INTERPRETIVE SUMMARY

Effects of concentrate crude protein contents on nutrient digestibility, energy utilization and methane emissions of lactating dairy cows fed fresh-cut perennial grass. By Hynes et al. Livestock associated methane emissions have huge environmental implications. The aim of the study was to investigate the effect of concentrate protein content and animal genotype on methane emissions and energy utilization of dairy cows fed fresh grass. Reducing concentrate protein content (18.1 to 14.1%) did not affect methane yields, energy utilization or partitioning in dairy cows. In comparison to Holstein crossbreds, Holstein cows had a greater energy intake and incorporated more energy into milk, but had no effects on energy utilization efficiency or methane emission rates. Grazing cows can be offered low protein concentrates without compromising energy utilization efficiency, although this approach may not be an effective strategy in alleviating methane emissions.

RUNNING HEAD: CONCENTRATE PROTEINS' EFFECT ON METHANE EMISSIONS

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

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ABSTRACT

Although many studies have investigated mitigation strategies for methane (CH_4) output from dairy cows fed a wide variety of diets, research on effects of concentrate crude protein (**CP**) content on CH_4 emissions from dairy cows offered fresh grass is limited. The present study was therefore designed to evaluate effects of cow genotype and concentrate CP level on nutrient digestibility, energy utilization and CH_4 emissions of dairy cows offered fresh grass based diets. Twelve multiparous lactating dairy cows (6 Holstein and 6 Holstein × Swedish Red) were blocked into 3 groups within each breed and assigned to low, medium or high CP concentrate diet (14.1, 16.1 and 18.1 % on dry matter (**DM**) basis), respectively, in a 3-period changeover study (25-d / 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. No significant interaction between concentrate CP level and cow genotype on any parameter was observed. 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 CH_4 emission (g/d), CH_4 per kg feed intake or nutrient digestibility. The crossbreeding 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 effects on energy utilization efficiency or CH_4 parameters. Furthermore, the present study yielded a value for gross energy lost as CH_4 (5.6 %) on fresh grass-based diets that is lower than the widely accepted value of 6.5 %. The present findings indicate reducing concentrate CP content from 18.1 to 14.1 % may not be a successful approach to alleviate CH_4 emissions from lactating dairy cows offered good quality fresh grass, however 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 (CH_4), and responsible for 13.5 % of total greenhouse gas emissions globally (IPCC et al., 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 CO_2 equivalent annually or 29 % of total annual greenhouse gas emissions (Salisbury et al., 2015). Methane emissions do not only raise environmental concerns but also form a sizable loss of feed energy intake from dairy and beef cows, which ranges from 2 to 12 % (Johnson and Johnson, 1995). Therefore alleviating CH_4 emissions may increase ME available and thus improve energy utilization efficiency in ruminant systems. The extent of CH_4 emission rates are influenced by a range of diet and animal factors, such as, feed intake, diet quality and nutrient utilization effiency (Johnson and Johnson, 1995; Kebreab et al., 2006; Muñoz et al., 2015). A large range of mitigation strategies have been investigated for dairy cows offered ensiled forages, but there is lack of such information for grazing cows.

Pasture-based dairy systems are widely used in Ireland and many countries of similar climatic conditions, whereby 89 % of agricultural land is allocated for grazing swards (Hart et al., 2009). For example, promising mitigation strategies, as stated by a number of 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 alternatively replacing high protein feed components of the concentrate (e.g. soyabean meal, rapeseed extract) with high starch feed components (e.g. corn, wheat feed). However, replacing CP content of concentrate with starch on pasture based diets, a successful strategy for alleviating N excretion, has not been investigated. In a meta-analysis of indirect calorimetry data of dairy cows offered perennial ryegrass silage-based diets, Yan and Mayne (2007) found a negative relationship between CH_4 / kg DMI and dietary CP concentration. This effect is likely not solely dependent on dietary CP concentrations, but a result of the subsequent change in other dietary factors (e.g., fiber and starch concentrations). Indeed, Stergiadis et al. (2016) found increasing grass CP and water soluble carbohydrate (**WSC**) concentrations increased CH_4 / kg DMI in dry cows offered fresh perennial ryegrass only diets at maintenance feeding levels. Therefore, the effects of dietary CP contents on CH_4 emissions and energy utilization merit investigation in studies with dairy cows offered fresh forage based diets.

