Effects of concentrate crude protein content on nutrient digestibility, energy utilization, and methane emissions in lactating dairy cows fed fresh-cut perennial grass

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ABSTRACT

Although many studies have investigated mitigation strategies for methane (CH₄) output from dairy cows fed a wide variety of diets, research on the effects of concentrate crude protein (CP) content on CH₄ emissions from dairy cows offered fresh grass is limited. The present study was designed to evaluate the effects of cow genotype and concentrate CP level on nutrient digestibility, energy utilization, and CH₄ emissions in dairy cows offered fresh-grass diets. Twelve multiparous lactating dairy cows (6 Holstein and 6 Holstein × Swedish Red) were blocked into 3 groups for each breed and assigned to a low-, medium-, or high-CP concentrate diet [14.1, 16.1, and 18.1% CP on a dry matter (DM) basis, respectively], in a 3-period changeover study (25 d per period). Total diets contained (DM basis) 32.8% concentrates and 67.2% perennial ryegrass, which was harvested daily. All measurements were undertaken during the final 6 d of each period: digestibility measurements for 6 d and calorimetric measurements in respiration chambers for 3 d. Feed intake and milk production data were reported in a previous paper. We observed no significant interaction between concentrate CP level and cow genotype on any parameter. Concentrate CP level had no significant effect on any energy utilization parameter, except for urinary energy output, which was positively related to concentrate CP level. Similarly, concentrate CP content had no effect on CH₄ emission (g/d), CH₄ per kg feed intake, or nutrient digestibility. Cross breeding of Holstein cows significantly reduced gross energy, digestible energy, and metabolizable energy intake, heat production, and milk energy output. However, cow genotype had no significant effect on

energy utilization efficiency or CH₄ parameters. Furthermore, the present study yielded a value for gross energy lost as CH₄ (5.6%) on fresh grass-based diets that was lower than the widely accepted value of 6.5%. The present findings indicate that reducing concentrate CP content from 18.1 to 14.1% may not be a successful way of alleviating CH₄ emissions from lactating dairy cows offered good-quality fresh grass, but grazing cows could be offered a low-CP concentrate without compromising energy utilization efficiency. Further research is needed to investigate whether larger differences in dietary CP content may yield positive results.

Key words: dairy cow, energy utilization, methane, fresh grass

INTRODUCTION

The agricultural industry is a major contributor of atmospheric methane (CH₄) and is responsible for 13.5% of total greenhouse gas emissions globally (IPCC, 2007). A large proportion of these emissions (80%) come from livestock production systems (FAO, 2006). In Northern Ireland, agriculture is responsible for the emission of 6.49 Mt of CO₂ equivalents annually or 29% of total annual greenhouse gas emissions (Salisbury et al., 2015). Methane emissions not only raise environmental concerns but also form a sizable loss of feed energy intake from dairy and beef cows, from 2 to 12% (Johnson and Johnson, 1995). Alleviating CH₄ emissions may increase the ME available and improve energy utilization efficiency in ruminant systems. Rates of CH₄ emission are influenced by a range of diet and animal factors, such as feed intake, diet quality, and nutrient utilization efficiency (Johnson and Johnson, 1995; Kebreab et al., 2006; Muñoz et al., 2015). Many mitigation strategies have been investigated for dairy cows offered ensiled forage, but information for grazing cows is lacking.

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Pasture-based dairy systems are widely used in Ireland and in other countries with similar climatic conditions; 89% of agricultural land is allocated for grazing swards (Hart et al., 2009). A promising mitigation strategy reported in several studies (Aguerre et al., 2011; Haque et al., 2014) appears to be the increase of dietary starch content, either by increasing concentrate input (which increases feed costs) or by replacing high-protein feed components (e.g., soybean meal, rapeseed extract) with high-starch components (e.g., corn, wheat). However, replacing the CP content of concentrate with starch in pasture-based diets—a successful strategy for alleviating N excretion—has not been investigated. In a meta-analysis of indirect calorimetry data from dairy cows offered perennial ryegrass silage-based diets, Yan and Mayne (2007) found a negative relationship between CH₄/kilogram of DMI and dietary CP concentration. This effect was likely not solely dependent on dietary CP concentrations, but a result of changes in other dietary factors (e.g., fiber and starch concentrations). Indeed, Stergiadis et al. (2016) found increasing grass CP and water-soluble carbohydrate concentrations increased CH₄/kilogram of DMI in dry cows offered diets of only fresh perennial ryegrass at maintenance feeding levels. Therefore, the effects of dietary CP contents on CH₄ emissions and energy utilization merit investigation in studies with dairy cows offered fresh-forage diets.

