

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

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Hynes, D. N., Stergiadis, S. ORCID: <https://orcid.org/0000-0002-7293-182X>, Gordon, A. and Yan, T. (2016) Effects of concentrate crude protein content on nutrient digestibility, energy utilization, and methane emissions in lactating dairy cows fed fresh-cut perennial grass. *Journal of Dairy Science*, 99 (11). pp. 8858-8866. ISSN 0022-0302 doi: <https://doi.org/10.3168/jds.2016-11509> Available at <https://centaur.reading.ac.uk/66250/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.3168/jds.2016-11509>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in

the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

## 1 **INTERPRETIVE SUMMARY**

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

13

## 14 **RUNNING HEAD: CONCENTRATE PROTEINS' EFFECT ON METHANE EMISSIONS**

15

### 16 **Effects of concentrate crude protein contents on nutrient digestibility, energy utilization** 17 **and methane emissions of lactating dairy cows fed fresh-cut perennial grass.**

18

19 **D. N. Hynes,\*† S. Stergiadis, ‡, A. Gordon, § and T. Yan\***

20

21 \*Sustainable Agri-Food Sciences Division, Agriculture Branch, Agri-Food and Biosciences  
22 Institute, Large Park, Hillsborough, County Down, BT26 6DR, UK

23 † Institute for Global Food Security, School of Biological Sciences, Queens University  
24 Belfast, University Road, Belfast, County Antrim, BT7 1NN, UK

25 ‡ Animal, Dairy and Food Chain Sciences Division, Centre for Dairy Research, University of  
26 Reading, School of Agriculture, Policy and Development, Earley Gate, PO Box 237,  
27 Reading, Berkshire, RG6 6AR, UK

28 § Finance and Corporate Affairs Division, Biometrics and Information Systems Branch,  
29 Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast, County Antrim, BT9 5PX,  
30 UK

31 Corresponding author: Tianhai Yan, Agri-Food and Biosciences Institute, Large Park,  
32 Hillsborough, County Down, BT26 6DR, UK. Phone: 0044 28 9268 0555. Fax: 0044 28 9268  
33 9594. Email: [tianhai.yan@afbini.gov.uk](mailto:tianhai.yan@afbini.gov.uk)

## ABSTRACT

Although many studies have investigated mitigation strategies for methane (CH<sub>4</sub>) output from dairy cows fed a wide variety of diets, research on effects of concentrate crude protein (CP) content on CH<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> emission (g/d), CH<sub>4</sub> 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<sub>4</sub> parameters. Furthermore, the present study yielded a value for gross energy lost as CH<sub>4</sub> (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<sub>4</sub> 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

64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89

The agricultural industry is a major contributor of atmospheric methane (CH<sub>4</sub>), 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<sub>2</sub> 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<sub>4</sub> emissions may increase ME available and thus improve energy utilization efficiency in ruminant systems. The extent of CH<sub>4</sub> emission rates 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). 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<sub>4</sub> / 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

90 dietary factors (e.g., fiber and starch concentrations). Indeed, Stergiadis et al. (2016) found  
91 increasing grass CP and water soluble carbohydrate (**WSC**) concentrations increased CH<sub>4</sub> /  
92 kg DMI in dry cows offered fresh perennial ryegrass only diets at maintenance feeding levels.  
93 Therefore, the effects of dietary CP contents on CH<sub>4</sub> emissions and energy utilization merit  
94 investigation in studies with dairy cows offered fresh forage based diets.

95 Animal genetic factors have been found to play a significant role in influence of energy  
96 utilization efficiency and CH<sub>4</sub> emissions from ruminants (Pinares-Patino et al., 2009; Clark,  
97 2013). It is well documented improving productivity can lead to a reduction in CH<sub>4</sub> emissions  
98 per unit of produce (Chagunda et al., 2009; Wall et al., 2010; Cottle et al., 2011) while  
99 simultaneously making mitigation strategies appealing to producers. Beecher et al. (2014)  
100 and Palladino et al. (2010) showed that Holstein-Friesian cows on perennial ryegrass silage  
101 diets offered at maintenance level and grazing perennial ryegrass respectively may exhibit  
102 differences in production efficiency when compared with Jersey and Jersey × Holstein-  
103 Friesian. However, comparisons on CH<sub>4</sub> emissions between Holstein and other breeds under  
104 grazing or zero-grazing conditions are limited with literature focusing on ensiled forage (Xue  
105 et al., 2011; Arndt et al., 2015).

106 The present study was thus designed to address these knowledge gaps as identified  
107 previously, by evaluating the effects of reducing concentrate CP contents (with little  
108 influence on starch and fiber contents), cow genotype and their interactions on nutrient  
109 digestibilities, energy utilization efficiency and CH<sub>4</sub> emissions in lactating dairy cows offered  
110 fresh perennial ryegrass diets so that practices are widely applicable in pasture-based  
111 systems.

112

## MATERIALS AND METHODS

113  
114 All scientific procedures described were carried out under experimental license from the  
115 Department of Health, Social Services and Public Safety of Northern Ireland in accordance  
116 with the Animal (Scientific Procedures) Act (Home Office, 1986).

