ab]	4.20[b]	*0.155*	*0.70*	*0.014*	*<0.001*	*0.02*	*0.89*
Protein	3.55	3.44	*0.059*	3.44[a]	3.52[c]	3.54[c]	3.48[b]	*0.043*	*0.24*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Lactose	4.65	4.41	*0.070*	4.54	4.55	4.54	4.49	*0.054*	*0.06*	*0.05*	*<0.001*	*0.011*	*0.31*
MUN[3], mg/dL	11.23	9.80	*0.563*	7.46[a]	9.36[b]	11.68[c]	13.47[c]	*0.510*	*0.13*	*<0.001*	*0.10*	*0.001*	*0.56*
Milk component yields, kg/d													
Fat	0.967	0.817	*0.023*	0.853	0.898	0.909	0.908	*0.030*	*0.004*	*0.67*	*0.43*	*0.29*	*0.53*
Protein	0.823	0.698	*0.021*	0.73	0.775	0.785	0.751	*0.028*	*0.008*	*0.61*	*0.94*	*0.67*	*0.20*
Lactose	1.085	0.914	*0.031*	0.973	1.012	1.027	0.986	*0.039*	*0.009*	*0.83*	*0.92*	*0.93*	*0.36*

Means in a row with different superscripts for the dietary CP levels are significantly different at $P < 0.05$.

[1]Eff. is gross feed use efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.

[2]ECM = energy-corrected milk yield.

[3]MUN = milk urea nitrogen.

(20.3 ± 0.57 kg/d) group. The effects of dietary CP level and its interaction with efficiency background on both milk and ECM yields were not significant ($P > 0.1$).

Milk fat and protein contents were not affected by the efficiency background, but milk lactose content tended to be greater ($P = 0.064$) for the HEFF cows than the LEFF cows. The interaction effects of dietary CP level and efficiency background were significant for milk fat, protein, and lactose content ($P < 0.0001$). As such, increasing dietary CP levels from 130 to 175 g/kg DM resulted in an increment of 0.46% and 0.04% fat in the HEFF and LEFF cows, respectively. Similar interaction effects indicated that milk protein content increased with increasing dietary CP levels up to 160 g/kg DM in the HEFF cows before declining at 175 g/kg DM. For the LEFF cows, milk protein content increased only with the first increment in CP level (quadratic effect, $P < 0.0001$). The MUN was not affected by either the efficiency background or its interaction with CP level. However, MUN significantly increased with increasing dietary CP level ($P < 0.0001$).

Mean milk protein, fat, and lactose yields were greater for HEFF than LEFF cows. However, milk component yields were not affected by dietary CP level or its interaction with efficiency background.

Rumen Fermentation Parameters

Summarized rumen pH data are presented in Table 4, whereas fluctuation in rumen pH subject to treatments (dietary CP level and efficiency background) and other dietary characteristics is presented in Fig. 1. Mean rumen pH recorded continuously over 24-h cycles was not affected by the efficiency background ($P > 0.1$) and its interaction effects with level of dietary CP and time relative to meals. Dietary CP tended to affect rumen pH ($P = 0.078$), whereby the lowest CP level resulted in marginally higher pH values. Furthermore, there were strong effects of meals ($P < 0.016$) and time relative to meals ($P < 0.001$) on rumen pH; rumen pH peaked in the hours leading to morning (0630 h) and afternoon (1400 h) meals.

Summary for rumen NH_3-N and VFA is presented in Table 4. Dietary CP level significantly influenced rumen NH_3-N concentration ($P < 0.0001$). Ignoring meal effects and over the time intervals where rumen fluid samples were taken (i.e., 0.5- to 11.5-h postfeeding), NH_3-N concentration increased with increasing dietary CP until 1.5-h postfeeding. Then after, it decreased before reaching nadir for all CP levels at 9.5-h postfeeding. The rate of decline was different between dietary CP levels as indicated by the CP and time relative to meal interaction effect ($P < 0.001$). Overall, the observed mean daily rumen fluid NH_3-N concentration at the lowest CP level was about one-third (53.1 mg/L) of that observed at the highest CP level (161.8 mg/L) with a linear increment over the range of CP tested (linear trend; $P < 0.0001$).

Rumen fluid total VFA concentration (mmol/L) was not affected ($P > 0.05$) by either the dietary

Table 4. Rumen ammonia nitrogen (NH_3-N; mg/L), total volatile fatty acid (VFA; mM), molar proportions of acetate (Ac), propionate (Pr), butyrate (Bu) and valeriate (Val), isobutyrate (IsoBu), isovalerate (IsoVal), and nonglucogenic to glucogenic VFA ratio (NGR) from 2 groups of dairy cows (HEFF vs. LEFF) at different sampling time points of a day when fed on total mixed ration with CP levels of 130, 145, 160, and 175 g/kg DM

			Volatile fatty acids									
Treatments		NH_3-N	Total VFA	Ac	Pr	Bu	Val	IsoBu	IsoVal	NGR[1]	Ac/Pr	Rumen pH
Efficiency[2]	HEFF	113.8	108.3	64.92	19.50	13.01	1.21	0.64	0.75	4.32	3.41	6.31
	LEFF	110.2	106.7	65.70	19.65	12.09	1.13	0.64	0.79	4.28	3.45	6.26
	SE	*5.24*	*1.82*	*0.264*	*0.348*	*0.285*	*0.029*	*0.008*	*0.040*	*0.096*	*0.076*	*0.034*
Dietary CP	130	53.1[a]	104.6	65.26	19.52	12.55	1.16	0.67[c]	0.81	4.28	3.43	6.40[a]
	145	97.7[b]	106.8	65.43	19.47	12.48	1.16	0.64[b]	0.82	4.35	3.49	6.24[b]
	160	135.5[c]	110.2	65.37	19.62	12.45	1.13	0.64[b]	0.76	4.27	3.41	6.27[b]
	175	161.8[d]	108.4	65.17	19.69	12.73	1.22	0.61[a]	0.68	4.29	3.40	6.23[b]
	SE	*7.14*	*2.45*	*0.352*	*0.452*	*0.317*	*0.036*	*0.010*	*0.046*	*0.123*	*0.099*	*0.042*
Statistics: effects of[3]												
Efficiency		*0.49*	*0.47*	*0.10*	*0.69*	*0.060*	*0.11*	*0.96*	*0.47*	*0.73*	*0.84*	*0.24*
Dietary CP		*<0.001*	*0.45*	*0.88*	*0.95*	*0.88*	*0.35*	*0.010*	*0.078*	*0.95*	*0.85*	*0.078*
Meal[4]		*0.001*	*0.90*	*<0.001*	*<0.001*	*0.35*	*<0.001*	*0.070*	*0.018*	*<0.001*	*<0.001*	*0.016*
TRF[5]		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.003*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Dietary CP × TRF		*<0.001*	*0.97*	*0.99*	*0.97*	*0.94*	*0.45*	*0.96*	*0.28*	*0.99*	*0.98*	*0.53*
Efficiency × dietary CP		*0.99*	*0.40*	*0.98*	*0.95*	*0.50*	*0.91*	*0.19*	*0.13*	*0.98*	*0.85*	*0.15*
Contrast for dietary CP levels												
Linear		*<0.001*	*0.26*	*0.72*	*0.65*	*0.68*	*0.36*	*0.001*	*0.026*	*0.86*	*0.59*	*0.042*
Quadratic		*0.46*	*0.34*	*0.50*	*0.77*	*0.54*	*0.19*	*0.74*	*0.27*	*0.77*	*0.66*	*0.16*

