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

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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 (CH₄) 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, CH₄ emission, and its intensity (g CH₄/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 CH₄ 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 CH₄ emission intensity compared with the LEFF cows. In the absence of interaction effects between efficiency background and dietary CP level, our results suggest that CH₄ 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

INTRODUCTION

Recent greenhouse gas (GHG) emission from livestock supply chains is estimated at 7.1 Gt CO₂-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\textsubscript{4}) and nitrous oxide (N\textsubscript{2}O) 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\textsubscript{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\textsubscript{4} generated during enteric fermentation and proper management of manure to reduce CH\textsubscript{4} and N\textsubscript{2}O released during storage (Gerber et al., 2013b). Enteric CH\textsubscript{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\textsubscript{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 Favor 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

| Ingredients in TMR | Dietary Treatments | 130 | 145 | 160 | 175 |
|-------------------|-------------------|-----|-----|-----|-----|
| Grass silage      |                   | 500.0| 500.0| 492.5| 492.5|
| Concentrate¹      |                   | 425.0| 425.0| 425.0| 415.0|
| Barley pellet     |                   | 65.0 | 37.5 | 17.5 | 0.0 |
| Protein supplement² |                 | 10.0 | 37.5 | 65.0 | 92.5|

Chemical composition of TMR (analyzed/estimated)

|                          | DM content, g/kg fresh | OM | Ash | CP | Starch | aNDFom³ | pdNDF⁴, g/kg NDF | iNDF⁵ | ADF⁶ | Crude fat | FPF⁷ | RestCHO⁸ | NEₚ, MJ/kg DM |
|--------------------------|------------------------|-----|-----|----|--------|---------|-----------------|-------|------|-----------|------|----------|----------------|
|                          | 411.0                  | 939.1 | 60.9 | 118.2 | 227.3 | 399.2 | 797.8          | 80    | 241.2 | 24.8      | 15.0 | 159.0    | 6.7            |
|                          | 411.0                  | 939.0 | 61.0 | 134.0 | 221.8 | 392.1 | 803.4          | 77.1  | 238.1 | 24.0      | 15.0 | 153.0    | 6.6            |
|                          | 415.0                  | 938.5 | 61.5 | 149.1 | 211.2 | 391.8 | 802.0          | 78.2  | 239.1 | 24.9      | 14.5 | 139.0    | 6.6            |
|                          | 415.0                  | 938.0 | 62.0 | 166.7 | 211.2 | 391.8 | 809.8          | 74.4  | 239.3 | 22.8      | 14.8 | 134.0    | 6.6            |
|                          | 415.0                  | 938.0 | 62.0 | 166.7 | 211.2 | 391.8 | 809.8          | 74.4  | 239.3 | 22.8      | 14.8 | 134.0    | 6.6            |

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

¹Commercial 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).

²Protein 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).

³NDF corrected for ash.

⁴Potentially degradable NDF.

⁵Indigestible NDF.

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

⁷Residual carbohydrates corrected for low-molecular-weight fractions (urea and NH₃-N) as in the Nordic feed evaluation system (NorFor, 2011).

⁸Calculated NEₚ based on the proportion of ingredients and their energy values in the TMR.

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 Kjeltte 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 ANKOM²⁰ 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 Donnem 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-nitropropane-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 (NH₃-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 NH₃-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) H₂SO₄ 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 CH₄ production was estimated using sulfur hexafluoride (SF₆) as a marker (Johnson et al., 1994). Brass permeation tubes filled with SF₆ gas (mean ± SD = 2338 ± 148.9 mg) and predetermined mean (± SD) release rate of 4.614 (± 0.228; r² = 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 CH₄ 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 CH₄. 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 CH₄ and an electron capture detector for SF₆ analysis. Daily enteric CH₄ emission was calculated according to McGinn et al. (2006):

\[
Q_{CH₄} = \frac{C_{CH₄} - C_{CH₄}^b}{C_{SF₆} - C_{SF₆}^b} \frac{MW_{CH₄}}{MW_{SF₆}}
\]

where \(Q_{CH₄}\) is daily enteric methane emission (g/d); \(Q_{SF₆}\) is predetermined marker release rate (g/d); \(C_{CH₄}\) and \(C_{SF₆}\) are CH₄ and SF₆ mixing ratios in the yokes (µmol/mol); \(C_{CH₄}^b\) and \(C_{SF₆}^b\) are background CH₄ and SF₆ levels in air samples from the barn; and \(MW_{CH₄}/MW_{SF₆}\) 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