### 117 *Experimental Design*

118 The current study presents observations from a calorimetry experiment performed at Agri-  
119 Food and Biosciences Institute (Hillsborough, Northern Ireland, UK), using 12 multiparous  
120 lactating (6 Holstein and 6 Holstein × Swedish Red (50:50) crossbred) cows on diets of fresh-  
121 cut perennial ryegrass and concentrate feeds during the 2014 grazing season. Details of  
122 animals, experiment design and diets were reported in a companion paper (Hynes et al.,  
123 2016). A brief description of the design and measurement procedures follows. Animals were  
124 offered 3 dietary treatments with different concentrate CP contents (2 cows within each  
125 genotype/diet) in a changeover study with three (25-d) periods. All measurements were taken  
126 during the final 6-d of each period; 3-d digestibility units and 3-d in indirect open-circuit  
127 respiration calorimeter chambers, with continuation of digestibility measurements in the  
128 respiration chambers. Diets composed of zero-grazed perennial ryegrass and concentrate  
129 feeds of differing CP content; low CP concentrate diet (LCP, 14.1 % DM), medium CP  
130 concentrate diet (MCP, 16.1 % DM) and high CP concentrate diet (HCP, 18.1 % DM) fed at  
131 32.8 % DMI in combination with perennial ryegrass fed at 67.2 % DMI. The low and high  
132 CP concentrates were formulated to possess the same dietary components and similar  
133 chemical composition with the exception of CP level, while the medium CP concentrate was  
134 produced by mixing the low and high CP concentrates in equal proportions. This resulted in 3  
135 concentrate feeds which were comparable in regard to ME, fermentable ME and fiber  
136 content. Concentrates were offered at milking, 50 % at 0700 and 50 % at 1500, and fresh  
137 herbage at 1000 (ad libitum) each morning. The zero-grazed herbage was harvested (from a



138 single sward) each morning using a Haldrup 1500, boxed loosely to avoid nutrient  
139 degradation and perennial ryegrass' temperature was monitored for the duration of the study.  
140 Herbage regrowth intervals (initially 22-d regrowth with incremental increases up to 30-d  
141 from June to September) and fertilization practises (within 3-d of harvesting at 35 kg N / ha)  
142 were determined based on common routine practices, in order to ensure perennial ryegrass of  
143 a similar quality was being offered to animals for the duration of experimental work.  
144 Concentrate rations were calculated based on the average DMI of the previous 7-d and  
145 animals had free access to water throughout the study.

#### 146 *Digestibility and Calorimeter Chamber Measurements*

147 All procedures for records of feed intake, feces and urine excretion and milk production, and  
148 all sample measurements during the final 6-d of each period were reported by Hynes et al.  
149 (2016). In brief, perennial ryegrass and concentrate were analyzed for DM, N, gross energy  
150 (GE), NDF, ADF, ash, WSC (perennial ryegrass only) and starch (concentrate only) and DM,  
151 N, NDF, ADF and ash contents in feces and N in urine was assessed. In addition, analysis of  
152 GE was conducted in the present study, using a Parr 6300 oxygen bomb calorimeter (Parr  
153 Instrument Company, Illinois, USA), on fecal and urine samples on a dry and fresh basis  
154 respectively, as described in Jiao et al. (2013). Gaseous exchange (O<sub>2</sub> consumption and CO<sub>2</sub>  
155 and CH<sub>4</sub> production) was measured in the final 48 h of the 72 h calorimetric-chamber stage.  
156 Two indirect open-circuit respiration calorimeter chambers consisting of a climatic control  
157 unit, an air flow and measurement system and 3 gas analyzers were utilized. Chambers were  
158 maintained at 16 ± 1°C and 60 % relative humidity via air conditioning unit including a  
159 Vaisala PTA 427 digital barometer and Vaisala HUMICAP sensor probes (Delta-T devices,  
160 Cambridge, UK). Air was dehumidified, heated or cooled to 13-15°C and re-humidified, if  
161 necessary, prior to entering the chambers. Chambers were run under a slight negative  
162 pressure and possessed airlock systems for entry and feeding to ensure against leakage. Each

163 chambers' flow system consisted of 2 inlet ambient air tubes and 3 extraction tubes fitted  
164 with turbine flow meters (GH flow Automation Ltd. Andover, UK). Suction pumps were set  
165 to perform 3.4 (75 m<sup>3</sup> / h flowrate / 22 m<sup>3</sup> total chamber volume) air exchanges / h.  
166 Measurement of flow rate and concentration of ambient and extraction air allowed for  
167 calculation of CH<sub>4</sub> output. All gases were measured by ADC MGA3000 Multi gas analyzer  
168 (ADC Gas Analysis Ltd., Hertfordshire, UK), CH<sub>4</sub> and CO<sub>2</sub> concentrations by  
169 electrochemical sensors and O<sub>2</sub> concentrations by paramagnetic sensor. The analyzer  
170 switched between both chambers and span gases every 75 s and completed a full rotation  
171 every 225 s. Data were then transferred onto a 16-bit digital converter (Strawberry tree model  
172 ACPC -16, Adepth Scientific Mirco System Ltd., Letchworth, UK). All equipment,  
173 procedures, analytical methods and calculations used in the calorimetric experiments were as  
174 reported by Gordon et al. (1995) and calibration of the chambers by Yan et al. (2000).

175

### 176 *Calculations and Statistical Analysis*

177 Prior to analysis a number of energy utilization parameters were calculated using the  
178 equations in Table 1. Heat production (**HP**) was calculated based on O<sub>2</sub> consumption, CO<sub>2</sub>  
179 and CH<sub>4</sub> production and urinary N excretion using the equation of Brouwer (1965). Retained  
180 energy was calculated by subtracting HP and milk energy from ME intake (MEI). The ME  
181 requirement for maintenance (**ME<sub>m</sub>**) and subsequently the efficiency of ME use for lactation  
182 (**ki**) was calculated according to Agnew et al. (2004).

183 Means of individual animals' variables over the two 3-d collection phases (with the exception  
184 of the calorimetric data collected over one 3-d phase) were used for statistical analysis.  
185 Experimental data were analyzed using Genstat statistical package (VSN International, 2013).  
186 Linear mixed model methodology with REML estimation (Gilmour et al., 1995) was implied  
187 with dietary treatment and genotype as fixed factors and cow and date (of entry to collection

188 stage) fitted as random effects. Orthogonal contrasts were used to test for linear and quadratic  
189 effects of treatment as described by Hynes et al. (2016). Residuals conveyed no deviation  
190 from normality. Differences between treatments, genotypes and interactions were assessed  
191 with 5 degrees of significance, non-significant ( $P > 0.10$ ) and significance at  $P < 0.05$ ,  $P <$   
192  $0.01$  and  $P < 0.001$ , while tendencies were declared at  $0.05 < P < 0.10$ .

