

*Effects of diet forage source and neutral detergent fiber content on milk production of dairy cattle and methane emissions determined using GreenFeed and respiration chamber techniques*

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Accepted Version

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Hammond, K. J., Jones, A. K., Humphries, D. J., Crompton, L. A. and Reynolds, C. K. (2016) Effects of diet forage source and neutral detergent fiber content on milk production of dairy cattle and methane emissions determined using GreenFeed and respiration chamber techniques. *Journal of Dairy Science*, 99 (10). pp. 7904-7917. ISSN 0022-0302 doi: <https://doi.org/10.3168/jds.2015-10759> Available at <https://centaur.reading.ac.uk/66153/>

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To link to this article DOI: <http://dx.doi.org/10.3168/jds.2015-10759>

Publisher: Elsevier

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1 **Interpretive Summary**

2 **Effects of diet forage source and neutral-detergent fiber content on milk production of**

3 **dairy cattle and methane emissions determined using GreenFeed and respiration**

4 **chamber techniques**

5

6 Hammond

7 Replacing grass silage (GS) with maize silage (MS) in dairy cow diets decreased methane per  
8 unit of feed consumed (yield), in part due to higher feed and starch intakes, which also  
9 increased milk yield and protein concentration. Additional neutral-detergent fiber increased  
10 methane yield for higher MS diets, but not higher GS diets. This was attributable to the higher  
11 starch concentration of the higher MS diet, and was associated with increased milk fat  
12 concentration, emphasising the importance of dietary carbohydrate source and type. GreenFeed  
13 and respiration chamber methods were able to detect similar dietary treatment effects on  
14 methane emission from dairy cattle.

15 MILK PRODUCTION, METHANE AND MEASUREMENT TECHNIQUE

16

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21 **Effects of diet forage source and neutral-detergent fiber content on milk production of**  
22 **dairy cattle and methane emissions determined using GreenFeed and respiration**  
23 **chamber techniques**

24

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26

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34 **ABSTRACT**

35 Strategies to mitigate greenhouse gas emissions from dairy cattle are unlikely to be adopted if  
36 production or profitability is reduced. The primary objective of this study was to examine the  
37 effects of high maize silage (MS) vs. high grass silage (GS) diets, without or with added  
38 neutral-detergent fiber (NDF) on milk production and methane emission of dairy cattle, using  
39 GreenFeed (GF) or respiration chamber (RC) techniques for methane emission measurements.  
40 Experiment 1 was 12-wks in duration with a randomized block continuous design and 40  
41 Holstein cows (74 d in milk; DIM) in free-stall housing, assigned to 1 of 4 dietary treatments  
42 (n = 10 per treatment), according to calving date, parity and milk yield. Milk production and  
43 dry matter intake (DMI) were measured daily, and milk composition measured weekly, with  
44 methane yield (g/kg DMI) estimated using a GF unit (wks 10 to 12). Experiment 2 was a 4 × 4  
45 Latin Square Design with 5-wk periods and 4 dairy cows (114 DIM) fed the same 4 dietary  
46 treatments as in experiment 1. Measurements of DMI, milk production and composition  
47 occurred in wk 4, and DMI, milk production and methane yield were measured for 2 d in RC  
48 during wk 5. Dietary treatments for both experiments were fed as TMRs offered ad libitum and  
49 containing 500 g silage/kg DM comprised of either 75:25 MS:GS (MS) or 25:75 MS:GS (GS),  
50 without or with added NDF from chopped straw and soy hulls (+47 g NDF/kg DM; MSNDF  
51 and GSNDF). In both experiments, compared to high GS, cows fed high MS had a higher ( $P =$   
52 0.01) DMI, greater ( $P = 0.01$ ) milk production, and lower ( $P = 0.02$ ) methane yield (24% lower  
53 in experiment 1 using GF and 8% lower in experiment 2 using RC). Added NDF increased (or  
54 tended to increase) methane yield for high MS, but not high GS diets ( $P = 0.02$  for experiment  
55 1 and  $P = 0.10$  for experiment 2, forage type × NDF interaction). In the separate experiments  
56 the GF and RC methods detected similar dietary treatment effects on methane emission  
57 (expressed as g/d and g/kg DMI), although the magnitude of the difference varied between  
58 experiments for dietary treatments Overall methane emission and yield were 448 g/d and 20.9

59 g/kg DMI using GF for experiment 1 using GF and 458 g/d and 23.8 g/kg DMI for experiment  
60 2 using RC, respectively.