Means with different superscripts in a column for the dietary CP levels are significantly different from each other at $P < 0.05$.

[1]NGR = [Ac + 2× Bu + Bc]/[Pr + Bc], where Bc stands for valeriate and branched chain fatty acids (Morvay et al., 2011); Ac/Pr = acetate to propionate ratio.

[2]Efficiency is gross feed use efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.

[3]Three-way interaction effects (Eff. × Dietary CP × TRF) were not significant and hence not provided here.

[4]Meal is daily DM allowance offered in 3 portions a day (as 50%, 30% and 20% at 0630, 1400, and 1830 h, respectively).

[5]TRF is time relative to meal (feeding at 0630, 1400, and 1830 h) in minutes.

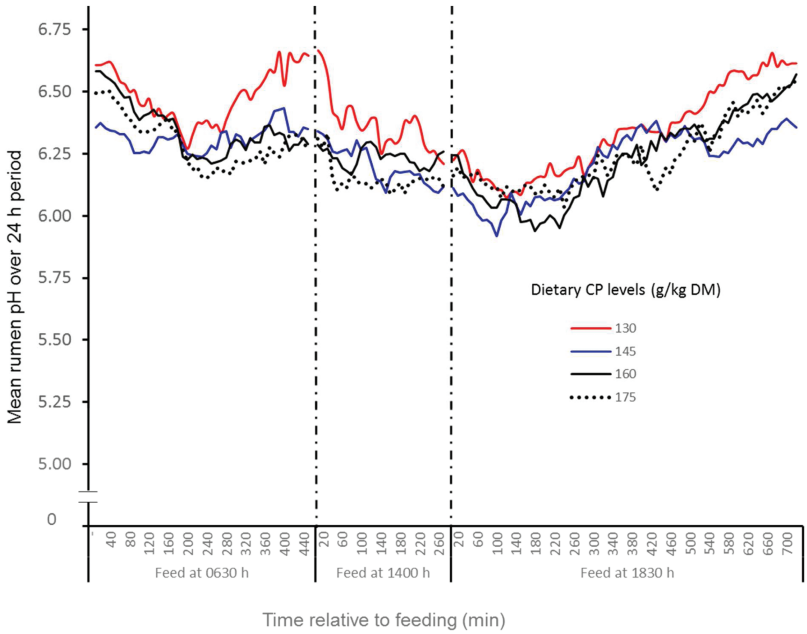

Figure 1. Rumen pH logged continuously over four 24-h periods with Norwegian Red dairy cows fed diets varying in CP concentration from 130 to 175 g/kg DM. Feed was offered in 3 portions at 0630, 1400, and 1830 h as 50%, 30%, and 20% of the daily ad libitum allowance, respectively. The pH was logged every 10-min interval over the recording days and is presented in minutes relative to the 3 meals (as indicated in the figure).

CP level, efficiency background, or interactions thereof. Similar patterns were observed when the 3 main VFA (acetate, propionate, and butyrate) were expressed in molar proportions (% of total VFA; Table 4). Isobutyrate ($P = 0.010$) and isovalerate ($P = 0.078$) decreased with increasing dietary CP level both following a linear trend ($P < 0.05$).

Meal and time relative to meals had strong influence on molar proportions of acetate, propionate, valeriate, isobutyrate, and isovalerate. However, the total VFA and molar proportion of butyrate were not affected by meal. Furthermore, the interaction effects of dietary CP and time relative to meals, dietary CP and efficiency background, and the 3-way interaction effect between level of dietary CP, efficiency background, and time relative to meals were not significant for the total and specific VFA.

The ratios of nonglucogenic to glucogenic VFA (NGR) and acetate to propionate (Ac/Pr) were not influenced by dietary CP, efficiency background, and their interactions ($P > 0.1$). However, both NGR and Ac/Pr ratio were significantly affected by meal and time relative to meals ($P < 0.001$). As a result, both parameters increased with increasing time relative to meals.

Methane Production

Enteric methane emission and its intensity data are presented in Table 5 and Fig. 2. Mean daily enteric methane production was not affected ($P > 0.1$) by either the efficiency background or level of dietary CP offered or interactions thereof. Similarly, dietary CP level, efficiency background, and interactions thereof did not influence methane production intensity expressed per kilogram DMI or OM intake ($P > 0.1$). However, methane production intensity expressed per kilogram milk yield and kilogram ECM was significantly lower ($P < 0.01$) for the HEFF than for the LEFF cows. Enteric methane emission intensity per kilogram milk was significantly greater on the highest level of dietary CP compared with the other 3 dietary treatments (effect of CP; $P < 0.05$).

Dietary Protein Utilization and Nitrogen Excretion

Mean daily nitrogen intake and excretion pattern are presented in Table 6. Furthermore, NUE in relation to daily quantitative crude protein intake is presented in Fig. 3. Nitrogen excreted (g/d) into milk and feces were greater for the HEFF than the LEFF cows. However, daily quantitative nitrogen excreted in milk was not affected by increasing dietary CP level from 130 to 175 g/kg DM. Fecal nitrogen excretion tended to increase ($P = 0.063$) with increasing dietary CP level. However, daily quantitative nitrogen excretion in urine increased in a linear fashion with increasing dietary CP level ($P < 0.0001$).