## 193 **RESULTS**

194 There was no significant interaction between concentrate CP level and cow genotype on any  
195 parameter evaluated in terms of digestibility, CH<sub>4</sub> emissions or energy intake, output or  
196 utilization efficiency. Therefore, only results of main factors were presented in the present  
197 study. The results on dietary composition, feed intake and milk production were reported by  
198 Hynes et al. (2016) viz. concentrate CP contents did not affect DMI, milk yield (MY) or milk  
199 composition. Concentrate feeds had similar chemical compositions only varying in CP  
200 content, consequently total dietary CP levels were 16.9, 17.6 and 18.3 % (DM basis) for the  
201 LCP, MCP and HCP treatments respectively.

### 202 ***Nutrient Apparent Whole-tract Digestibility***

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

### 207 ***Energy Utilization***

208 Findings on the effects of concentrate CP levels and cow genotype on energy utilization  
209 variables are displayed in Table 3. Analysis showed there was no significant effect of dietary  
210 treatment on energy intake (GE, digestible energy (**DE**) or ME), retained energy or energy  
211 partition in feces, CH<sub>4</sub>, HP or milk, although there was a positive linear effect of concentrate

212 CP observed urine energy output. We found no significant effect of treatment on DE/GE,  
213 ME/GE, HP/MEI or  $k_1$ .

214 In comparison to Crossbreds, Holstein cows had significantly higher GE, DE and ME intakes  
215 and consequently higher HP and milk energy output. Cow genotype had no significant effect  
216 on DE/GE, ME/GE, HP/MEI or  $k_1$ .

### 217 *Methane Emissions*

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

## 223 **DISCUSSION**

224 Grazing systems are extensively used in areas with cool and moist climates, which allow a  
225 long grazing season and high forage production, thus providing a low-cost feeding approach  
226 for ruminant production systems (Peyraud and Delagarde, 2013). Hence profitability of  
227 dairying in these areas is fundamentally linked to forage utilization, for example in Ireland  
228 every extra tonne of forage yield per ha (DM basis) is worth 161 euro (Shalloo, 2009).  
229 Although previous work on CH<sub>4</sub> emissions in grazing animals predominantly relied on the  
230 SF<sub>6</sub> tracer method to measure CH<sub>4</sub> emissions (Pinares-Patiño et al., 2007; Cavanagh et al.,  
231 2008), in the current study indirect open-circuit calorimetry chambers were used. These  
232 chambers measured gaseous exchanges including CH<sub>4</sub> which allowed for the calculation of  
233 HP a variable which could not be measured by SF<sub>6</sub> technique. Although the lack of energy  
234 expenditure at pasture due to grazing cannot be assessed when animals are in the chambers,

235 results from the current study may be highly applicable to pasture-based systems, due to the  
236 zero-grazing practices used, and compliment results from studies using SF<sub>6</sub> tracer techniques.

### 237 *Nutrient Digestibility and Energy Utilization Efficiency*

238 Due to the relatively high apparent digestibilities in the present study, DMI were high across  
239 all treatments; the positive association between highly digestible feed and DMI has been  
240 previously demonstrated (NRC, 2001). Apparent DM digestibility of 0.76 in the present study  
241 is comparable with published figures (0.76-0.78) of dairy cows on similar diets (Whelan et  
242 al., 2012). Lack of effect of dietary treatment on digestibility parameters obtained in the  
243 present study is in agreement with results from a study by Moorby et al. (2006) in dairy cows  
244 offered diets containing 65 % ryegrass silage and 35 % concentrate. The present N  
245 digestibility values were similar to those observed in studies under a wide variety of dietary  
246 regimes (Huhtanen et al., 2008), including fresh-forage diets (van Vuuren et al., 1992).  
247 Increasing N digestibility with increasing dietary CP concentration, as tended to occur in the  
248 present study, reflects the increased urine N loss with increasing concentrate CP content  
249 while treatment had no effect on milk N output or retained N (Hynes et al., 2016). However,  
250 NDF (0.725) and organic matter (OM; 0.792) digestibility values obtained in the present  
251 study were higher than previously recorded figures (Nousiainen et al., 2004; Huhtanen et al.,  
252 2008) which averaged at 0.622 and 0.726 respectively. This may be explained by the good  
253 quality perennial ryegrass offered during the present study which may have improved feed  
254 OM digestibility (Stergiadis et al., 2015).

255

256 Energy (GE, DE and ME) intakes and outputs did not differ across dietary treatments with the  
257 exception of urine energy outputs. The observed differences in urinary energy partitioning are  
258 in agreement with previous work (Ramin and Huhtanen, 2013), which reported urinary  
259 energy was positively associated with dietary CP content on a wide range of dietary

260 treatments (n = 207). This may be due to the associated excess N in urine that increases urine  
261 energy content as found to be the case in Holstein steers on concentrate based diets (Mwenya  
262 et al., 2004). The lack of effect of diet treatments on energy intake, utilization efficiency and  
263 nutrient digestibility values obtained in the present study may imply that the total dietary CP  
264 content (16.9 %) of the LCP treatment may be sufficient to supply degradable CP for rumen  
265 microbial activity and MP for milk production. Indeed, the present study found that  
266 increasing concentrate CP levels had no significant effect on total DMI, MY or composition  
267 or N utilization efficiency in terms of N excretion in feces, urine or milk as a proportion of N  
268 intake (Hynes et al., 2016). However, increasing concentrate CP levels significantly increased  
269 N excretion in urine and urine N/manure N. It is a common practice in dairy farming in  
270 Northern Ireland to feed dairy cows grazing diets and winter diets containing CP content of  
271 approximately 18 % (DM basis). However, the present study clearly demonstrated the  
272 grazing diet at a CP content of 17 % (DM basis) is enough to sustain milk production as  
273 reported by Hynes et al. (2016) and energy digestibility, metabolizability and  $k_I$ . Further  
274 investigation into the long term effects on production efficiency and other functional traits  
275 (e.g., fertility) would also need to be evaluated. Feeding dairy cows low CP diets may save on  
276 feed cost of high priced protein feeds (e.g. soybean meal), and also reduce environmental  
277 footprint (urinary N excretion).