\[
\text{ECM} = \frac{\text{Calculated ECM} - \text{Cow Gen Balance \_ ECM}}{100} \times \text{Total Yield (Kg)}
\]
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 CH4 emission were assessed using the following model:

\[ Y_{ijklmn} = \mu + \alpha_i + \beta_j + \theta_k + C_{l(j)} + D_{m(k)} + (\alpha \beta)_ij + \epsilon_{ijklmn} \]

where \( Y_{ijklmn} \) is response variable, \( \mu \) is the overall mean, \( \alpha \) is the effect level of dietary CP protein, \( \beta \) is the effect of efficiency background (block), \( \theta \) 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 \( \epsilon_{ijklmn} \) is residual error term.

Rumen fluid VFA and NH3-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:

\[ Y_{ijklmn} = \mu + \alpha_i + \beta_j + \theta_k + C_{l(j)} + F_m + TRF_{n(m)} + (\alpha \beta)_ij + \epsilon_{ijklmn} \]

where \( Y_{ijklmn} \) is response variable, \( \mu \) is the overall mean for a response variable, \( \alpha \) is the effect level of dietary CP, \( \beta \) is the effect of efficiency background (block), \( \theta \) 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, \( \beta TRF \) is the interaction effect of efficiency background and time relative to feeding, \( \alpha TRF \) 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 \( \epsilon_{ijklmn} \) 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 (BW0.75), 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 BW0.75) 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

| Parameters                  | Eff. | 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     |
| CP                          | 2.81 | 2.69 | 0.09| 2.22^a|2.67^a|2.92^c|3.18^b|0.17| 0.25 | <0.001| 0.27     | <0.001 | 0.14     |
| 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                       | 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

| Parameters                  | Eff. | LEFF | SE  | 130 | 145 | 160 | 175 | SE  | Eff. | CP   | CP × Eff. | Linear | Quadratic |
|-----------------------------|------|------|-----|-----|-----|-----|-----|-----|------|------|----------|--------|----------|
| 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     |
| CP                          | 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     |

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

1Eff. is gross feed efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.
2aNDF corrected for ash.
3Metured 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

| Parameters                  | Eff. | 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^c                       | 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^b|4.04^b|4.20^a|0.155| 0.70  | 0.014| <0.001   | 0.02   | 0.89     |
| Protein                     | 3.55 | 3.44 | 0.059| 3.44^a|3.52^a|3.54^b|3.48^b|0.043| 0.24  | <0.001| <0.001   | <0.001 | <0.001   |
| Lactose                     | 4.65 | 4.41 | 0.070| 4.54| 4.54 | 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^a|11.68^a|13.47^a|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.

1Eff. is gross feed use efficiency background with HEFF for high-efficiency cows and LEFF for low-efficiency cows.
2ECM = energy-corrected milk yield.
3MUN = 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 NH₃-N and VFA is presented in Table 4. Dietary CP level significantly influenced rumen NH₃-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), NH₃-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 NH₃-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 contrast for dietary CP levels. Dietary CP level significantly influenced rumen NH₃-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), NH₃-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 NH₃-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).

Table 4. Rumen ammonia nitrogen (NH₃-N; mg/L), total volatile fatty acid (VFA; mM), molar proportions of acetate (Ac), propionate (Pr), butyrate (Bu) and valerate (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

| Treatments | NH₃-N | Total VFA | Ac | Pr | Bu | Val | IsoBu | IsoVal | NGR | Ac/Pr | Rumen pH |
|------------|-------|-----------|----|----|----|-----|-------|--------|-----|-------|----------|
| Efficiency |       |           |    |    |    |      |       |        |     |       |          |
| 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        | 53.1a | 104.6     | 65.26| 19.52| 12.55| 1.16| 0.67c | 0.81   | 4.28| 3.43  | 6.40a    |
| 145        | 97.7b | 106.8     | 65.43| 19.47| 12.48| 1.16| 0.64b | 0.82   | 4.35| 3.49  | 6.24b    |
| 160        | 135.5c| 110.2     | 65.37| 19.62| 12.45| 1.13| 0.64b | 0.76   | 4.27| 3.41  | 6.27c    |
| 175        | 161.8b| 108.4     | 65.17| 19.69| 12.73| 1.22| 0.61b | 0.68   | 4.29| 3.40  | 6.23b    |
| 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

| Efficiency |       |           |    |    |    |      |       |        |     |       |          |
|------------|-------|-----------|----|    |    |      |       |        |     |       |          |
| 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| 0.35  | 0.010  | 0.95| 0.85  | 0.078    |
| Meal       | 0.001 | 0.90      | <0.001| <0.001| 0.35 | <0.001| 0.070 | 0.018  | <0.001| <0.001| 0.016    |
| TRF        | <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 | 0.028  | 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.