Animal genetic factors have been found to play a significant role in influence of energy utilization efficiency and CH_4 emissions from ruminants (Pinares-Patino et al., 2009; Clark, 2013). It is well documented improving productivity can lead to a reduction in CH_4 emissions per unit of produce (Chagunda et al., 2009; Wall et al., 2010; Cottle et al., 2011) while simultaneously 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 level and grazing perennial ryegrass respectively may exhibit differences in production efficiency when compared with Jersey and Jersey × Holstein-Friesian. However, comparisons on CH_4 emissions between Holstein and other breeds under grazing or zero-grazing conditions are limited with literature focusing on ensiled forage (Xue et al., 2011; Arndt et al., 2015).

The present study was thus designed to address these knowledge gaps as identified previously, by evaluating the effects of reducing concentrate CP contents (with little influence on starch and fiber contents), cow genotype and their interactions on nutrient digestibilities, energy utilization efficiency and CH_4 emissions in lactating dairy cows offered fresh perennial ryegrass diets so that practices are widely applicable in 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 animals, experiment design and diets were 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 within each genotype/diet) in a changeover study with three (25-d) periods. All measurements were taken during the final 6-d of each period; 3-d digestibility units and 3-d in indirect open-circuit respiration calorimeter chambers, with continuation of digestibility measurements in the respiration chambers. Diets composed of zero-grazed perennial ryegrass and concentrate feeds of differing CP content; low CP concentrate diet (LCP, 14.1 % DM), medium CP concentrate diet (MCP, 16.1 % DM) and high CP concentrate diet (HCP, 18.1 % DM) fed at 32.8 % DMI in combination 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 with the exception of CP level, while the medium CP concentrate was produced by mixing the low and high CP concentrates in equal proportions. This resulted in 3 concentrate feeds which were comparable in regard to ME, fermentable ME and fiber content. Concentrates were offered at milking, 50 % at 0700 and 50 % at 1500, and fresh herbage at 1000 (ad libitum) each morning. The zero-grazed herbage was harvested (from a single sward) each morning using a Haldrup 1500, boxed loosely to avoid nutrient degradation and perennial ryegrass' temperature was monitored for the duration of the study. Herbage regrowth intervals (initially 22-d regrowth with incremental increases up to 30-d from June to September) and fertilization practises (within 3-d of harvesting at 35 kg N / ha) were determined based on common routine practices, in order to ensure perennial ryegrass of a similar quality was being offered to animals for the duration of experimental work. Concentrate rations 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 records of feed intake, feces and urine excretion and milk production, and all sample measurements during the final 6-d of each period were reported by Hynes et al. (2016). In brief, perennial ryegrass and concentrate were analyzed for DM, N, gross energy (GE), NDF, ADF, ash, WSC (perennial ryegrass only) and starch (concentrate only) and DM, N, NDF, ADF and ash contents in feces and N in urine was assessed. In addition, analysis of GE was conducted in the present study, using a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Illinois, USA), 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 air flow and measurement system and 3 gas analyzers were utilized. Chambers were maintained at 16 ± 1°C and 60 % relative humidity via air conditioning unit including a Vaisala PTA 427 digital barometer and Vaisala HUMICAP sensor probes (Delta-T devices, Cambridge, UK). Air was dehumidified, heated or cooled to 13-15°C and re-humidified, if necessary, prior to entering the chambers. Chambers were run under a slight negative pressure and possessed airlock systems for entry and feeding to ensure against leakage. Each chambers' 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 perform 3.4 (75 m^3 / h flowrate / 22 m^3 total chamber volume) air exchanges / h. Measurement of flow rate and concentration of ambient and extraction air allowed for calculation of CH_4 output. All gases were measured by ADC MGA3000 Multi gas analyzer (ADC Gas Analysis Ltd., Hertfordshire, UK), CH_4 and CO_2 concentrations by electrochemical sensors and O_2 concentrations by paramagnetic sensor. The analyzer switched between both 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, Adepth Scientific Mirco 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 by Yan et al. (2000).