Animal genetic factors have been found to play a significant role in energy utilization efficiency and CH₄ emissions from ruminants (Pinares-Patiño et al., 2009; Clark, 2013). It is well documented that improving productivity can lead to a reduction in CH₄ emissions per unit of produce (Chagunda et al., 2009; Wall et al., 2010; Cottle et al., 2011) while making mitigation strategies appealing to producers. Beecher et al. (2014) and Palladino et al. (2010) showed that Holstein-Friesian cows on perennial ryegrass silage diets offered at maintenance levels and grazing perennial ryegrass, respectively, may exhibit differences in production efficiency compared with Jersey and Jersey × Holstein-Friesian cows. However, comparisons of CH₄ emissions between Holstein and other breeds under grazing or zero-grazing conditions have been limited, with the literature focusing on ensiled forage (Xue et al., 2011; Arndt et al., 2015).

The present study was designed to address these knowledge gaps by evaluating the effects of reducing concentrate CP content (with little influence on starch and fiber content), cow genotype, and their interaction on nutrient digestibility, energy utilization efficiency, and CH₄ emissions in lactating dairy cows offered fresh perennial ryegrass diets, so that practices are widely applicable to pasture-based systems.

MATERIALS AND METHODS

All scientific procedures described were carried out under experimental license from the Department of Health, Social Services and Public Safety of Northern Ireland in accordance with the Animal (Scientific Procedures) Act (Home Office, 1986).

Experimental Design

The current study presents observations from a calorimetry experiment performed at Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK), using 12 multiparous lactating (6 Holstein and 6 Holstein × Swedish Red 50:50 crossbred) cows on diets of fresh-cut perennial ryegrass and concentrate feeds during the 2014 grazing season. Details of the animals, experiment design, and diets are reported in a companion paper (Hynes et al., 2016). A brief description of the design and measurement procedures follows.

Animals were offered 3 dietary treatments with different concentrate CP contents (2 cows in each genotype/diet) in a changeover study with 3 periods of 25 d. All measurements were taken during the final 6 d of each period: 3 d in digestibility units and 3 d in indirect open-circuit respiration calorimeter chambers, with continuation of digestibility measurements in the respiration chambers. Diets were composed of zero-grazed perennial ryegrass and concentrate feeds of differing CP content: low-CP concentrate (14.1% DM), medium-CP concentrate (16.1% DM), and high-CP concentrate (18.1% DM) fed at 32.8% DMI combined with perennial ryegrass fed at 67.2% DMI. The low- and high-CP concentrates were formulated to possess the same dietary components and similar chemical composition except for CP level, and the medium-CP concentrate was produced by mixing the low- and high-CP concentrates in equal proportions. This resulted in 3 concentrate feeds that were comparable in terms of ME, fermentable ME, and fiber content. Concentrates were offered at milking (50% at 0700 h and 50% at 1500 h), and fresh herbage was offered ad libitum at 1000 h each morning. The zero-grazed herbage was harvested from a single sward each morning using a Haldrup 1500 (Plot Combine, Haldrup, UK) and boxed loosely to avoid nutrient degradation. The temperature of the perennial ryegrass was monitored for the duration of the study. Herbage regrowth intervals (initially 22 d of regrowth with incremental increases up to 30 d from June to September) and fertilization practices (within 3 d of harvesting at 35 kg of N/ha) were determined based on common routine practices to ensure perennial ryegrass of a similar quality was being offered to animals for

the duration of the experimental work. Concentrations were calculated based on the average DMI of the previous 7 d, and animals had free access to water throughout the study.