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

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

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

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

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

Methane emission and nitrogen use efficiency background. 

Methane emission and nitrogen use efficiency background.
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...
Methane emission and nitrogen use efficiency

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:

\[
UN \ (g/d) = 15.07 \times MUN \ (SE = 0.563; \ P-value < 0.001; \ r^2 = 0.958)
\]

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

\[
UN \ (gd) = 0.02232 \times MUN \times BW \ (SE = 0.0007; \ P < 0.0001; \ r^2 = 0.968)
\]

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 \(4 \times 4\) 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 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

| Parameters                      | Eff. \(^1\) | Dietary CP level | Effects (P-value) | Contrast for CP |
|---------------------------------|-------------|------------------|-------------------|-----------------|
|                                 | HEFF       | LEFF             | 130  | 145  | 160  | 175  | SE         | Eff. | CP | CP × Eff. | Linear | Quadratic |
| CH\(_4\) yield, g/d             | 482.3      | 465.7            | 13.55 |      |      |      |           | 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  |      |      |      |           |      |      |      |      |      |
| OMI                             | 26.0       | 26.0             | 0.63  |      |      |      |           |      |      |      |      |      |
| Milk                            | 20.4       | 24.1             | 0.54  |      |      |      |           |      |      |      |      |      |
| ECM\(^2\)                       | 19.9       | 24.3             | 0.54  |      |      |      |           |      |      |      |      |      |

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.
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 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

| Parameters | Eff.1 | Dietary CP level | Effects (P-value) | Contrast for CP |
|------------|-------|------------------|------------------|----------------|
|            | HEFF | LEFF | SE | 130 | 145 | 160 | 175 | SE | Eff. | CP | Eff. | Linear | Quadratic |
| N intake, g/d | 450.4 | 422.5 | 8.20 | 360.1b | 416.1b | 461.9c | 507.7c | 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 | 94.5a | 135.6b | 182.0c | 211.5c | 10.55 | 0.94 | <0.001 | 0.96 | <0.001 | 0.59 |
| Total recovered | 415.5 | 392.2 | 8.47 | 333.2a | 387.1a | 435.8b | 459.1c | 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 |

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

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

2N 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.

3Apparent 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 (γ = −0.041 × N intake + 44.3; r = −0.442) and solid line for the HEFF group (γ = −0.48 × N intake + 50.8; r = −0.679).
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.) (Agniew 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 (Agniew 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 requirement, 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

| 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$^3$| 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 $\sqrt{\frac{\sum (\text{Predicted} - \text{Observed})^2}{\text{Number of observations}}}$.

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

Models from Jonker et al. (1998); Kauffman and St-Pierre (2001); Kohn et al. (2002); ‘Our own data.'

| 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$^3$| 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 $\sqrt{\frac{\sum (\text{Predicted} - \text{Observed})^2}{\text{Number of observations}}}$.

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

Models from Jonker et al. (1998); Kauffman and St-Pierre (2001); Kohn et al. (2002); ‘Our own data.'
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; Prestøløkken, 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 a similar level of DMI and similar level of daily enteric CH4 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 CH4 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 CH4 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 H2 (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 CH4 pool on a temporal scale. Therefore, enteric CH4 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 NH3-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 NH3-N falls short of the minimum level of rumen NH3 (235 mg/L) concentration for maximal rate of fermentation (Mehrez et al., 1977). However, rumen NH3-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 NH3-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 CH₄ emission, and CH₄ 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 CH₄ 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 CH₄ emission intensity compared with their low-efficiency contemporaries regardless of the level of dietary CP. This would imply that enteric CH₄ 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 expensive and useful tool, under Nordic conditions where ordinary milk analysis comes with MUN estimates, for assessing UN excretion from dairy cows to the environment.

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