Calculations and Statistical Analysis

Prior to analysis a number of 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 **(ME_m)** and subsequently the efficiency of ME use for lactation **(k_l)** was calculated according to Agnew et al. (2004).

Means of individual animals' variables over the two 3-d collection phases (with the exception of the calorimetric data collected over one 3-d phase) were used for statistical analysis. Experimental data were analyzed using Genstat statistical package (VSN International, 2013). Linear mixed model methodology with REML estimation (Gilmour et al., 1995) was implied 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 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, non-significant ($P > 0.10$) and significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, while tendencies were declared at $0.05 < P < 0.10$.

RESULTS

There was no significant interaction between concentrate CP level and cow genotype on any parameter evaluated in terms of digestibility, CH_4 emissions or energy intake, output or utilization efficiency. Therefore, only results of main factors were presented in the present study. The results on dietary composition, feed intake and milk production were reported by Hynes et al. (2016) viz. concentrate CP contents did not affect DMI, milk yield (MY) or milk composition. Concentrate feeds had similar chemical compositions only varying in CP content, consequently total dietary CP levels were 16.9, 17.6 and 18.3 % (DM basis) for the LCP, MCP and HCP 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 a tendency of N digestibility to linearly increase with increasing concentrate CP content, was observed.

Energy Utilization

Findings on the effects of concentrate CP levels and cow genotype on energy utilization variables are displayed in Table 3. Analysis showed there was no significant effect of dietary treatment on energy intake (GE, digestible energy (**DE**) or ME), retained energy or energy partition in feces, CH_4, HP or milk, although there was a positive linear effect of concentrate

CP observed urine energy output. We found no significant effect of treatment on DE/GE, ME/GE, HP/MEI or k_l.

In comparison to Crossbreds, Holstein cows had significantly higher GE, DE and ME intakes and consequently higher HP and milk energy output. Cow genotype had no significant effect on DE/GE, ME/GE, HP/MEI or k_l.

Methane Emissions

Enteric CH_4 emission data are shown in Table 4. Neither concentrate CP level nor cow genotype had significant effect on any CH_4 emission factor, in terms of total emission (g/d), or CH_4 emissions as a proportion of feed intake, MY, or CH_4 energy (**CH_4-E**) as a proportion of GE intake (**GEI**). The ratio of CH_4-E as a proportion of GE, DE and ME intakes had mean values of 0.056, 0.076 and 0.089 (MJ/MJ) respectively.

DISCUSSION

Grazing systems are extensively used 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). Hence profitability of dairying in these areas is fundamentally linked to forage utilization, for example in Ireland every extra tonne of forage yield per ha (DM basis) is worth 161 euro (Shalloo, 2009). Although previous work on CH_4 emissions in grazing animals predominantly relied on the SF_6 tracer method to measure CH_4 emissions (Pinares-Patiño et al., 2007; Cavanagh et al., 2008), in the current study indirect open-circuit calorimetry chambers were used. These chambers measured gaseous exchanges including CH_4 which allowed for the calculation of HP a variable which could not be measured by SF_6 technique. Although the lack of energy expenditure at pasture due to grazing cannot be assessed when animals are in the chambers, results from the current study may be highly applicable to pasture-based systems, due to the zero-grazing practices used, and compliment results from studies using SF_6 tracer techniques.

Nutrient Digestibility and Energy Utilization Efficiency

Due to the relatively high apparent digestibilities in the present study, DMI were high across all treatments; the positive association between highly digestible feed and DMI has been previously demonstrated (NRC, 2001). Apparent DM digestibility of 0.76 in the present study is comparable with published figures (0.76-0.78) of dairy cows on similar diets (Whelan et al., 2012). Lack of effect of dietary treatment on digestibility parameters obtained in the present study is 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 present N digestibility values were similar to those observed in studies under a wide variety of dietary regimes (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, reflects the increased urine N loss with increasing concentrate CP content while treatment had no effect on milk N output or retained N (Hynes et al., 2016). However, NDF (0.725) and organic matter (OM; 0.792) digestibility values obtained in the present study were higher than previously recorded figures (Nousiainen et al., 2004; Huhtanen et al., 2008) which averaged at 0.622 and 0.726 respectively. This may be explained by the good quality perennial ryegrass offered during the present study which may have improved feed OM digestibility (Stergiadis et al., 2015).