61

62 **Keywords:** forage, fiber, milk production, methane emission

63

64

## INTRODUCTION

65 The current United Kingdom (UK) National Greenhouse Gas (GHG) Inventory largely  
66 estimates emissions from agriculture using the most simplified approach (Tier 1) to accounting  
67 (IPCC, 2007). This approach uses generic assumptions and factors about livestock  
68 management to estimate GHG emissions, and there is a lack of methane emission factors from  
69 livestock in different farming systems fed a variety of diets. Analyses of calorimetry data (Mills  
70 et al., 2001) have shown that enteric methane emission is affected by dietary concentrations of  
71 starch relative to fiber. Previous comparisons have found replacing grass silage (GS) with  
72 maize silage (MS) increases milk production from dairy cows, mostly through increased feed  
73 intake for MS compared to GS (Kliem et al., 2008, O'Mara et al., 1998, Phipps et al., 1988,  
74 1992 and 1995). Enteric methane emission was also found to be variably lower with MS  
75 compared to GS diets (Reynolds et al., 2010; Hammond et al., 2015b), although this is not  
76 always consistent (Livingstone et al., 2015; Hammond et al., 2015b). An explanation for  
77 differences (and also lack of difference) in ruminant methane emission with high MS vs. high  
78 GS diets may be the physical and chemical attributes of these silages, along with digestive  
79 processes associated with the quantity of feed eaten. In the study of Reynolds et al. (2010),  
80 high MS and high GS diets were formulated to be similar in starch and neutral-detergent fiber  
81 (NDF) concentrations by manipulation of the concentration proportion of the diet. It was  
82 concluded that observed differences in high MS vs. high GS diets on methane emission was  
83 attributed to differences in the rate and extent of degradation of carbohydrate components.

84 Intakes of fibrous diets (i.e., GS or diets with high NDF concentration) are not expected to be  
85 as high as diets comprising higher proportions of readily fermentable carbohydrates (i.e., MS  
86 or diets with high starch concentration) because of increased rumen fill and extended time  
87 required to chew and reduce the particle size of fiber to enable passage from the rumen.  
88 Considering that MS and GS diets are applicable to rations based on typical UK forages, further  
89 work is warranted to examine the effects of forage type and composition on milk production  
90 and methane emission from ruminant livestock.

91

92 Dietary manipulation can be effective for mitigation of methane emission from dairy cattle,  
93 and alternative methods to respiration chambers (**RC**) are being introduced as a less intrusive  
94 way to measure enteric methane emission. Particularly lacking is the capability to accurately  
95 measure individual methane emission from multiple animals in a production environment over  
96 a long period of time without interference to daily routine. The GreenFeed (**GF**) system (C-  
97 Lock Inc., Rapid City, USA) is a portable sampling unit that is used to estimate individual daily  
98 methane emission by integrating measurements of airflow, gas concentration, and detection of  
99 head position during each animals visit to the unit. The animal is free to move and voluntarily  
100 enters a hood where an enticement, usually in the form of a feed supplement, is delivered, and  
101 while eating a sample of the animal's breath is analyzed for methane emission. Depending on  
102 GreenFeed set up animals can be free to visit GreenFeed at any time of the day or access can  
103 be dictated by the investigators. Measurements of methane emission by GF are typically over  
104 short periods (3 to 7 min) at several variable times within a day, over a number of days, so that  
105 ultimately a 24 h individual methane emission profile is estimated based on extrapolation from  
106 repeated short-term measurements. An in-depth description of the GF system for measurement  
107 of enteric methane can be obtained from Zimmerman and Zimmerman (2012), Hristov et al.  
108 (2015), Huhtanen et al. (2015) and Hammond et al. (2016).

109 With increasing use of GF, more studies have compared GF estimates with methane  
110 measurements using other techniques, however comparisons with RC are difficult as  
111 measurements are not simultaneous. In a summary of GreenFeed publications by Hammond et  
112 al. (2016), under a variety of conditions, GF, RC and sulphur hexafluoride (**SF<sub>6</sub>**) techniques  
113 are shown to give a similar estimate of daily enteric methane emission for cattle on most  
114 occasions. However, it was concluded that suitability of the GF system will be affected by the  
115 experimental objectives and design. An example is Hammond et al. (2015a) who used dairy  
116 cattle to compare RC, GF and SF<sub>6</sub> measurement techniques. Although techniques were  
117 comparable for measurement of methane emission, it was concluded that further work was  
118 needed to determine how to best deploy the GF system to detect significant changes in methane  
119 emission attributable to individual animals and treatments, and that future studies should  
120 include a greater number of animals per treatment than is required for RC studies.