Expressed as a percentage of intake, nitrogen excreted in milk protein (gross NUE) was greater for the HEFF than LEFF cows ($P = 0.007$). Both NUE and fecal nitrogen (as % of intake) decreased

Table 5. Mean daily enteric methane production and intensity parameters of 2 groups of dairy cows (HEFF vs. LEFF) fed a total mixed ration with CP levels of 130, 145, 160, and 175 g/kg DM

	Eff.[1]			Dietary CP level					Effects (*P*-value)			Contrast for CP	
Parameters	HEFF	LEFF	SE	130	145	160	175	SE	Eff.	CP	CP × Eff.	Linear	Quadratic
CH_4 yield, g/d	482.3	465.7	*13.55*	457.7	481.0	462.5	494.9	*15.72*	*0.41*	*0.29*	*0.97*	*0.18*	*0.77*
CH_4 emission intensity, g/kg intake or product													
DMI	24.7	24.4	0.59	24.4	25.0	23.1	25.7	*0.74*	*0.72*	*0.11*	*0.14*	*0.57*	*0.20*
OMI	26.3	26.0	0.63	26.05	26.64	24.65	27.40	*0.80*	*0.73*	*0.11*	*0.14*	*0.57*	*0.20*
Milk	20.4	24.1	0.54	21.49[a]	22.30[a]	21.10[a]	24.06[b]	*0.71*	*0.013*	*0.032*	*0.18*	*0.064*	*0.16*
ECM[2]	19.9	24.3	0.54	21.76	21.91	20.75	23.5	*0.71*	*0.007*	*0.073*	*0.72*	*0.22*	*0.09*

Means in a row with different superscripts for the dietary CP levels are significantly different at $P < 0.05$.

[1]Eff. is gross feed use efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.

[2]ECM = energy-corrected milk yield.

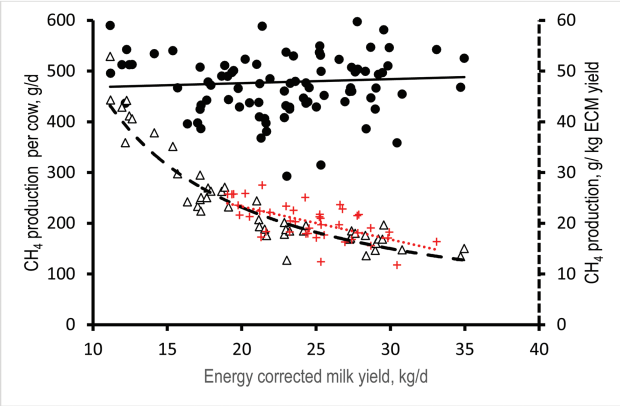

Figure 2. Mean daily enteric methane production and partial intensities in relation to milk yield in Norwegian Red dairy cows in their mid- to late-lactation and exhibiting divergence in gross feed use efficiency (• = daily methane production with a solid trend line; Δ = g CH_4/kg ECM for LEFF cows with broken trend line; and + = g CH_4/kg ECM for HEFF cows with a dotted trend line).

with increasing dietary CP level in a linear fashion ($P < 0.001$) in the range of CP tested. On the contrary, UN excretion (both in g/d and as % of intake) increased with increasing CP level ($P < 0.001$) following a linear fashion. The interaction effects of efficiency background and dietary CP level were not significant for the above parameters.

In the absence of interaction effects between efficiency background and dietary CP levels for the observed nitrogen partitioning, it was possible to make a simple predictive model for UN excretion based on measured UN, MUN, and cow BW. As such, UN excretion calculated as a function of MUN (mg/dL) alone yielded the following equation:

$$\text{UN (g/d)} = 15.07 \times \text{MUN (SE} = 0.563;\ P\text{-value} < 0.001;\ r^2 = 0.958)$$

Whereas daily UN excretion calculated as a function of MUN (mg/dL) and cow average BW (kg) yielded the following equation:

$$\text{UN (g/d)} = 0.02232 \times \text{MUN} \times \text{BW (SE} = 0.0007;\ P < 0.0001;\ r^2 = 0.968)$$

Mean observed and predicted values from the above equations and other existing UN excretion prediction models for other dairy breeds (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Kohn et al., 2002) are presented in Table 7.

DISCUSSION

The interactive effects of FUE background and levels of dietary CP were tested on milk production, enteric CH_4 emission, and NUE using 8 rumen cannulated NRF dairy cows in a 4 × 4 Latin square design experiment. The planned changes in dietary CP levels were achieved through slight changes in ingredient composition without altering the energy density of the diets tested. The objective of the experiment was to assess whether selecting NRF dairy cows for gross feed efficiency would improve NUE and reduce enteric CH_4 emission in subsequent lactations and whether these improvements were to be maintained under different dietary CP levels.

Dry Matter Intake, Milk Yield, and Its Chemical Composition

Mean intake of DM and other nutrients, except for the planned difference in CP, were not different between dietary treatments, suggesting that the lowest level dietary CP did not restrict intake parameters. Similar pattern of DMI and intake per unit metabolic BW observed for both efficiency backgrounds, in the absence of interaction effects with dietary CP level, allowed discussing the observed effects in relation to dietary CP or efficiency background. Furthermore, in the absence of

Table 6. Mean daily nitrogen (N) intake and its excretion patterns in milk, feces, and urine in 2 groups of dairy cows (HEFF vs. LEFF) fed a total mixed ration with CP levels of 130, 145, 160, and 175 g/kg DM