Energy (GE, DE and ME) intakes and outputs did not differ across dietary treatments with the exception of urine energy outputs. The observed differences in urinary energy partitioning are in agreement with previous work (Ramin and Huhtanen, 2013), which reported urinary energy was positively associated with dietary CP content on 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 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 LCP 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, MY 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 the grazing diet at a CP content of 17 % (DM basis) is enough to sustain milk production as reported by Hynes et al. (2016) and energy digestibility, metabolizability and k_l. Further investigation into the long term effects on production efficiency and other functional traits (e.g., fertility) would also need to be evaluated. Feeding dairy cows low CP diets may save on feed cost of high priced protein feeds (e.g. soybean meal), and also reduce environmental footprint (urinary N excretion).

The present study demonstrated that crossbreeding of Holstein cows with Swedish Red sires had no effects on nutrient and energy digestibility, energy metabolizability or k_l when cows were offered fresh perennial ryegrass-based diets, although Holstein cows had significantly greater GE, DE and ME intakes. A number of previous studies also found a similar result when offered ensiled forage. For example, Xue et al. (2011) observed no difference in energy metabolizability or k_l between Holstein and Jersey-Holstein cows offered perennial ryegrass silage diets containing either 30 % or 70 % of 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 current study indicate that the cross-breeding of Holstein cows with Swedish Red or Jersey sires has negligible influence on the potential of high production efficiency of the Holstein breed. Swedish Red cows have been traditionally selected for milk production and other functional traits (e.g. fertility, disease resistance) and thus have a longer service term than Holstein cows (Swalve, 2007). Consequently, Swedish Red sires have been widely used to improve reproductive performance and health status of Holstein cows. The present study indicates that although the crossbred cows had a lower feed intake and MY as reported by Hynes et al. (2016), energy digestibility, energy metabolizability and k_l traits were not compromised when compared to pure Holstein cow offered fresh perennial ryegrass based diets.

Methane Emissions

The present findings, that dietary CP concentration did not affect CH_4 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 CH_4 / kg DMI and dietary CP concentration. Conversely, Stergiadis et al. (2016) found increasing perennial ryegrass CP and WSC contents increased CH_4 / kg DMI in dry cows offered fresh perennial ryegrass only diets at maintenance level. Arndt et al. (2015) suggested a quadratic relationship between CH_4 (g/d, g/kg DMI and MJ/MJ GEI) and dietary CP when different ratios of alfalfa silage to corn silage were fed. It is difficult to determine the root cause of changes in CH_4 yields, but Hassanat et al. (2013) suggested it may be due to increasing dietary starch content with decreasing CP content resulting in a drop in pH, protozoa and methanogens. Similarly, Dijkstra et al. (2011) speculated yields of CH_4 may decrease when starch increased at the expense of CP content due to fermentation of fiber, producing higher volumes of VFA, acetate and butyrate which yield H_2, a precursor of methanogenesis, in comparison to starch which results in higher volumes of propionate, a reaction which utilizes H_2. Although this may imply the resultant altered fiber / starch concentration can affect enteric CH_4 outputs, in addition to a reduction in urinary N output when dietary N content decreases (Külling et al., 2001; Weiss et al., 2009, Arndt et al., 2015), the outcome of present study did not confirm this hypothesis. In the present study the formulation of concentrate supplements did not alter their NDF and ADF concentrations. Although increasing CP contents decreased starch contents in the 3 concentrates, the differences in starch contents were relatively small (21.1 to 23.2 % DM basis) between the 3 concentrates and negligible (6.9 to 7.6 %) between total diets. Therefore, the present study suggests that increasing concentrate CP contents resulting in a concomitant increase in total dietary CP content from 16.9 to 18.3 % had no effects on enteric CH_4 emission rates on perennial ryegrass and concentrate base diets.