278

279 The present study demonstrated that crossbreeding of Holstein cows with Swedish Red sires  
280 had no effects on nutrient and energy digestibility, energy metabolizability or  $k_I$  when cows  
281 were offered fresh perennial ryegrass-based diets, although Holstein cows had significantly  
282 greater GE, DE and ME intakes. A number of previous studies also found a similar result  
283 when offered ensiled forage. For example, Xue et al. (2011) observed no difference in energy  
284 metabolizability or  $k_I$  between Holstein and Jersey-Holstein cows offered perennial ryegrass

285 silage diets containing either 30 % or 70 % of concentrates. Heins et al. (2008) also reported  
286 that the feed efficiency for d 4 to 150 of lactation was similar for Jersey-Holstein and pure  
287 Holstein cows offered diets containing alfalfa hay and corn silage. These results along with  
288 those from the current study indicate that the cross-breeding of Holstein cows with Swedish  
289 Red or Jersey sires has negligible influence on the potential of high production efficiency of  
290 the Holstein breed. Swedish Red cows have been traditionally selected for milk production  
291 and other functional traits (e.g. fertility, disease resistance) and thus have a longer service  
292 term than Holstein cows (Swalve, 2007). Consequently, Swedish Red sires have been widely  
293 used to improve reproductive performance and health status of Holstein cows. The present  
294 study indicates that although the crossbred cows had a lower feed intake and MY as reported  
295 by Hynes et al. (2016), energy digestibility, energy metabolizability and  $k_1$  traits were not  
296 compromised when compared to pure Holstein cow offered fresh perennial ryegrass based  
297 diets.

### 298 *Methane Emissions*

299 The present findings, that dietary CP concentration did not affect  $CH_4$  emissions, is in  
300 agreement with van Dorland et al. (2007). However, in a meta-analysis of calorimetry data,  
301 Yan and Mayne (2007) found a negative relationship between  $CH_4$  / kg DMI and dietary CP  
302 concentration. Conversely, Stergiadis et al. (2016) found increasing perennial ryegrass CP  
303 and WSC contents increased  $CH_4$  / kg DMI in dry cows offered fresh perennial ryegrass only  
304 diets at maintenance level. Arndt et al. (2015) suggested a quadratic relationship between  
305  $CH_4$  (g/d, g/kg DMI and MJ/MJ GEI) and dietary CP when different ratios of alfalfa silage to  
306 corn silage were fed. It is difficult to determine the root cause of changes in  $CH_4$  yields, but  
307 Hassanat et al. (2013) suggested it may be due to increasing dietary starch content with  
308 decreasing CP content resulting in a drop in pH, protozoa and methanogens. Similarly,  
309 Dijkstra et al. (2011) speculated yields of  $CH_4$  may decrease when starch increased at the

310 expense of CP content due to fermentation of fiber, producing higher volumes of VFA,  
311 acetate and butyrate which yield H<sub>2</sub>, a precursor of methanogenesis, in comparison to starch  
312 which results in higher volumes of propionate, a reaction which utilizes H<sub>2</sub>. Although this  
313 may imply the resultant altered fiber / starch concentration can affect enteric CH<sub>4</sub> outputs, in  
314 addition to a reduction in urinary N output when dietary N content decreases (Külling et al.,  
315 2001; Weiss et al., 2009, Arndt et al., 2015), the outcome of present study did not confirm  
316 this hypothesis. In the present study the formulation of concentrate supplements did not alter  
317 their NDF and ADF concentrations. Although increasing CP contents decreased starch  
318 contents in the 3 concentrates, the differences in starch contents were relatively small (21.1 to  
319 23.2 % DM basis) between the 3 concentrates and negligible (6.9 to 7.6 %) between total  
320 diets. Therefore, the present study suggests that increasing concentrate CP contents resulting  
321 in a concomitant increase in total dietary CP content from 16.9 to 18.3 % had no effects on  
322 enteric CH<sub>4</sub> emission rates on perennial ryegrass and concentrate base diets.

323

324 The present study found that crossbreeding of Holstein cows with Swedish Red sires had no  
325 significant effect on CH<sub>4</sub> / kg DMI, CH<sub>4</sub> / kg OM intake or CH<sub>4</sub>-E / GEI (MJ/MJ), with CH<sub>4</sub> /  
326 kg ECM yield being identical between the 2 genotypes. Although there were no comparable  
327 calorimetry data with fresh ryegrass, Yan and Mayne (2009) observed a similar result when  
328 compared between Holstein and Jersey × Holstein cows offered diets containing perennial  
329 ryegrass silage and either 30 % or 70 % concentrates. A number of recent studies have  
330 assessed the potential association between enteric CH<sub>4</sub> emissions and microbial ecology of  
331 ruminal methanogens. Using the culture-independent methods, Zhou et al. (2009; 2010)  
332 reported that while there was no significant difference in the total population of methanogens  
333 between cattle with different feed efficiencies, their rumen methanogenesis capacity was  
334 highly related to changes in feed intake and dietary composition. The abundance of



335 predominant methanogenic species obtained on the low energy density diet shifted to a  
336 community containing a more diverse range of predominant species with the high energy  
337 density diet (Zhou et al., 2010). These results indicated that enteric CH<sub>4</sub> emission rate in  
338 cattle is mainly driven by feed intake and dietary nutrient composition and cow genotypes  
339 based on the Holstein breed may have little effect on the inherent genetic capacity for the  
340 rumen methanogenesis. The heritability for CH<sub>4</sub> emissions of Holstein cows is low (Lassen  
341 and Løvendahl, 2016). Hence rather than breeding for reduced CH<sub>4</sub> (g/d) or CH<sub>4</sub> / kg DMI,  
342 Cottle et al. (2011) suggested that a breeding approach for improved feeding efficiency would  
343 be more successful and in line with current breeding objectives, so as to minimise risk of  
344 undesirable trade-offs.