	Eff.[1]			Dietary CP level					Effects (*P*-value)			Contrast for CP	
Parameters	HEFF	LEFF	SE	130	145	160	175	SE	Eff.	CP	CP × Eff.	Linear	Quadratic
N intake, g/d	450.4	422.5	*8.20*	360.1^{a}	416.1^{b}	461.9^{c}	507.7^{d}	*11.60*	*0.031*	*<0.001*	*0.45*	*<0.001*	*0.67*
N recovered (g/d)[2]													
Milk	126.4	108.9	*1.94*	114.4	117.4	123.1	115.8	*2.75*	*<0.001*	*0.17*	*0.54*	*0.45*	*0.10*
Feces	132.7	127.8	*1.61*	124.3	134.1	130.7	131.8	*2.80*	*0.047*	*0.063*	*0.40*	*0.091*	*0.080*
Urine	156.4	155.5	*7.47*	94.5^{a}	135.6^{b}	182.0^{c}	211.5^{d}	*10.55*	*0.94*	*<0.001*	*0.96*	*<0.001*	*0.59*
Total recovered	415.5	392.2	*8.47*	333.2^{a}	387.1^{b}	435.8^{c}	459.1^{c}	*11.97*	*0.072*	*<0.001*	*0.70*	*<0.001*	*0.22*
N not recovered	34.9	30.3	*9.39*	26.9	28.9	26.1	48.6	*13.27*	*0.73*	*0.58*	*0.70*	*0.32*	*0.46*
N recovered as % of N intake													
Milk (NUE)[3]	28.6	26.3	*0.51*	31.7^{c}	28.2^{b}	26.9^{b}	23.0^{a}	*0.72*	*0.007*	*<0.001*	*0.13*	*<0.001*	*0.74*
Feces	30.0	30.6	*0.55*	34.7^{a}	32.2^{a}	28.3^{b}	25.9^{b}	*0.78*	*0.42*	*<0.001*	*0.38*	*<0.001*	*0.91*
Urine	34.1	35.8	*1.60*	26.0^{a}	32.5^{ab}	39.6^{bc}	41.6^{c}	*2.47*	*0.48*	*0.001*	*0.98*	*<0.001*	*0.33*
Total	92.7	92.7	*1.93*	92.5	92.9	94.8	90.4	*2.73*	*0.99*	*0.71*	*0.72*	*0.74*	*0.39*
N not recovered	7.3	7.3	*1.93*	7.5	7.1	5.2	9.6	*2.73*	*0.99*	*0.71*	*0.72*	*0.74*	*0.39*

Means in a row with different superscripts for the dietary CP levels are significantly different at $P < 0.05$.

[1]Eff. is gross feed use efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.

[2]N recovered is amount of nitrogen accounted for in milk, feces, and urine, whereas N not recovered is nitrogen invested in BW changes and hair losses.

[3]Apparent nitrogen use efficiency.

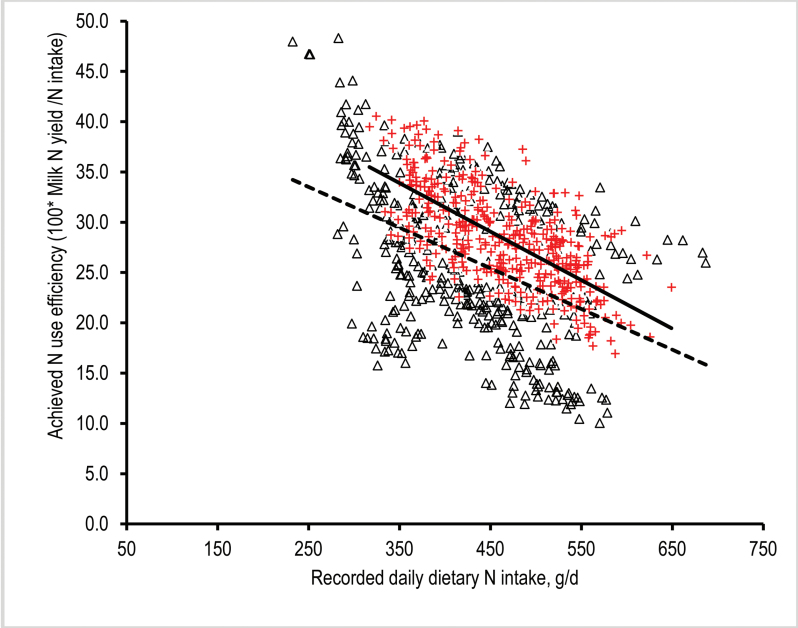

Figure 3. Gross nitrogen use efficiency (100 × milk protein N/N intake) in Norwegian Red dairy cows in their mid- to late-lactation and exhibiting divergence in gross feed use efficiency (Δ = LEFF; + = HEFF) in relation to daily quantitative N intake. Linear trend lines: broken line for the LEFF cows ($Y = -0.041 \times$ N intake + 44.3; $r = -0.442$) and solid line for the HEFF group ($Y = -0.48 \times$ N intake + 50.8; $r = -0.679$).

differences in DM and nutrient intake, we allude the observed effects between dietary treatments to the level of achieved CP intake. As such, the absence of the effects of dietary CP levels in milk and its component yields was not surprising in view of the above intake parameters and the often variable and weak marginal milk yield response to dietary CP level (Broderick, 2003; Bach, 2013). Monteils et al. (2002) report similar findings among 3 groups of cows fed diets differing in CP (130, 145, and 160 g/kg DM). The lack of difference in feed intake, milk, and its component yields suggests that dietary CP

Table 7. Observed and predicted urinary nitrogen (UN, g/d) excretion using our data and different existing models based on milk urea nitrogen (MUN, mg/dL) and cow BW (kg)

Model	Mean	Mean bias	Residual error	RMSPE
Observed UN	157.6	(SD = 59.7)	—	—
Predictions				
12.54 × MUN[1]	132.3	−25.3	35.7	43.7
17.64 × MUN[2]	186.2	28.5	33.5	44.0
0.0259 × MUN × BW[2]	184.1	26.4	29.5	39.6
0.026 × MUN × BW[3]	184.8	27.1	29.5	40.1
15.07 × MU[4]	159.4	1.7	33.8	33.9
0.0223 × MU × BW[4]	158.8	1.1	29.5	29.5

Mean bias was calculated as $\frac{\sum(\text{Predicted} - \text{Observed})}{\text{Number of observations}}$.

RMSPE = root mean square prediction error and calculated as $\sqrt{\left[\frac{\sum(\text{Predicted} - \text{Observed})^2}{\text{Number of observations}}\right]}$.

Residual error was calculated as $\sqrt{[\text{RMSPE}^2 - (\text{Mean bias})^2]}$.

Models from [1]Jonker et al. (1998); [2]Kauffman and St-Pierre (2001); [3]Kohn et al. (2002); [4]Our own data.

level of 130 g/kg DM, even though marked with lowest levels of rumen fluid NH_3-N levels (see the Rumen Fermentation Parameters section) relative to other groups, fulfilled minimum requirements for microbial growth and feed degradation in the rumen. Under such low CP diets, it is expected that the higher turnover rate of urea N with reduced clearance in the kidneys and increased clearance from the digestive tract (Marini and Van Amburgh, 2003) would compensate for the low level of dietary CP for rumen microbes (Brake et al., 2010).