The present study found that crossbreeding of Holstein cows with Swedish Red sires had no significant effect on CH_4 / kg DMI, CH_4 / kg OM intake or CH_4-E / GEI (MJ/MJ), with CH_4 / kg ECM yield being identical between the 2 genotypes. Although there were no comparable calorimetry data with fresh ryegrass, Yan and Mayne (2009) observed a similar result when compared between Holstein and Jersey × Holstein cows offered diets containing perennial ryegrass silage and either 30 % or 70 % concentrates. A number of recent studies have assessed the potential association between enteric CH_4 emissions and microbial ecology of ruminal methanogens. Using the culture-independent methods, Zhou et al. (2009; 2010) reported that while there was 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 CH_4 emission rate in cattle is mainly driven by feed intake and dietary nutrient composition and cow genotypes based on the Holstein breed may have little effect on the inherent genetic capacity for the rumen methanogenesis. The heritability for CH_4 emissions of Holstein cows is low (Lassen and Løvendahl, 2016). Hence rather than breeding for reduced CH_4 (g/d) or CH_4 / kg DMI, Cottle et al. (2011) suggested that a breeding approach for improved feeding efficiency would be more successful and in line with current breeding objectives, so as to minimise risk of undesirable trade-offs.

In the present study, GEI lost as CH_4-E was on average 5.6 %. This figure is very close to the simulated prediction (5.8 %) by Bannink et al. (2010) with lactating dairy cows on a similar DMI and fresh forage: concentrate ratio, and similar to that (averaging 5.7 %) of grazing dairy cows with CH_4 emissions measured using the SF_6 technique (O'Neill et al., 2012; Jiao et al., 2014). However, these CH_4/GEI data are all lower that of 6.5 % recommended by IPCC (2006) to calculate enteric CH_4 emission inventory for a region where local CH_4 emission data are not available. Therefore it is possible utilizing the IPCC default value for inventory purposes would overestimate CH_4 production in grazing systems, especially for countries where grazing management regimes are a major component of dairy production, such as in Ireland, UK, New Zealand and Australia. This issue merits further investigation.

CONCLUSION

The results from the current study suggest reducing concentrate CP content from 18.1 to 14.1% does not affect energy utilization efficiency or enteric CH_4 emission rates in lactating dairy cows on fresh-cut perennial ryegrass based diets. Crossbreeding Holstein cows with

Swedish Red sires had no significant effect on energy utilization efficiency or enteric CH_4 emission rates, although Holstein cows had higher energy intakes and milk energy outputs. Hence these findings suggest concentrates with CP levels as low as 14.1 % can be offered in combination with good quality perennial ryegrass without any negating effect on CH_4 emissions or energy partitioning for production, although sustainability of production would have to be confirmed on a long-term study. Feeding grazing cows with low CP concentrates not only reduces feed costs but is also environmentally beneficial with lower urinary nitrogen excretion.

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TABLES

Table 1. Equations used for the calculations of heat production**,** ME requirement for maintenance and efficiency of ME for lactation[1,2]

Estimated variable	Equation	Reference
HP (MJ/d)	$= [(16.18 \times O_2) + (5.16 \times CO_2) - (2.42 \times CH_4) - (5.9 \times UN)] / 1000$	(Brouwer, 1965)
ME_m (when Eg < 0)	$= HP - (1/ k_{l\ (AFRC)} - 1) \times E_l - (1/k_t - 2) \times E_g - (1/k_p - 1) \times E_p$	(AFRC, 1993)
ME_m (when Eg > 0)	$= HP - (1/ k_{l\ (AFRC)} - 1) \times E_l - (1/k_g - 2) \times E_g - (1/k_p - 1) \times E_p$	
$E_{l(0)}$ (when Eg < 0)	$= E_l + 0.84 \times E_g$	(AFRC, 1993)
$E_{l(0)}$ (when Eg > 0)	$= E_l + 1/k_g \times E_g$	
k_l	$= E_{l(0)} / (ME_{int} - ME_m)$	(AFRC, 1993)

[1] CH_4 = methane produced (L/d), CO_2 = carbon dioxide produced (L/d), O_2 = oxygen consumed (L/d), UN = urinary nitrogen excreted (g/d).

[2] E_g = net energy for BW change (MJ/d), E_l = milk energy output (MJ/d), $E_{l(0)}$ = milk energy output adjusted to zero energy balance (MJ/d), E_p = net energy requirement for pregnancy, HP = heat production (MJ/d), ME_{int} = ME intake (MJ/d), ME_m = ME requirement for maintenance (MJ/d), k_g = efficiency of ME use for weight gain, k_l = efficiency of ME use for lactation, $k_{l\ (AFRC)}$ = efficiency of ME use for lactation calculated from AFRC (1993), k_p = efficiency of ME use for pregnancy, k_t = efficiency of utilization of mobilized energy for lactation.