345

346 In the present study, GEI lost as CH<sub>4</sub>-E was on average 5.6 %. This figure is very close to the  
347 simulated prediction (5.8 %) by Bannink et al. (2010) with lactating dairy cows on a similar  
348 DMI and fresh forage: concentrate ratio, and similar to that (averaging 5.7 %) of grazing  
349 dairy cows with CH<sub>4</sub> emissions measured using the SF<sub>6</sub> technique (O'Neill et al., 2012; Jiao  
350 et al., 2014). However, these CH<sub>4</sub>/GEI data are all lower than that of 6.5 % recommended by  
351 IPCC (2006) to calculate enteric CH<sub>4</sub> emission inventory for a region where local CH<sub>4</sub>  
352 emission data are not available. Therefore it is possible utilizing the IPCC default value for  
353 inventory purposes would overestimate CH<sub>4</sub> production in grazing systems, especially for  
354 countries where grazing management regimes are a major component of dairy production,  
355 such as in Ireland, UK, New Zealand and Australia. This issue merits further investigation.

356

## CONCLUSION

357 The results from the current study suggest reducing concentrate CP content from 18.1 to  
358 14.1% does not affect energy utilization efficiency or enteric CH<sub>4</sub> emission rates in lactating  
359 dairy cows on fresh-cut perennial ryegrass based diets. Crossbreeding Holstein cows with

360 Swedish Red sires had no significant effect on energy utilization efficiency or enteric CH<sub>4</sub>  
361 emission rates, although Holstein cows had higher energy intakes and milk energy outputs.  
362 Hence these findings suggest concentrates with CP levels as low as 14.1 % can be offered in  
363 combination with good quality perennial ryegrass without any negating effect on CH<sub>4</sub>  
364 emissions or energy partitioning for production, although sustainability of production would  
365 have to be confirmed on a long-term study. Feeding grazing cows with low CP concentrates  
366 not only reduces feed costs but is also environmentally beneficial with lower urinary nitrogen  
367 excretion.

368

### **ACKNOWLEDGEMENTS**

369 This study was funded by the department of agriculture, food and the marine of Republic of  
370 Ireland as part of the stimulus funded project (1S105). Authors would like to acknowledge  
371 technical assistance from staff of the Agri-Food and Bioscience Institute Hillsborough  
372 Ruminant Nutrition Unit and Laboratory.

373

375

376 Agnew, R. E., T. Yan, J. France, E. Kebreab, and C. Thomas. 2004. Energy requirement and  
377 supply. Pages 11–20 in *Feed into Milk: A New Applied Feeding System for Dairy*  
378 *Cows*. C. Thomas, ed. Nottingham Univ. Press, Nottingham, UK.

379 Aguerre, M. J., M. A. Wattiaux, J. M. Powell, G. A. Broderick, and C. Arndt. 2011. Effect of  
380 forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide,  
381 and ammonia, lactation performance, and manure excretion. *J. Dairy Sci.*, 94: 3081-  
382 3093.

383 Arndt, C., J. M. Powell, M. J. Aguerre, and M. A. Wattiaux. 2015. Performance, digestion,  
384 nitrogen balance, and emission of manure ammonia, enteric methane, and carbon  
385 dioxide in lactating cows fed diets with varying alfalfa silage-to-corn silage ratios. *J.*  
386 *Dairy Sci.*, 98: 418-430.

387 Bannink, A., M. C. J. Smits, E. Kebreab, J. A. N. Mills, J. L. Ellis, A. Klop, J. France, and J.  
388 Dijkstra. 2010. Simulating the effects of grassland management and grass ensiling on  
389 methane emission from lactating cows. *J. Agric. Sci.*, 148: 55-72.

390 Beecher, M., F. Buckley, S. M. Waters, T. M. Boland, D. Enriquez-Hidalgo, M. H. Deighton,  
391 M. O'Donovan, and E. Lewis. 2014. Gastrointestinal tract size, total-tract digestibility,  
392 and rumen microflora in different dairy cow genotypes. *J. Dairy Sci.*, 97: 3906-3917.

393 Benchaar, C., F. Hassanat, R. Gervais, P. Y. Chouinard, C. Julien, H. V. Petit, and D. I.  
394 Massé. 2013. Effects of increasing amounts of corn dried distillers grains with  
395 solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N  
396 balance, and milk production. *J. Dairy Sci.*, 96: 2413-2427.

397 Benchaar, C., F. Hassanat, R. Gervais, R. Y. Chouinard, H. V. Petit, and D. I. Massé. 2014.  
398 Methane production, digestion, ruminal fermentation, nitrogen balance, and milk  
399 production of cows fed corn silage- or barley silage-based diets. *J. Dairy Sci.*, 97:  
400 961-974.

401 Brouwer, E. 1965. Report of the sub-committee on constants and factors. Pages 441–443 in  
402 *Energy Metabolism of Farm Animals*. K. L. Blaxter, ed. European Association of  
403 *Animal Production Publication No. 11*. Academic Press, London, UK.

404 Buddle, B. M., M. Denis, G. T. Attwood, E. Altermann, P. H. Janssen, R. S. Ronimus, C. S.  
405 Pinares-Patiño, S. Muetzel, and D. Neil Wedlock. 2011. Strategies to reduce methane  
406 emissions from farmed ruminants grazing on pasture. *Vet. J.*, 188: 11-17.

407 Cavanagh, A., L. McNaughton, H. Clark, C. Greaves, J. M. Gowan, C. Pinares-Patiño, D.  
408 Dalley, B. Vlaming, and G. Molano. 2008. Methane emissions from grazing Jersey x  
409 Friesian dairy cows in mid lactation. *Aust. J. Exp. Agr.*, 48: 230-233.