Digestibility and Calorimeter Chamber Measurements

All procedures for recording feed intake, feces and urine excretion, and milk production, and all sample measurements during the final 6 d of each period have been reported by Hynes et al. (2016). In brief, perennial ryegrass and concentrate were analyzed for DM, N, gross energy (**GE**), NDF, ADF, ash, water-soluble carbohydrates (perennial ryegrass only) and starch (concentrate only). The DM, N, NDF, ADF, and ash contents in feces and N in urine were assessed. Analysis of GE was conducted for the present study using a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL), on fecal and urine samples on a dry and fresh basis, respectively, as described in Jiao et al. (2013). Gaseous exchange (O_2 consumption and CO_2 and CH_4 production) was measured in the final 48 h of the 72-h calorimetric-chamber stage. Two indirect open-circuit respiration calorimeter chambers consisting of a climatic control unit, an airflow and measurement system, and 3 gas analyzers were used. Chambers were maintained at $16 \pm 1^\circ C$ and 60% relative humidity via air conditioning, including a Vaisala PTA 427 digital barometer and Vaisala HUMICAP sensor probes (Delta-T devices, Cambridge, UK). Air was dehumidified, heated or cooled to 13 to $15^\circ C$, and rehumidified if necessary before entering the chambers. Chambers were run under a slight negative pressure and possessed airlock systems for entry and feeding to prevent leakage. Each chamber's flow system consisted of 2 inlet ambient air tubes and 3 extraction tubes fitted with turbine flow meters (GH flow Automation Ltd., Andover, UK). Suction pumps were set to per-

form 3.4 air exchanges per hour ($75 \text{ m}^3/\text{h}$ flow rate, 22-m^3 total chamber volume). Measurement of flow rate and concentration of ambient and extraction air allowed for calculation of CH_4 output. All gases were measured using an ADC MGA3000 Multi gas analyzer (ADC Gas Analysis Ltd., Hoddesdon, UK)— CH_4 and CO_2 concentrations by electrochemical sensors, and O_2 concentrations by paramagnetic sensor. The analyzer switched between chambers and span gases every 75 s and completed a full rotation every 225 s. Data were then transferred onto a 16-bit digital converter (Strawberry tree model ACPC-16; Adept Scientific Micro System Ltd., Letchworth, UK). All equipment, procedures, analytical methods, and calculations used in the calorimetric experiments were as reported by Gordon et al. (1995), and calibration of the chambers was as reported by Yan et al. (2000).

Calculations and Statistical Analysis

Prior to analysis, several energy utilization parameters were calculated using the equations in Table 1. Heat production (**HP**) was calculated based on O_2 consumption, CO_2 and CH_4 production, and urinary N excretion using the equation of Brouwer (1965). Retained energy was calculated by subtracting HP and milk energy from ME intake (MEI). The ME requirement for maintenance and the efficiency of ME use for lactation (k_l) were calculated according to AFRC (1993).

Means of individual animal variables over both 3-d collection phases (except for the calorimetric data collected over a single 3-d phase) were used for statistical analysis. Experimental data were analyzed using the Genstat statistical package (VSN International, 2013). A linear mixed model methodology with REML estimation (Gilmour et al., 1995) was applied, with dietary treatment and genotype as fixed factors and cow and date of entry to collection stage fitted as random effects. Orthogonal contrasts were used to test for linear

Table 1. Equations used to calculate heat production, ME requirement for maintenance, and efficiency of ME for lactation