In addition to the above intake parameters, the achieved level of total tract DM digestibility, BW change, and body condition score (A. Kidane et al., unpublished data) were similar between the 2 efficiency groups. Despite these similarities, cows from the HEFF group produced higher milk, energy-corrected milk, and milk component yields than cows from the LEFF group. Therefore, the observed differences could be attributed to differences in efficiency of partitioning the absorbed nutrients into different bodily functions (maintenance, milk production, pregnancy, BW gain, etc.) (Agnew and Yan, 2000). Our animals were at very early part of pregnancy and showed similar BW gain where differences associated to resource allocations to these sinks would be minimal and thus could be ignored, even though such assumption of constant level of energy allocation per unit BW gain has inherent limitations (Agnew and Yan, 2000). However, at around 3× maintenance feeding which is observed in our trial, maintenance requirement would be assumed a large nutrient sink. Furthermore, the latter is often assumed fixed for kg $BW^{0.75}$ (INRA, 1989), or a function of $BW^{0.75}$ with some additional factors for activity and class of an animal (NorFor, 2011). However, maintenance requirements are not fixed. For example, maintenance energy requirement increases with increasing feed intake as indicated by Dong et al. (2015). The authors argue that current feeding systems, which assume single fixed maintenance requirements, may underestimate energy requirements for high yielding dairy cows. Even though, Dong et al. (2015) observed no differences in energetic efficiency between breeds/groups, there was a large variation in ME_m requirement (about 0.4 to 0.9 MJ/kg $BW^{0.75}$) between individual cows. Here, we further argue that because of such large variations between individuals in maintenance requirements, differences in milk yields can be partially attributed to partitioning part of this assumed maintenance intake into milk production at similar level of energy intake.

Rumen Fermentation Parameters

The overall recorded rumen pH values were in the physiological range for dairy cows (5.5 to 7.0) and showed indifference to the efficiency background. With regard to diurnal fluctuations, rumen pH values for each group peaked before morning and afternoon feeding with temporal nadir attained at about 2- to 4-h postfeeding, depending on meals. This relatively elevated pH before feeding compared with postfeeding is coherent with other reports (Galyean et al., 1981; Belanche et al., 2012) and could be the effect of long hours postfeeding (fasting), mirroring the decreasing rumen

VFA concentration as discussed later. This was evident from our result that the AM (0630 h) and PM (1400 h) meals that were 12 and 7.5 h, respectively, after previous feedings resulted in elevated rumen pH recordings. On the contrary, the evening (1830 h) meal with short duration (4.5 h) from previous feeding did not produce similar influence on rumen pH.

The greater pH for the lowest dietary CP group compared with the other dietary treatments suggested somewhat weaker buffering capacity of the marginally lower rumen VFA, probably resulting from a decreased carbohydrate degradation. However, the overall picture contradicts the reports of Haaland et al. (1982) where increasing dietary CP (110, 140, and 170 g/kg) increased rumen pH with nonlactating Holstein-Friesian cows.

Rumen fluid VFA concentration and molar proportions of the main VFA (acetate, propionate, and butyrate) in samples taken at frequent intervals showed indifference to the efficiency groups with butyrate only tending to be greater for HEFF than LEFF cows. Similarly, these parameters were not affected by dietary CP levels. Colmenero and Broderick (2006) reported comparable results when feeding diets ranging in CP from 135 to 194 g/kg DM to lactating Holstein-Friesian dairy cows. Furthermore, Belanche et al. (2012) reported lack of difference in the main VFA between dairy cows fed high- and low-protein diets. However, the decrease in molar proportions of isobutyrate and isovalerate with increasing dietary CP level contradicts the recent report of Belanche et al. (2012) where lower dietary CP level was associated with lower levels of these VFA. These VFA were expected to originate mainly from AA (leucine and valine) metabolism (Menahan and Schiltz, 1964; Zarling and Ruchim, 1987), especially under excess CP supply. Therefore, the decreasing molar proportions of these VFA with increasing dietary CP levels could be due to the concentration of these AA from the incremental protein supplement. First, the incorporation of urea in the diet, which increased with increasing CP level, comes without any contribution to this AA pool and, therefore, could be seen as a diluting factor. Furthermore, the yeast-based microbial crude protein (**DEMP**) which substituted barley at higher levels of CP comes with relatively lower valine and leucine concentration (Watson, 1976) compared with barley (Shewry et al., 1983; Prestløkken, 1999).

Rumen fluid VFA and the relative proportion in which each specific VFA is produced depend to a large extent on substrate composition, its availability, rumen microbial species present and rumen pH attained (Dijkstra, 1994). Here, efforts were made to minimize differences in diet gross composition between the 4 dietary treatments, except for the CP level. Therefore, the observed results would indicate that as long as its level was not limiting intake, reduction in dietary CP to 130 g/kg DM might not be detrimental for rumen microbial function and, as such, fiber digestion. Indeed, improved fiber digestibility is associated with increasing dietary CP level in dairy cows diets (Huhtanen et al., 2009). Conversely, reducing dietary N from 3.4% to 1.44% (equivalent to 213 to 90 g CP/kg DM) depressed fiber digestibility with Holstein-Friesian heifers (Marini and Van Amburgh, 2003). However, this does not seem to be the case in the ranges of CP we tested here.

Enteric Methane Emission

Manipulating the nutrient composition of ruminant diets is one of the options to reduce CH_4 emissions without lowering animal production (Grainger and Beauchemin, 2011). Nevertheless, the outcomes are often complex and variable. We did not observe any reduction in enteric methane production by increasing dietary CP. Our observed mean daily enteric CH_4 emission values are close to recent reports for dairy cows (Alstrup et al., 2013; Basarab et al., 2013) but higher than what was reported for relatively high yielding cows consuming similar level of DM (Brask et al., 2013; Niu et al., 2016). However, Niu et al. (2016) fed diets lower in dietary forage to concentrate ratio (mean alfalfa hay to compound feed at 45:55), lower in NDF (mean: 276 g/kg DM), and relatively higher in crude fat (mean: 37 g/kg DM) compared to our diets. Similarly, Brask et al. (2013) fed diets higher in crude fat (mean: 54.3 g/kg DM) and lower NDF (mean: 327 g/kg DM) than what we report here. Therefore, such differences as dietary forage to concentrate feed ratio, diet chemical composition, stage of lactation and associated DMI levels, and methods of CH_4 measurement could justify some of the differences (Johnson et al., 1994; Kebreab et al., 2006; Grainger and Beauchemin, 2011; Alstrup et al., 2013; Niu et al., 2016).