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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	lin	quad	Holstein	Crossbred[2]	SEM	P-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
DOMD	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
GE	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

DOMD = Digestible OM in DM, GE = gross energy.

[1] Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

[2] Crossbred 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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	lin	quad	Holstein	Crossbred[2]	SEM	P-value
Energy intake and output (MJ/d)										
GE intake	372	383	375	8.2	0.498	0.197	390	364	6.5	<0.001
DE intake	276	286	278	7.3	0.290	0.055	291	268	6.0	0.002
ME intake	237	246	238	7.1	0.426	0.108	250	231	6.3	0.017
Faecal energy	96	97	96	2.2	0.888	0.742	98	95	2.2	0.392
Urinary energy	17.9	17.9	19.8	0.98	0.004	0.292	19.1	17.9	1.02	0.378
CH_4 energy	21.1	21.6	20.8	1.15	0.724	0.467	22.4	20.0	1.15	0.164
Heat production	138	133	134	4.1	0.452	0.679	140	130	3.1	0.019
Milk energy	85	85	85	2.9	0.562	0.616	91	79	2.8	0.012
Retained energy	14.7	26.8	17.6	7.35	0.561	0.193	16.8	22.6	7.29	0.519
Energy utilization (MJ/MJ)										
DE / GE	0.740	0.745	0.741	0.0060	0.611	0.324	0.746	0.738	0.0057	0.236
ME / GE	0.636	0.642	0.632	0.0082	0.761	0.165	0.640	0.633	0.0093	0.602
Heat production / ME	0.587	0.546	0.569	0.0229	0.423	0.237	0.570	0.565	0.0215	0.840
Milk energy / ME	0.362	0.344	0.364	0.0159	0.833	0.108	0.367	0.346	0.0153	0.227
Retained energy /ME	0.052	0.108	0.066	0.0313	0.582	0.130	0.062	0.089	0.0310	0.475
k_l	0.623	0.628	0.624	0.0035	0.854	0.519	0.628	0.621	0.0025	0.121

GE = gross energy, DE = digestible energy, k_l = efficiency of ME use for lactation

[1] Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

[2] Crossbred 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

	Concentrate CP level				P-value[1]		Cow genotype			
	Low	Medium	High	SEM	lin	quad	Holstein	Crossbred[2]	SEM	P-value
CH_4 (g/d)	381.6	391.3	377.3	20.8	0.724	0.467	405	362	20.9	0.164
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	0.593
CH_4 / OMI	20.15	20.25	20.00	1.13	0.923	0.779	20.6	19.7	1.17	0.598
CH_4 / Digestible DMI	24.20	24.04	23.90	1.41	0.809	0.972	24.4	23.7	1.49	0.737
CH_4 / Digestible OMI	25.86	25.74	25.60	1.51	0.831	0.863	26.1	25.3	1.60	0.724
CH_4 / Milk yield	14.35	14.85	14.30	1.01	0.372	0.366	13.8	15.2	0.95	0.225
CH_4 / ECMY	13.49	14.15	13.33	0.90	0.480	0.240	13.3	14.0	0.90	0.575
CH_4-E / energy intake (MJ/MJ)										
CH_4-E / GEI	0.056	0.056	0.056	0.0031	0.972	0.755	0.057	0.055	0.0032	0.605
CH_4-E / DEI	0.077	0.076	0.076	0.0045	0.712	0.923	0.077	0.075	0.0048	0.724
CH_4-E / MEI	0.090	0.089	0.089	0.0059	0.776	0.969	0.091	0.088	0.0063	0.759

OMI = OM intake, ECMY = ECM yield (ECMY =Milk GE content (MJ/Kg) × MY (kg/d) / 3.0968, as shown by Tyrrell and Reid, (1965)), CH_4-E = methane energy output, GEI = gross energy intake, DEI = digestible energy intake, MEI = ME intake.

[1] Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

[2] Crossbred cows were crosses between Holstein and Swedish Red.