Estimated variable ¹	Equation ²	Reference
HP (MJ/d)	$= [(16.18 \times O_2) + (5.16 \times CO_2) - (2.42 \times CH_4) - (5.9 \times UN)]/1,000$	(Brouwer, 1965)
ME _m (when $E_g < 0$)	$= HP - (1/k_l \text{ (AFRC)} - 1) \times E_l - (1/k_t - 2) \times E_g - (1/k_p - 1) \times E_p$	(AFRC, 1993)
ME _m (when $E_g > 0$)	$= HP - (1/k_l \text{ (AFRC)} - 1) \times E_l - (1/k_g - 2) \times E_g - (1/k_p - 1) \times E_p$	
$E_{l(0)}$ (when $E_g < 0$)	$= E_l + 0.84 \times E_g$	(AFRC, 1993)
$E_{l(0)}$ (when $E_g > 0$)	$= E_l + 1/k_g \times E_g$	
k_l	$= E_{l(0)}/(ME_{int} - ME_m)$	(AFRC, 1993)

¹HP = heat production (MJ/d), ME_m = ME requirement for maintenance (MJ/d), E_g = net energy for BW change (MJ/d), $E_{l(0)}$ = milk energy output adjusted to zero energy balance (MJ/d), k_l = efficiency of ME use for lactation.

² O_2 = oxygen consumed (L/d), CO_2 = carbon dioxide produced (L/d), CH_4 = methane produced (L/d), UN = urinary nitrogen excreted (g/d), $k_l \text{ (AFRC)}$ = efficiency of ME use for lactation calculated from AFRC (1993), E_l = milk energy output (MJ/d), k_t = efficiency of utilization of mobilized energy for lactation, k_p = efficiency of ME use for pregnancy, k_g = efficiency of ME use for weight gain, E_p = net energy requirement for pregnancy, ME_{int} = ME intake (MJ/d).

and quadratic effects of treatment as described by Hynes et al. (2016). Residuals conveyed no deviation from normality. Differences between treatments, genotypes, and interactions were assessed with 5 degrees of significance; nonsignificance was declared at $P > 0.10$, significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, and tendencies at $0.05 < P < 0.10$.

RESULTS

We observed no significant interaction between concentrate CP level and cow genotype on any parameter evaluated in terms of digestibility, CH₄ emissions, energy intake, energy output, or energy utilization efficiency. Therefore, only results of the main factors are presented here. The results for dietary composition, feed intake, and milk production were reported by Hynes et al. (2016); in brief, concentrate CP contents did not affect DMI, milk yield, or milk composition. Concentrate feeds had similar chemical compositions, varying only in CP content, so that total dietary CP levels were 16.9, 17.6, and 18.3% (DM basis) for the low-, medium-, and high-CP concentrate treatments, respectively.

Nutrient Apparent Whole-Tract Digestibility

Data on nutrient digestibility are presented in Table 2. Findings conveyed no significant effects of dietary treatment or genotype on any apparent whole-tract digestibility parameter (DM, OM, GE, NDF, ADF, or digestible OM in total DM), but we did observe a tendency for N digestibility to linearly increase with increasing concentrate CP content.

Energy Utilization

Findings on the effects of concentrate CP levels and cow genotype on energy utilization variables are presented in Table 3. Analysis showed no significant effect

of dietary treatment on energy intake [GE, digestible energy (**DE**) or ME], retained energy, or energy partition in feces, CH₄, HP, or milk, but we did observe a positive linear effect of concentrate CP on urine energy output. We found no significant effect of treatment on DE/GE, ME/GE, HP/MEI, or k_i.

Compared with the crossbreds, Holstein cows had significantly higher GE, DE, and MEI, and consequently higher HP and milk energy output. Cow genotype had no significant effect on DE/GE, ME/GE, HP/MEI, or k_i.

Methane Emissions

Enteric CH₄ emission data are shown in Table 4. Neither concentrate CP level nor cow genotype had a significant effect on any CH₄ emission factor in terms of total emission (g/d) or CH₄ emissions as a proportion of feed intake, milk yield, or CH₄ energy (**CH₄-E**) as a proportion of GE intake (**GEI**). The ratio of CH₄-E as a proportion of GE, DE, and MEI had mean values of 0.056, 0.076, and 0.089 (MJ/MJ), respectively.