410 Chagunda, M. G. G., D. A. M. Roemer and D. J. Roberts. 2009. Effect of genotype and  
411 feeding regime on enteric methane, non-milk nitrogen and performance of dairy cows  
412 during the winter feeding period. *Livest. Sci.*, 122; 323-332.

413 Clark, H. 2013. Nutritional and host effects on methanogenesis in the grazing ruminant.  
414 *Animal*, 7: 41-48.

415 Cottle, D. J., J. V. Nolan and S. G. Wiedemann. 2011. Ruminant enteric methane mitigation:  
416 a review. *Anim. Prod. Sci.*, 51: 491-514.

417 Dijkstra, J., O. Oenema, and A. Bannink. 2011. Dietary strategies to reducing N excretion  
418 from cattle: implications for methane emissions. *Curr. Opin. Environ. Sustain.*, 3:  
419 414-422.

420 FAO 2006. *Livestock's role in climate change and air pollution. Livestock's long shadow.*  
421 *Environmental issues and options*. Food and Agriculture Organization of the United  
422 Nations. Accessed April 03, 2016. <ftp://ftp.fao.org/docrep/fao/010/a0701e/a0701e.pdf>

- 423 Gilmour, A. R., R. Thompson and B. R. Cullis. 1995. Average information REML: An  
424 efficient algorithm for variance parameter estimation in linear mixed models.  
425 *Biometrics*, 51: 1440-1450.
- 426 Gordon, F. J., M. G. Porter, C. S. Mayne, E. F. Unsworth, and D. J. Kilpatrick. 1995. Effect  
427 of forage digestibility and type of concentrate on nutrient utilization for lactating dairy  
428 cattle. *J. Dairy Res.* 62:15–27.
- 429 Haque, M. N., C. Cornou, and J. Madsen. 2014. Estimation of methane emission using the  
430 CO<sub>2</sub> method from dairy cows fed concentrate with different carbohydrate  
431 compositions in automatic milking system. *Livest. Sci.*, 164: 57-66
- 432 Hart, K. J., P. G. Martin, P. A. Foley, D. A. Kenny, and T. M. Boland. 2009. Effect of sward  
433 dry matter digestibility on methane production, ruminal fermentation, and microbial  
434 populations of zero-grazed beef cattle. *J. Anim. Sci.*, 87: 3342-3350.
- 435 Hassanat, F., R. Gervais, C. Julien, D. I. Massé, A. Lettat, P. Y. Chouinard, H. V. Petit, and  
436 C. Benchaar. 2013. Replacing alfalfa silage with corn silage in dairy cow diets:  
437 Effects on enteric methane production, ruminal fermentation, digestion, N balance,  
438 and milk production. *J. Dairy Sci.*, 96: 4553-4567.
- 439 Heins, B. J., L. B. Hansen, A. J. Seykora, A. R. Hazel, D. G. Johnson and J. G. Linn. 2008.  
440 Crossbreds of Jersey x Holstein compared with pure Holsteins for body weight, body  
441 condition score, dry matter intake, and feed efficiency during the first one hundred  
442 fifty days of first lactation. *J. Dairy Sci.*, 91: 3716-3722.
- 443 Home Office, 1986. Guidance on the operation of the animals (scientific procedures) act  
444 1986. Her Majesty's Stationery Off., London, UK.
- 445 Huhtanen, P., J. I. Nousiainen, M. Rinne, K. Kytölä, and H. Khalili. 2008. Utilization and  
446 partition of dietary nitrogen in dairy cows fed grass silage-based diets. *J. Dairy Sci.*,  
447 91: 3589-3599.
- 448 Hynes, D. N., S. Stergiadis, A. Gordon and T. Yan. 2016. Effects of crude protein levels in  
449 concentrate supplements on animal performance and nitrogen utilization of lactating  
450 dairy cows fed fresh-cut perennial grass. *J. Dairy Sci.*, (accepted).
- 451 IPCC 2006. 2006 IPCC guidelines for national greenhouse gas inventories, Vol. 4:  
452 Agriculture, Forestry and Other Land Use, Intergovernmental Panel on Climate  
453 Change. Accessed Apr. 03, 2016. [http://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/](http://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_10_Ch10_Livestock.pdf)  
454 [4\\_Volume4/V4\\_10\\_Ch10\\_Livestock.pdf](http://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_10_Ch10_Livestock.pdf)
- 455 IPCC, 2007. Climate change 2007: IPCC Fourth Assessment Report. Cambridge University  
456 Press, Uk.
- 457 Jiao, H. R., A. J. Dale, A. F. Carson, S. Murray, A. W. Gordon, and C. P. Ferris. 2014. Effect  
458 of concentrate feed level on methane emissions from grazing dairy cows. *J. Dairy*  
459 *Sci.*, 97: 7043-7053.
- 460 Jiao, H. P., T. Yan, D. A. McDowell, A. F. Carson, C. P. Ferris, D. L. Easson and D. Wills.  
461 2013. Enteric methane emissions and efficiency of use of energy in Holstein heifers  
462 and steers at age of six months. *J. Anim. Sci.*, 91: 356-362.
- 463 Johnson, K. A. and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.*, 73:  
464 2483-2492.
- 465 Kebreab, E., K. Clark, C. Wagner-Riddle, and J. France. 2006. Methane and nitrous oxide  
466 emissions from Canadian animal agriculture: A review. *Can. J. Anim. Sci.*, 86: 135-  
467 158.
- 468 Külling, D. R., H. Menzi, T. F. Kröber, A. Neftel, F. Sutter, P. Lischer, and M. Kreuzer.  
469 2001. Emissions of ammonia, nitrous oxide and methane from different types of dairy  
470 manure during storage as affected by dietary protein content. *J. Agr. Sci.*, 137: 235-  
471 250.