121

122 The primary objective of the present study was to examine the effect of feeding forages  
123 differing in MS and GS proportions to lactating dairy cattle, with or without supplemental  
124 NDF, on feed intake, milk production and composition, and methane emission. Methane  
125 emission was measured using RC in experiment 2 and GF in experiment 1, as an alternative  
126 method to RC for measuring dietary effects on methane emission. It was hypothesized that feed  
127 intake and milk production would be greater, and methane yield (g/kg DMI) lower for cows  
128 fed higher MS diets and diets without additional NDF, compared to higher GS diets and diets  
129 with higher NDF concentration.

130

131

## **MATERIALS AND METHODS**

132

### ***Experimental Design***



133 Two experiments using the same dietary treatments were undertaken simultaneously at the  
134 University of Reading's Centre for Dairy Research (**CEDAR**, Arborfield, UK). All procedures  
135 were approved and monitored under the UK Home Office Animals (Scientific Procedures) Act  
136 1986. Experiment 1 was a 12-wk randomized block continuous design experiment. Forty  
137 lactating Holstein dairy cows were blocked into 4 treatment groups (10 cows each) based on  
138 calving date, parity and milk yield determined in the 3 wks prior to the experiment commencing  
139 (wks -3 to -1, covariate period) when cows were fed a common commercial TMR. For the  
140 entire experiment, cows were loose-housed in a yard with sand-bedded cubicles, weighed twice  
141 weekly, and fed using an electronic Calan Broadbent individual feeding system allowing  
142 measurement of individual cow feed intake (American Calan, Northwood, New Hampshire,  
143 USA). During a 3 wk training period prior to the covariate period (wks -6 to -4) and from wks  
144 9 to 12, cows had variable and voluntary access to a GF unit, however, GF measurements of  
145 methane were only considered for analysis between wks 10 to 12. Measurements from cows of  
146 experiment 1 included diet composition, feed intake, BW and milk yield and composition  
147 during wks 1 to 8 (production period), and diet composition, feed intake, milk yield and  
148 methane emission during wks 10 to 12 (methane measurement period).

149

150 Experiment 2 used 4 lactating Holstein cows surgically fitted with rumen cannulae (type #1 C,  
151 100 mm centre diameter, Bar Diamond Inc., Parma, Idaho, USA) in a previous lactation.  
152 Experiment 2 was a  $4 \times 4$  Latin square design balanced for carry-over effects with 5-wk  
153 treatment periods. From wks 1 to 3 animals were group-housed with access to cubicles bedded  
154 with rubber mats and wood shavings, fed TMR diets ad libitum, and milked twice daily. During  
155 this time, animals were adapted to dietary treatments with feed intake measured using a  
156 roughage intake control feeding system (Insentec B.V., Marknesse, The Netherlands). During  
157 wk 4, animals were moved to individual tie stalls and in wk 5 animals were staggered in pairs

158 to 2 individual RC for 2 consecutive days of methane measurements. Measurements included  
159 diet composition, feed intake and milk yield and composition during wk 4, and DMI, milk yield  
160 and methane emission whilst in RC during wk 5. Cows were weighed weekly and before and  
161 after measurements in RC.

162

### 163 *Animals and Dietary Treatments*

164 In experiment 1, cows averaged ( $\pm$  SEM)  $74 \pm 16.2$  DIM at the start of the experiment and a  
165 BW of  $670 \pm 4.0$  kg throughout the experiment. In experiment 2, cows averaged ( $\pm$  SEM)  $114$   
166  $\pm 3.3$  DIM at the start of the experiment and  $678 \pm 10.5$  kg BW throughout the experiment.

167

168 Cows in both experiments were fed for ad libitum DMI (5% refusals). In experiment 1, cows  
169 were fed once daily between 07:00 and 09:00 h, and milked twice daily between 06:00 and  
170 07:00 h, and 15:00 and 16:00 h. Feed refusals were collected thrice weekly (Monday,  
171 Wednesday and Friday) for estimates of individual daily DMI. In experiment 2, diets were fed  
172 twice daily at 10:00 and 16:00 h from wks 1 to 3, and thereafter (wks 4 to 5) were fed 4 times  
173 daily at 05:00, 11:00, 17:00 and 22:00 h. Feed refusals were collected once daily at 08:00 h,  
174 and cows were milked twice daily between 06:00 and 07:00 h, and 15:00 and 16:00 h.