The intensity of CH_4 emission generated per unit output is often described as a useful metric (Gerber et al., 2013a). Our calculated partial emission intensities for HEFF vs. LEFF groups and for the 4 dietary treatments fall very close to recent reports by Alstrup et al. (2013) or within the range of values reported by Grainger and Beauchemin (2011). Enteric CH_4 emission presented as a portion of

estimated gross energy intake (i.e., ~7.0% observed here) is within the range of what is expected [2% to 12%; (Czerkawski, 1986)] but higher than values for cattle fed high grain diets typical of feedlot operations, that is, ~2% to 4% (Johnson et al., 1994).

The HEFF cows produced more milk at similar level of DMI and similar level of daily enteric CH_4 yield. This has resulted in a lower partial emission intensity (expressed per unit kg milk or ECM) for HEFF than for LEFF cows. The outcome suggested that attempts to mitigate GHG emission could benefit more from strategies that target gross feed use efficiencies (Gerber et al., 2013b).

The absence of the effects of dietary CP level on either daily methane production or partial emission intensity over the ranges of CP tested was initially not expected as DMI, DM digestibility, rumen fermentation parameters, and milk yield were expected to differ between the different dietary treatments. However, we did not observe these differences, and under such conditions, the outcome of methane emission was not surprising.

The increasing NGR, Ac/Pr, and rumen pH with increasing time postfeeding suggested that if methane emissions were to be influenced by diet fermentation pattern, rumen pH, and VFA profile (Wolin, 1960; Russel, 1998), the diurnal pattern of enteric CH_4 emission may not be constant. Recent reports by Danielsson et al. (2017) and Doreau et al. (2018) clearly indicate this diurnal fluctuation. This is expected because of the differences in rate and extent of fermentation of different dietary components into different VFA. As such, the amount of CH_4 formed per unit of feed fermented depends on the relative activities of the species of microbes using each of the possible fermentation pathways producing different kinds of VFA and amounts of H_2 (Jansen, 2010). Here, the rapidly degradable carbohydrates, like starch, are fermented largely to propionate, whereas the relatively slowly degrading cellulose and hemicellulose from the dietary fiber are fermented largely to acetate contributing differently to the CH_4 pool on a temporal scale. Therefore, enteric CH_4 sampling techniques that fail to cover 24-h cycle or sampling techniques with time points that are not distributed uniformly throughout the day may produce data that are not representative of true emissions.

Nitrogen Metabolism and Excretion

We observed a substantial portion (average across treatments = 65%) of dietary N consumed excreted in urine and feces. Niu et al. (2016) report comparable level of N loss with dairy cows fed dietary CP levels of 152 and 185 g/kg DM. Such a loss has many implications. First, it is a wasted resource because protein ingredients are often imported and come with added costs for milk production. Second, it has an unwanted environmental impacts (Castillo et al., 2000). Nitrogen excretion decreased with decreasing dietary CP from 175 g/kg DM to 130 g/kg DM, which is in agreement with other reports (Colmenero and Broderick, 2006; Powell et al., 2008, 2010; Rendon-Huerta et al., 2014). Furthermore, the positive linear response of rumen NH_3-N to increasing levels of CP in the diet, over the ranges tested, agrees well with other reports (Mehrez et al., 1977; Haaland et al., 1982; Colmenero and Broderick, 2006; Amaral et al., 2016). The observed range of mean values for rumen NH_3-N falls short of the minimum level of rumen NH_3 (235 mg/L) concentration for maximal rate of fermentation (Mehrez et al., 1977). However, rumen NH_3-N concentrations required for maximum microbial growth and maximum digestion may not be constant depending on diet fermentation characteristics (Erdman et al., 1986). The achieved similar level of rumen degradation of DM and other nutrients (A. Kidane et al., unpublished data), rumen total and specific VFA concentration against the observed large variation in rumen fluid NH_3-N concentrations further underlines the limitations of such minimum level recommendations.

The quantitative amount of N excreted in feces marginally increased with increasing dietary CP level. However, expressed as percentage of intake, this trend was reversed indicating the less sensitive nature of fecal N excretion in stark contrast to UN excretion. Similar outcome was reported with Holstein dairy cows fed diets varying in CP from 13.5% to 19.4% (Colmenero and Broderick, 2006). The implication here is that the level of dietary CP should be reduced in an attempt to improve NUE and minimize losses (Castillo et al., 2000; Sinclair et al., 2014). In addition, increasing N intake does not always lead to improved lactational performance (Santos et al., 1998; Monteils et al., 2002; Bach, 2013), especially at higher levels of CP intake (Broderick, 2003). The observed difference in milk and its component yields between the 2 efficiency backgrounds was bigger than the improvements brought about by gradually increasing dietary crude protein from 130 to 175 g/kg DM.

CONCLUSIONS

No interaction was observed between dietary CP level and FUE background on DMI, other nutrient intake, NUE, enteric CH_4 emission, and CH_4 emission intensity. Gradually decreasing dietary CP from 175 to 130 g/kg DM did not affect DMI, milk yield, energy-corrected milk yield, milk component yield, and daily enteric CH_4 emission. However, decreasing dietary CP increased NUE and reduced UN excretion (both in quantitative terms and as proportion of N intake).

Cows with higher FUE showed improved NUE and decreased enteric CH_4 emission intensity compared with their low-efficiency contemporaries regardless of the level of dietary CP. This would imply that enteric CH_4 emission intensity and UN excretions can be reduced by selecting dairy cows with higher FUE and reducing dietary CP level, respectively, independent of one another.

Furthermore, UN excretion predictions based on MUN and cow BW for NRF cows produced very close estimates to recorded values. This requires larger data set for validation for application under a large scale. However, it at least promises an inexpensive and useful tool, under Nordic conditions where ordinary milk analysis comes with MUN estimates, for assessing UN excretion from dairy cows to the environment.