472 Lassen J. and P. Løvendahl. 2016. Heritability estimates for enteric methane emissions from  
473 Holstein cattle measured using noninvasive methods. *J. Dairy Sci.*, 99: 1959-1967  
474 Moorby, J. M., R. J. Dewhurst, R. T. Evans, and J. L. Danelón. 2006. Effects of dairy cow  
475 diet forage proportion on duodenal nutrient supply and urinary purine derivative  
476 excretion. *J. Dairy Sci.*, 89: 3552-3562.  
477 Muñoz, C., S. Hube, J. M. Morales, T. Yan, and E. M. Ungerfeld. 2015. Effects of  
478 concentrate supplementation on enteric methane emissions and milk production of  
479 grazing dairy cows. *Livest. Sci.*, 175: 37-46.  
480 Mwenya, B., X. Zhou, B. Santoso, C. Sar, Y. Gamo, T. Kobayashi, and J. Takahashi. 2004.  
481 Effects of probiotic-vitacogen and beta 1-4 galacto-oligosaccharides supplementation  
482 on methanogenesis and energy and nitrogen utilization in dairy cows. *Asian*  
483 *Australas. J. Anim. Sci.*, 17: 349-354.  
484 Nousiainen, J., K. J. Shingfield, and P. Huhtanen. 2004. Evaluation of milk urea nitrogen as a  
485 diagnostic of protein feeding. *J. Dairy Sci.*, 87: 386-398.  
486 NRC (National Research Council). 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed.  
487 Natl. Acad. Press, Washington, DC.  
488 O'Neill, B. F., M. H. Deighton, B. M. O'Loughlin, N. Galvin, M. O'Donovan, and E. Lewis.  
489 2012. The effects of supplementing grazing dairy cows with partial mixed ration on  
490 enteric methane emissions and milk production during mid to late lactation. *J. Dairy*  
491 *Sci.*, 95: 6582-6590.  
492 Palladino, R. A., F. Buckley, R. Prendiville, J. J. Murphy, J. Callan, and D. A. Kenny. 2010.  
493 A comparison between Holstein-Friesian and Jersey dairy cows and their F<sub>1</sub> hybrid on  
494 milk fatty acid composition under grazing conditions. *J. Dairy Sci.*, 93: 2176-2184.  
495 Peyraud, J. L. and R. Delagarde. 2013. Managing variations in dairy cow nutrient supply  
496 under grazing. *Animal*, 7: 57-67.  
497 Pinares-Patiño, C. S., P. D'Hour, J. P. Jouany, and C. Martin. 2007. Effects of stocking rate  
498 on methane and carbon dioxide emissions from grazing cattle. *Agr. Ecosyst. Environ.*,  
499 121: 30-46.  
500 Pinares-Patiño, C. S., G. C. Waghorn, R. S. Hegarty, and S. O. Hoskin. 2009. Effects of  
501 intensification of pastoral farming on greenhouse gas emissions in New Zealand. *New*  
502 *Zeal. Vet. J.*, 57: 252-261.  
503 Ramin, M. and P. Huhtanen. 2013. Development of equations for predicting methane  
504 emissions from ruminants. *J. Dairy Sci.*, 96: 2476-2493.  
505 Salisbury, E. T., G. Young, K. Cardenas, L. Thomson, A. 2015. Report: Greenhouse Gas  
506 Inventories for England, Scotland, Wales and Northern Ireland: 1990-2013. Accessed  
507 Apr. 03, 2016. [http://naei.defra.gov.uk/reports/reports?report\\_id=810](http://naei.defra.gov.uk/reports/reports?report_id=810)  
508 Shalloo, L. 2009. Pushing the barriers of milk production costs/outputs. Pages 19-38 in *Proc.*  
509 *Natl. Dairy Conf.*, Mullingar and Kilarney, Ireland. Teagasc, Oak Park, Carlow,  
510 Ireland.  
511 Stergiadis, S., M. Allen, X. J. Chen, D. Wills, and T. Yan. 2015. Prediction of nutrient  
512 digestibility and energy concentrations in fresh grass using nutrient composition. *J.*  
513 *Dairy Sci.*, 98: 3257-3273.  
514 Stergiadis, S., C. X. Zou, X. J. Chen, M. Allen, D. Wills and T. Yan. 2016. Equations to  
515 predict methane emissions from cows fed at maintenance energy level in pasture-  
516 based systems. *Agr. Ecosyst. Environ.* 220: 8-20.  
517 Swalve, H. H., 2007. Crossbreeding in dairy cattle: international trends and results from  
518 crossbreeding data in Germany. *Lohmann Inf.* 42:38-46.  
519 Tyrrell, H. F. and J. T. Reid. 1965. Prediction of the Energy Value of Cow's Milk. *J. Dairy*  
520 *Sci.*, 48: 1215-1223.

521 Van Dorland, H. A., H. R. Wettstein, H. Leuenberger, and M. Kreuzer. 2007. Effect of  
522 supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane  
523 emission of dairy cows. *Livest. Sci.*, 111: 57-69.

524 van Vuuren, A. M., F. Krolkramer, R. A. Vanderlee and H. Corbijn. 1992. Protein digestion  
525 and intestinal amino-acids in dairy-cows fed fresh *Lolium-perenne* with different  
526 nitrogen contents. *J. Dairy Sci.*, 75: 2215-2225.

527 VSN International, 2013. GenStat for Windows 16th Edition. VSN International, Hemel 729  
528 Hempstead, UK.

529 Wall, E., G. Simm and D. Moran. 2010. Developing breeding schemes to assist mitigation of  
530 greenhouse gas emissions. *Animal* 4; 366-376.

531 Weiss, W. P., L. B. Willett, N. R. St-Pierre, D. C. Borger, T. R. McKelvey, and D. J. Wyatt.  
532 2009. Varying forage type, metabolizable protein concentration, and carbohydrate  
533 source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy  
534 cows. *J. Dairy Sci.*, 92: 5607-5619.

535 Whelan, S. J., K. M. Pierce, C. McCarney, B. Flynn, and F. J. Munigan. 2012. Effect of  
536 supplementary concentrate type on nitrogen partitioning in early lactation dairy cows  
537 offered perennial ryegrass-based pasture. *J. Dairy Sci.*, 95: 4468-4477.