DISCUSSION

Grazing systems are used extensively in areas with cool and moist climates, which allow a long grazing season and high forage production, thus providing a low-cost feeding approach for ruminant production systems (Peyraud and Delagarde, 2013). The profitability of dairying in these areas is fundamentally linked to forage utilization; for example, in Ireland, every extra tonne of forage yield per hectare (DM basis) is worth €161 (Shalloo, 2009). Although previous work on CH₄ emissions in grazing animals relied predominantly on the SF₆ tracer method to measure CH₄ emissions (Pinares-Patiño et al., 2007; Cavanagh et al., 2008), in the present study we used indirect open-circuit calorimetry chambers. These chambers measured gaseous exchanges, including CH₄, and allowed for the calculation

Table 2. Effect of concentrate CP level and cow genotype on total diet digestibility parameters (kg/kg)

Item	Concentrate CP level				<i>P</i> -value ¹		Cow genotype			
	Low	Medium	High	SEM	Linear	Quadratic	Holstein	Crossbred ²	SEM	<i>P</i> -value
DM	0.759	0.764	0.762	0.0060	0.585	0.615	0.766	0.757	0.0056	0.170
OM	0.780	0.785	0.782	0.0054	0.581	0.537	0.786	0.778	0.0051	0.179
Digestible OM in DM	0.710	0.714	0.711	0.0053	0.760	0.582	0.716	0.708	0.0049	0.185
N	0.655	0.668	0.673	0.0118	0.088	0.667	0.667	0.664	0.0134	0.880
Gross energy	0.740	0.745	0.741	0.0060	0.611	0.324	0.746	0.738	0.0057	0.236
NDF	0.699	0.701	0.705	0.0093	0.277	0.750	0.711	0.692	0.0090	0.106
ADF	0.681	0.687	0.684	0.0110	0.937	0.512	0.693	0.675	0.0108	0.205

¹Probability of a linear or quadratic effect of concentrate CP level in the diet.

²Crossbred cows were crosses between Holstein and Swedish Red.

Table 3. Effect of concentrate CP level and cow genotype on energy intake and output, and energy utilization efficiencies

Item ¹	Concentrate CP level				P-value ²			Cow genotype		
	Low	Medium	High	SEM	Linear	Quadratic		Holstein	Crossbred ³	P-value
Energy intake and output (MJ/d)										
GE intake	372	383	375	8.2	0.498	0.197		390	364	6.5
DE intake	276	286	278	7.3	0.290	0.055		291	268	6.0
ME intake	237	246	238	7.1	0.426	0.108		250	231	6.3
Fecal energy	96	97	96	2.2	0.888	0.742		98	95	2.2
Urinary energy	17.9	17.9	19.8	0.98	0.004	0.292		19.1	17.9	1.02
CH ₄ energy	21.1	21.6	20.8	1.15	0.724	0.467		22.4	20.0	1.15
Heat production	138	133	134	4.1	0.452	0.679		140	130	3.1
Milk energy	85	85	85	2.9	0.562	0.616		91	79	2.8
Retained energy	14.7	26.8	17.6	7.35	0.561	0.193		16.8	22.6	7.29
Energy utilization (MJ/MJ)										
DE/GE	0.740	0.745	0.741	0.0060	0.611	0.324		0.746	0.738	0.0057
ME/GE	0.636	0.642	0.632	0.0082	0.761	0.165		0.640	0.633	0.0093
Heat production/ME	0.587	0.546	0.569	0.0229	0.423	0.237		0.570	0.565	0.0215
Milk energy/ME	0.362	0.344	0.364	0.0159	0.833	0.108		0.367	0.346	0.0153
Retained energy/ME	0.052	0.108	0.066	0.0313	0.582	0.130		0.062	0.089	0.0310
k _i	0.623	0.628	0.624	0.0035	0.854	0.519		0.628	0.621	0.0025

¹GE = gross energy, DE = digestible energy, k_i = efficiency of ME use for lactation.
²Probability of a linear or quadratic effect of concentrate CP level in the diet.
³Crossbred cows were crosses between Holstein and Swedish Red.