LITERATURE CITED

Agnew, R. E., and T. Yan. 2000. Impact of recent research on energy feeding systems for dairy cattle. Livest. Prod. Sci. 66:197–215. doi:10.1016/S0301-6226(00)00161-5

Alstrup, L., M. R. Weisbjerg, and P. Lund, editors. 2013. Effect of fat supplementation and stage of lactation on methane emission in dairy cows. Energy and protein metabolism and nutrition in sustainable animal production. EAAP Publ. No. 134. Wageningen Acad. Publ., Wageningen, The Netherlands, p. 489–490.

Amaral, P. M., L. D. Mariz, P. D. Benedeti, L. G. Silva, E. M. Paula, H. F. Monteiro, T. Shenkoru, S. A. Santos, S. R. Poulson, and A. P. Faciola. 2016. Effects of static or oscillating dietary crude protein levels on fermentation dynamics of beef cattle diets using a dual-flow continuous culture system. PLoS One 11:e0169170. doi:10.1371/journal.pone.0169170

AOAC International (Association of Official Analytical Chemists). 2000. Official Method 973.18 Fiber (acid detergent) and lignin in animal feed. In: Horwitz, W., editor. Official methods of analysis of AOAC international, 17th edn. AOAC International, Gaithersburg, MD. p. 37–38.

AOAC International (Association of Official Analytical Chemists). 2002. Official methods of analysis of AOAC International. Method 2001.11. J AOAC Int. 85.

Bach, A. 2013. Key indicators for measuring dairy cow performance. In: H. P. S. Makkar and D. Beever, editors, Optimization of feed use efficiency in ruminant production systems. Proceedings of the FAO Symposium, 27 November 2012, Bangkok, Thailand. FAO Animal Production and Health Proceedings No. 16. FAO and Asian-Australasian Association of Animal Production Societies, Rome, Italy. p. 33–44.

Basarab, J. A., K. A. Beauchemin, V. S. Baron, K. H. Ominski, L. L. Guan, S. P. Miller, and J. J. Crowley. 2013. Reducing GHG emissions through genetic improvement for feed efficiency: Effects on economically important traits and enteric methane production. Animal 7(Suppl 2):303–315. doi:10.1017/S1751731113000888

Belanche, A., M. Doreau, J. E. Edwards, J. M. Moorby, E. Pinloche, and C. J. Newbold. 2012. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. J. Nutr. 142:1684–1692. doi:10.3945/jn.112.159574

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19:511–522.

Brake, D. W., E. C. Titgemeyer, M. L. Jones, and D. E. Anderson. 2010. Effect of nitrogen supplementation on urea kinetics and microbial use of recycled urea in steers consuming corn-based diets. J. Anim. Sci. 88:2729–2740. doi:10.2527/jas.2009-2641

Brask, M., P. Lund, M. R. Weisbjerg, A. L. F. Hellwing, M. Poulsen, M. K. Larsen, and T. Hvelplund. 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. J. Dairy Sci. 96:2356–2365. doi:10.3168/jds.2011–5239

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381. doi:10.3168/jds.S0022-0302(03)73721-7

Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci. 9:1–32. doi:10.22358/jafs/68025/2000

Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712. doi:10.3168/jds.S0022-0302(06)72238-X

Connor, E. E. 2015. Invited review: Improving feed efficiency in dairy production: Challenges and possibilities. Animal 9:395–408. doi:10.1017/S1751731114002997

Connor, E. E., J. L. Hutchison, H. D. Norman, K. M. Olson, C. P. Van Tassell, J. M. Leith, and R. L. Baldwin, 6th. 2013. Use of residual feed intake in Holsteins during early lactation shows potential to improve feed efficiency through genetic selection. J. Anim. Sci. 91:3978–3988. doi:10.2527/jas.2012-5977

Coppock, C. E., D. L. Bath, and B. Harris. 1981. From feeding to feeding systems. J. Dairy Sci. 64:1230–1249. doi:10.3168/jds.S0022-0302(81)82698-7

Czerkawski, J. W. 1986. An introduction to rumen studies. Pergamon Press, Oxford, UK. p. 236.

Danielsson, R., M. Ramin, J. Bertilsson, P. Lund, and P. Huhtanen. 2017. Evaluation of a gas in vitro system for predicting methane production in vivo. J. Dairy Sci. 100:8881–8894. doi:10.3168/jds.2017–12675

Dijkstra, J. 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39:61–69. doi:10.1016/0301-6226(94)90154-6

Dong, L. F., T. Yan, C. P. Ferris, and D. A. McDowell. 2015. Comparison of maintenance energy requirement and energetic efficiency between lactating Holstein-Friesian and other groups of dairy cows. J. Dairy Sci. 98:1136–1144. doi:10.3168/jds.2014–8629

Dønnem, I., Å. T. Randby, and M. Eknæs. 2011. Effects of grass silage harvesting time and level of concentrate supplementation on nutrient digestibility and dairy goat performance. Anim. Feed Sci. Technol. 163:150–160. doi:10.1016/j.anifeedsci.2010.10.018

Doreau, M., M. Arbre, Y. Rochette, C. Lascoux, M. Eugène, and C. Martin. 2018. Comparison of 3 methods for estimating enteric methane and carbon dioxide emission in nonlactating cows. J. Anim. Sci. 96:1559–1569. doi:10.1093/jas/sky033

EEA. 2016. Annual European Union greenhouse gas inventory 1990–2014 and inventory report 2016. European Environment Agency Report No. 15/2016. Publications Office of the European Union, Luxembourg. doi:10.2800/13607

Erdman, R. A., G. H. Proctor, and J. H. Vandersall. 1986. Effect of rumen ammonia concentration on in situ rate and extent of digestion of feedstuffs. J. Dairy Sci. 69:2312–2320. doi:10.3168/jds.S0022-0302(86)80670-1

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7–18. doi:10.2527/jas1981.5317

Gerber, P. J., A. N. Hristov, B. Henderson, H. Makkar, J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, et al. 2013a. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: A review. Animal 7:220–234. doi:10.1017/S1751731113000876

Gerber, P. J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio. 2013b. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Grainger, C., and K. A. Beauchemin. 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166–167:308–320. doi:10.1016/j.anifeedsci.2011.04.021

Haaland, G. L., H. F. Tyrrell, P. W. Moe, and W. E. Wheeler. 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentration of cattle. J. Anim. Sci. 55:943–950. doi:10.2527/jas1982.554943x

Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85:1479–1486. doi:10.2527/jas.2006-236

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. doi:10.3168/jds.2008-1834

INRA (Institut National de la Recherche Agronomique); Jarrige, R., editor. 1989. Ruminant nutrition: Recommended allowances and feed tables. John Libbey and Co. Ltd., London, UK. p. 389.