538 Xue, B., T. Yan, C. F. Ferris, and C. S. Mayne. 2011. Milk production and energy efficiency  
539 of Holstein and Jersey-Holstein crossbred dairy cows offered diets containing grass  
540 silage. *J. Dairy Sci.*, 94: 1455-1464.

541 Yan, T., R. E. Agnew, F. J. Gordon, and M. G. Porter. 2000. Prediction of methane energy  
542 output in dairy and beef cattle offered grass silage-based diets. *Livest. Prod. Sci.*  
543 64:253–263.

544 Yan, T. and Mayne, C. S. 2007. Mitigation strategies to reduce methane emission from dairy  
545 cows. Pages 345-348 in *Proc. Br. Soc. Anim. Sci.*, High Value Grassland: Providing  
546 Biodiversity, a Clean Environment and Premium Products. University of Keele,  
547 Staffordshire, UK.

548 Yan, T. and Mayne, C. S. 2009. Effect of level of concentrate supplementation on methane  
549 emission of Holstein and Holstein-Jersey dairy cows. Page 26 in *Proc. Br. Soc. Anim.*  
550 *Sci.*, British Society of Animal Science, Southend, England.

551 Zhou, M., E. Hernandez-Sanabria and L. L. Guan. 2009. Assessment of the microbial  
552 ecology of ruminal methanogens in cattle with different feed efficiencies. *Appl.*  
553 *Environ. Microb.* 45: 6524–6533.

554 Zhou, M., E. Hernandez-Sanabria and L. L. Guan. 2010. Characterization of variation in  
555 rumen methanogenic communities under different dietary and host feed efficiency  
556 conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis.  
557 *Appl. Environ. Microb.* 76: 3776–3786.  
558

## TABLES

Table 1. Equations used for the calculations of heat production, ME requirement for maintenance and efficiency of ME for lactation<sup>1,2</sup>

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 <sub>m</sub> (when E <sub>g</sub> < 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 <sub>m</sub> (when E <sub>g</sub> > 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 <sub>l(0)</sub> (when E <sub>g</sub> < 0)	$= E_l + 0.84 \times E_g$	(AFRC, 1993)
E <sub>l(0)</sub> (when E <sub>g</sub> > 0)	$= E_l + 1/k_g \times E_g$	
k <sub>l</sub>	$= E_{l(0)} / (ME_{int} - ME_m)$	(AFRC, 1993)

<sup>1.</sup> CH<sub>4</sub> = methane produced (L/d), CO<sub>2</sub> = carbon dioxide produced (L/d), O<sub>2</sub> = oxygen consumed (L/d), UN = urinary nitrogen excreted (g/d).

<sup>2.</sup> E<sub>g</sub> = net energy for BW change (MJ/d), E<sub>l</sub> = milk energy output (MJ/d), E<sub>l(0)</sub> = milk energy output adjusted to zero energy balance (MJ/d), E<sub>p</sub> = net energy requirement for pregnancy, HP = heat production (MJ/d), ME<sub>int</sub> = ME intake (MJ/d), ME<sub>m</sub> = ME requirement for maintenance (MJ/d), k<sub>g</sub> = efficiency of ME use for weight gain, k<sub>l</sub> = efficiency of ME use for lactation, k<sub>l(AFRC)</sub> = efficiency of ME use for lactation calculated from AFRC (1993), k<sub>p</sub> = efficiency of ME use for pregnancy, k<sub>t</sub> = 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			SEM	P-value <sup>1</sup>		Cow genotype		SEM	P-value
	Low	Medium	High		lin	quad	Holstein	Crossbred <sup>2</sup>		
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.

<sup>1</sup>Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

<sup>2</sup> 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 <sup>1</sup>		Cow genotype			
	Low	Medium	High	SEM	lin	quad	Holstein	Crossbred <sup>2</sup>	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 <sub>4</sub> 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 <sub>l</sub>	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<sub>l</sub> = efficiency of ME use for lactation

<sup>1</sup>Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

<sup>2</sup> 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 <sup>1</sup>		Cow genotype		SEM	P-value
	Low	Medium	High	SEM	lin	quad	Holstein	Crossbred <sup>2</sup>		
CH <sub>4</sub> (g/d)	381.6	391.3	377.3	20.8	0.724	0.467	405	362	20.9	0.164
CH <sub>4</sub> / feed intake or milk yield (g/kg)										
CH <sub>4</sub> / DMI	18.36	18.43	18.19	1.03	0.904	0.782	18.7	17.9	1.07	0.593
CH <sub>4</sub> / OMI	20.15	20.25	20.00	1.13	0.923	0.779	20.6	19.7	1.17	0.598
CH <sub>4</sub> / Digestible DMI	24.20	24.04	23.90	1.41	0.809	0.972	24.4	23.7	1.49	0.737
CH <sub>4</sub> / Digestible OMI	25.86	25.74	25.60	1.51	0.831	0.863	26.1	25.3	1.60	0.724
CH <sub>4</sub> / Milk yield	14.35	14.85	14.30	1.01	0.372	0.366	13.8	15.2	0.95	0.225
CH <sub>4</sub> / ECMY	13.49	14.15	13.33	0.90	0.480	0.240	13.3	14.0	0.90	0.575
CH <sub>4</sub> -E / energy intake (MJ/MJ)										
CH <sub>4</sub> -E / GEI	0.056	0.056	0.056	0.0031	0.972	0.755	0.057	0.055	0.0032	0.605
CH <sub>4</sub> -E / DEI	0.077	0.076	0.076	0.0045	0.712	0.923	0.077	0.075	0.0048	0.724
CH <sub>4</sub> -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<sub>4</sub>-E = methane energy output, GEI = gross energy intake, DEI = digestible energy intake, MEI = ME intake.

<sup>1</sup>Probability of a linear (lin) or quadratic (quad) effect of concentrate CP level in the diet.

<sup>2</sup> Crossbred cows were crosses between Holstein and Swedish Red.