Table 4. Effect of concentrate CP level and cow genotype on methane emissions in absolute terms or expressed as a proportion of production and energy efficiency

Item	Concentrate CP level				P-value ¹			Cow genotype		
	Low	Medium	High	SEM	Linear	Quadratic		Holstein	Crossbred ²	P-value
CH ₄ (g/d)	381.6	391.3	377.3	20.8	0.724	0.467		405	362	20.9
CH ₄ /feed intake or milk yield (g/kg)										
CH ₄ /DMI	18.36	18.43	18.19	1.03	0.904	0.782		18.7	17.9	1.07
CH ₄ /OM intake	20.15	20.25	20.00	1.13	0.923	0.779		20.6	19.7	1.17
CH ₄ /digestible DMI	24.20	24.04	23.90	1.41	0.809	0.972		24.4	23.7	1.49
CH ₄ /digestible OM intake	25.86	25.74	25.60	1.51	0.831	0.863		26.1	25.3	1.60
CH ₄ /milk yield	14.35	14.85	14.30	1.01	0.372	0.366		13.8	15.2	0.95
CH ₄ /ECM yield ³	13.49	14.15	13.33	0.90	0.480	0.240		13.3	14.0	0.90
CH ₄ -E/energy intake ⁴ (MJ/MJ)										
CH ₄ -E/GEI	0.056	0.056	0.056	0.0031	0.972	0.755		0.057	0.055	0.0032
CH ₄ -E/DEI	0.077	0.076	0.076	0.0045	0.712	0.923		0.077	0.075	0.0048
CH ₄ -E/MEI	0.090	0.089	0.089	0.0059	0.776	0.969		0.091	0.088	0.0063

¹Probability of a linear or quadratic effect of concentrate CP level in the diet.
²Crossbred cows were crosses between Holstein and Swedish Red.
³ECM yield = milk gross energy content (MJ/kg) × milk yield (kg/d)/3.0968, as shown by Tyrrell and Reid (1965).
⁴CH₄-E = methane energy output, DEI = digestible energy intake, GEI = gross energy intake, MEI = ME intake.

of HP—a variable that could not be measured by the SF₆ technique. Although energy expenditure at pasture due to grazing could not be assessed when animals were in the chambers, the results from the present study may be highly applicable to pasture-based systems, due to the zero-grazing practices used, and complement results from studies that use SF₆ tracer techniques.

Nutrient Digestibility and Energy Utilization Efficiency

Due to the relatively high apparent digestibilities in the present study, DMI was high across all treatments; the positive association between highly digestible feed and DMI has been demonstrated previously (NRC, 2001). An apparent DM digestibility of 0.76 in the present study is comparable with published findings (0.76–0.78) for dairy cows on similar diets (Whelan et al., 2012). The lack of effect of dietary treatment on digestibility parameters obtained in the present study was in agreement with results from a study by Moorby et al. (2006) in dairy cows offered diets containing 65% ryegrass silage and 35% concentrate. The N digestibility values in the present study were similar to those observed in studies under a wide variety of dietary regimens (Huhtanen et al., 2008), including fresh-forage diets (Van Vuuren et al., 1992). Increasing N digestibility with increasing dietary CP concentration, as tended to occur in the present study, reflected the increased urine N loss with increasing concentrate CP content, but treatment had no effect on milk N output or retained N (Hynes et al., 2016). The NDF (0.725) and OM (0.792) digestibility values obtained in the present study were higher than previously recorded (Nousiainen et al., 2004; Huhtanen et al., 2008), which averaged 0.622 and 0.726, respectively. This finding may be explained by the good-quality perennial ryegrass offered in the present study, which may have improved feed OM digestibility (Stergiadis et al., 2015).