Jansen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1–22. doi:10.1016/j.anifeedsci.2010.07.002

Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. Environ. Sci. Technol. 28:359–362. doi:10.1021/es00051a025

Jones, F. M., F. A. Phillips, T. Naylor, and N. B. Mercer. 2011. Methane emissions from grazing Angus beef cows selected for divergent residual feed intake. Anim. Feed Sci. Technol. 166–167:302–307. doi:10.1016/j.anifeedsci.2011.04.020

Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81:2681–2692. doi:10.3168/jds.S0022-0302(98)75825-4

Kauffman, A. J., and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. J. Dairy Sci. 84:2284–2294. doi:10.3168/jds.S0022-0302(01)74675-9

Kebreab, E., K. Clark, C. Wagner-Riddle, and J. France. 2006. Methane and nitrous oxide emissions from Canadian animal agriculture: A review. Can. J. Anim. Sci. 86:135–158. doi:10.4141/A05-010

Kidane, A., M. Øverland, L. T. Mydland, and E. Prestløkken. 2018. Milk production of Norwegian Red dairy cows on silages presumed either low or optimal in dietary crude protein content. Livest. Sci. 214:42–50. doi:10.1016/j.livsci.2018.05.011

Kohn, R. A., K. F. Kalscheur, and E. Russek-Cohen. 2002. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen1. J. Dairy Sci. 85:227–233. doi:10.3168/jds.S0022-0302(02)74071-X

Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545–552. doi:10.2527/2003.812545x.

McGinn, S. M., K. A. Beauchemin, A. D. Iwaasa, and T. A. McAllister. 2006. Assessment of the sulfur hexafluoride (SF6) tracer technique for measuring enteric methane emissions from cattle. J. Environ. Qual. 35:1686–1691. doi:10.2134/jeq2006.0054

Mehrez, A. Z., E. R. Orskov, and I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38:437–443. doi:10.1079/BJN19770108

Menahan, L. A., and L. H. Schiltz. 1964. Metabolism of leucine and valine with the rumen. J. Dairy Sci. 47:1080–1085. doi:10.3168/jds.S0022-0302(64)88849-4

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.

Monteils, V., S. Jurjanz, G. Blanchart, and F. Laurent. 2002. Nitrogen utilisation by dairy cows fed diets differing in crude protein level with a deficit in ruminal fermentable nitrogen. Reprod. Nutr. Dev. 42:545–557.

Morvay, Y., A. Bannink, J. France, E. Kebreab, and J. Dijkstra. 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. J. Dairy Sci. 94:3063–3080. doi:10.3168/jds.2010-3995

Niu, M., J. A. D. R. N. Appuhamy, A. B. Leytem, R. S. Dungan, and E. Kebreab. 2016. Effect of dietary crude protein and

forage contents on enteric methane emissions and nitrogen excretion from dairy cows simultaneously. Anim. Prod. Sci. 56:312–321. doi: 10.1071/An15498

NorFor. 2011. In: H. Volden, editor, NorFor – The Nordic feed evaluation system. Wageningen Acad. Publ., Wageningen, The Netherlands. p. 180.

Opio, C., P. Gerber, A. Mottet, A. Falcucci, G. Tempio, M. MacLeod, T. 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 (FAO), Rome, Italy.

Powell, J. M., C. J. P. Gourley, C. A. Rotz, and D. M. Weaver. 2010. Nitrogen use efficiency: A potential performance indicator and policy tool for dairy farms. Environ. Sci. Policy 13:217–228. doi:10.1016/j.envsci.2010.03.007

Powell, J. M., Y. Li, Z. Wu, G. A. Broderick, and B. J. Holmes. 2008. Rapid assessment of feed and manure nutrient management on confinement dairy farms. Nutr. Cycl. Agroecosys. 82:107–115. doi:10.1007/s10705-008-9173-3

Prestløkken, E. 1999. Ruminal degradability and intestinal digestibility of protein and amino acids in barley and oats expander-treated at various intensities. Anim. Feed Sci. Technol. 82:157–175. doi:10.1016/S0377-8401(99)00110-8

Rendon-Huerta, J. A., J. M. Pinos-Rodriguez, J. C. Garcia-Lopez, L. G. Yanez-Estrada, and E. Kebreab. 2014. Trends in greenhouse gas emissions from dairy cattle in Mexico between 1970 and 2010. Anim. Prod. Sci. 54:292–298. doi:10.1071/An12327

Russel, J. B. 1998. The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production in vitro. J. Dairy Sci. 81:3222–3230. doi:10.3168/jds.S0022-0302(98)75886-2

Santos, F. A., J. E. Santos, C. B. Theurer, and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182–3213. doi:10.3168/jds.S0022-0302(98)75884-9

Shewry, P. R., J. Franklin, S. Parmar, S. J. Smith, and B. J. Miflin. 1983. The effects of sulphur starvation on the amino acid and protein compositions of barley grain. J. Cereal Sci. 1:21–31. doi:10.1016/S0733-5210(83)80005-8

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

Sinclair, K. D., P. C. Garnsworthy, G. E. Mann, and L. A. Sinclair. 2014. Reducing dietary protein in dairy cow diets: Implications for nitrogen utilization, milk production, welfare and fertility. Animal 8:262–274. doi:10.1017/S1751731113002139

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, and O. Sirotenko. 2007. Agriculture. In: B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, and L. A. Meyer, editors, Climate change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Thiex, N. J., H. Manson, S. Andersson, and J. A. Persson. 2002. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: Collaborative study. J. AOAC Int. 85:309–317.

Watson, T. G. 1976. Amino-acid pool composition of *Saccharomyces cerevisiae* as a function of growth rate and amino-acid nitrogen source. J. Gen. Microbiol. 96:263–268. doi:10.1099/00221287-96-2-263

Wolin, M. J. 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43:1452–1459. doi:10.3168/jds.S0022-0302(60)90348-9

Zarling, E. J., and M. A. Ruchim. 1987. Protein origin of the volatile fatty acids isobutyrate and isovalerate in human stool. J. Lab. Clin. Med. 109:566–570.