Energy intakes and outputs (GE, DE, and ME) did not differ across dietary treatments, except for urine energy outputs. The observed differences in urinary energy partitioning were in agreement with previous work (Ramin and Huhtanen, 2013), which reported that urinary energy was positively associated with dietary CP content with a wide range of dietary treatments ($n = 207$). This may be due to the associated excess N in urine that increases urine energy content, as was found to be the case in Holstein steers on concentrate-based diets (Mwenya et al., 2004). The lack of effect of diet treatments on energy intake, utilization efficiency, and nutrient digestibility values obtained in the present study may imply that the total dietary CP content

(16.9%) of the low-CP concentrate treatment may be sufficient to supply degradable CP for rumen microbial activity and MP for milk production. Indeed, the present study found that increasing concentrate CP levels had no significant effect on total DMI, milk yield or composition, or N utilization efficiency in terms of N excretion in feces, urine, or milk as a proportion of N intake (Hynes et al., 2016). However, increasing concentrate CP levels significantly increased N excretion in urine and urine N/manure N. It is a common practice in dairy farming in Northern Ireland to feed dairy cows grazing diets and winter diets containing CP content of approximately 18% (DM basis). However, the present study clearly demonstrated that a grazing diet at a CP content of 17% (DM basis) was enough to sustain milk production as reported by Hynes et al. (2016), as well as energy digestibility, metabolizability, and k_f . Further investigation into the long-term effects on production efficiency and other functional traits (e.g., fertility) need to be evaluated. Feeding dairy cows low-CP diets may save on costs of high-priced protein feeds (e.g., soybean meal), and reduce the environmental footprint (urinary N excretion).

The present study demonstrated that cross-breeding Holstein cows with Swedish Red sires had no effects on nutrient and energy digestibility, energy metabolizability, or k_f when cows were offered diets of fresh perennial ryegrass, although Holstein cows had significantly greater GE, DE, and MEI. Several previous studies had similar results with ensiled forage. Xue et al. (2011) observed no difference in energy metabolizability or k_f between Holstein and Jersey-Holstein cows offered perennial ryegrass silage diets containing either 30 or 70% concentrates. Heins et al. (2008) also reported that the feed efficiency for d 4 to 150 of lactation was similar for Jersey-Holstein and pure Holstein cows offered diets containing alfalfa hay and corn silage. These results, along with those from the present study, indicate that cross breeding Holstein cows with Swedish Red or Jersey sires has negligible influence on the potential high-production efficiency of the Holstein breed. Swedish Red cows have traditionally been selected for milk production and other functional traits (e.g., fertility, disease resistance) and have a longer service term than Holstein cows (Swalve, 2007). Consequently, Swedish Red sires have been widely used to improve the reproductive performance and health status of Holstein cows. The present study indicates that although the crossbred cows had a lower feed intake and milk yield, as reported by Hynes et al. (2016), energy digestibility, energy metabolizability, and k_f traits were not compromised compared with pure Holstein cows offered diets of fresh perennial ryegrass.

Methane Emissions

The present finding—that dietary CP concentration did not affect CH₄ emissions—is in agreement with Van Dorland et al. (2007). However, in a meta-analysis of calorimetry data, Yan and Mayne (2007) found a negative relationship between CH₄/kg of DMI and dietary CP concentration. Conversely, Stergiadis et al. (2016) found that increasing perennial ryegrass CP and water-soluble carbohydrate contents also increased CH₄/kg of DMI in dry cows offered fresh perennial ryegrass-only diets at maintenance level. Arndt et al. (2015) suggested a quadratic relationship between CH₄ (g/d, g/kg of DMI, and MJ/MJ of GEI) and dietary CP with different ratios of alfalfa silage to corn silage. It is difficult to determine the root cause of changes in CH₄ yields, but Hassanat et al. (2013) suggested it might be due to increasing dietary starch content and decreasing CP content, resulting in a decrease in pH, protozoa, and methanogens. Similarly, Dijkstra et al. (2011) speculated that yields of CH₄ might decrease when starch increases at the expense of CP content, due to fermentation of fiber, producing higher volumes of VFA, acetate, and butyrate. These yield H₂, a precursor of methanogenesis, in comparison to starch, which results in higher volumes of propionate, a reaction that uses H₂. This hypothesis implies that altered fiber or starch concentration can affect enteric CH₄ outputs in addition to reduced urinary N output when dietary N content decreases (Külling et al., 2001; Weiss et al., 2009; Arndt et al., 2015), but the outcome of the present study did not confirm this. In the present study, the formulation of concentrate supplements did not alter NDF and ADF concentrations. Although increasing CP content led to decreased starch content in the 3 concentrates, the differences in starch content were relatively small (21.1 to 23.2% on a DM basis) between the 3 concentrates and negligible (6.9 to 7.6%) between total diets. The present study suggests that increasing concentrate CP content, resulting in a concomitant increase in total dietary CP content from 16.9 to 18.3%, had no effects on enteric CH₄ emission rates for perennial ryegrass and concentrate-based diets.

The present study found that cross breeding of Holstein cows with Swedish Red sires had no significant effect on CH₄/kg of DMI, CH₄/kg of OM intake, or CH₄-E/GEI (MJ/MJ); CH₄/kg of ECM yield was identical between the 2 genotypes. Although no comparable calorimetry data with fresh ryegrass were available, Yan and Mayne (2009) observed a similar result when comparing Holstein and Jersey × Holstein cows offered diets containing perennial ryegrass silage and either 30% or 70% concentrate. Several recent studies have assessed the potential association between enteric CH₄

emissions and the microbial ecology of ruminal methanogens. Using culture-independent methods, Zhou et al. (2009, 2010) reported that although they observed no significant difference in the total population of methanogens between cattle with different feed efficiencies, their rumen methanogenesis capacity was highly related to changes in feed intake and dietary composition. The abundance of predominant methanogenic species obtained on the low-energy-density diet shifted to a community containing a more diverse range of predominant species with the high-energy-density diet (Zhou et al., 2010). These results indicated that enteric CH₄ emission rates in cattle are driven mainly by feed intake and dietary nutrient composition, and that cow genotypes based on the Holstein breed may have little effect on inherent genetic capacity for rumen methanogenesis. Heritability for CH₄ emissions in Holstein cows is low (Lassen and Løvendahl, 2016). Hence, rather than breeding for reduced CH₄ (g/d) or CH₄/kg of DMI, Cottle et al. (2011) have suggested that a breeding approach for improved feeding efficiency would be more successful and in line with current breeding objectives, so as to minimize the risk of undesirable trade-offs.

In the present study, GEI lost as CH₄-E averaged 5.6%. This figure was very close to the simulated prediction (5.8%) by Bannink et al. (2010) with lactating dairy cows on a similar DMI and fresh forage-to-concentrate ratio, and similar to that (average 5.7%) of grazing dairy cows with CH₄ emissions measured using the SF₆ technique (O'Neill et al., 2012; Jiao et al., 2014). However, these CH₄/GEI data were all lower than that of 6.5% recommended by the IPCC (2006) to calculate enteric CH₄ emission inventory for a region where local CH₄ emission data are not available. Therefore, it is possible that using the Intergovernmental Panel on Climate Change default value for inventory purposes would overestimate CH₄ production in grazing systems, especially for countries where grazing management regimens are a major component of dairy production, such as in Ireland, the United Kingdom, New Zealand, and Australia. This issue merits further investigation.

CONCLUSIONS

The results from the current study suggest that reducing concentrate CP content from 18.1 to 14.1% did not affect energy utilization efficiency or enteric CH₄ emission rates in lactating dairy cows on diets of fresh-cut perennial ryegrass. Cross breeding Holstein cows with Swedish Red sires had no significant effect on energy utilization efficiency or enteric CH₄ emission rates, although Holstein cows had higher energy intake and milk energy output. These findings suggest that concentrates with CP levels as low as 14.1% can be offered

in combination with good-quality perennial ryegrass without any negative effect on CH₄ emissions or energy partitioning for production, although production sustainability would have to be confirmed in a long-term study. Feeding grazing cows with low-CP concentrates not only reduces feed costs but is also environmentally beneficial, with lower urinary N excretion.

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