175

176 Dietary treatments fed in both experiments were either a high MS (375 g/kg DM) and low GS  
177 (125 g/kg) TMR (MS), or the reverse proportions (GS), without or with additional chopped  
178 barley straw and soy hulls incorporated to increase concentration of NDF (+47 g/kg DM;  
179 MSNDF and GSNDF). For experiment 1 the TMR was prepared with a Mix Max 10 Paddle  
180 Feeder (Hi Spec Engineering Ltd, Bagenalstown, Republic of Ireland). For experiment 2 each  
181 TMR was prepared with a Dataranger (American Calan, Northwood, New Hampshire, USA).  
182 For both experiments ingredients were added in the order straw, concentrate mix, calf pellets,

183 limestone, grass silage, maize silage. Dietary treatments were formulated to be isonitrogenous  
184 and meet or exceed the recommendation for MP, minerals and vitamins based on Feed into  
185 Milk (Thomas 2007) recommendations (Table 1). The GF used for estimating methane  
186 emission from individual animals in experiment 1 required calf pellets as a form of enticement  
187 to encourage animals to enter the sampling hood. Therefore, dietary treatments were  
188 formulated for both experiments to include a commercial calf pellet (chemical composition  
189 [g/kg DM] of ash, 85.1; oil, 46.5; ADF, 174; NDF, 289; starch, 259; water soluble carbohydrate  
190 [WSCHO], 91.3; nitrogen [N], 27.3; CP, 171; and gross energy [GE; MJ/kg], 18.1) that was  
191 incorporated into the TMR to form 8.7% of the formulated TMR (DM basis). When the GF  
192 was used in experiment 1 (wks 9 to 12), pellets were excluded from the TMR and fed in the  
193 GF. Pellets were included in the TMR throughout experiment 2.

194

195 The MS was based on a mixture of maize varieties which were combined at harvest (22 October  
196 2012) and stored in clamps. Grass silage was made from a third cut (10 August 2012) *Lolium*  
197 *perenne* mixture of tetra and diploid ryegrass species. The ryegrass was wilted for 24 h and  
198 ensiled with an additive (GENUS ULV, Genus Breeding Ltd, Nantwich, UK; 40 ml per tonne).  
199 Both forage silages remained sealed in clamps for a minimum period of 6 wks before use.

200

### 201 ***Methane Emission***

202 For experiment 1, a single GF unit was used to estimate individual cow methane emission  
203 during wks 10 to 12. Details of the GF operation and use are given by Zimmerman and  
204 Zimmerman (2012), Hammond et al. (2015a), Hristov et al. (2015) and Huhtanen et al. (2015).  
205 Briefly, GF operation was initiated when an animal placed its head inside the GF hood. A radio  
206 frequency identification (RFID) reader identified the animal's ear tag and GF sampling was  
207 activated when the animals head (located by an infrared sensor) was located close to the

208 sampling inlet within the hood (muzzle within 30 cm of the sampling inlet as detailed by  
209 Huhtanen et al., 2015), and it was deemed that sufficient time had elapsed since the previous  
210 methane measurement for that animal. Position of the animals head within the hood was  
211 monitored using sensors to ensure complete breath collection. The GF unit was set up outside  
212 the end of a cubicle yard within a polytunnel that minimized the effect of wind on  
213 measurements. Gates were positioned to allow access to the GF by only 1 animal at a time.

214

215 The concentration of gas emitted by the animal was calculated using background gas  
216 concentration, the differential concentration of gas during the animal's time in the GF hood,  
217 and the calibration coefficient for concentration. See Huhtanen et al. (2015) for detailed  
218 calculations of GF-estimated methane emission which were used here. The calibration  
219 coefficient was based on nitrogen (N), carbon dioxide and methane gases used to calculate the  
220 response of the sensors. The GF analyzers were calibrated weekly using a zero baseline gas  
221 (oxygen-free N) and a span gas mixture of N containing 5000 ppm carbon dioxide and 1000  
222 ppm methane (BOC Ltd., Manchester, UK). This was to account for any drift in the calibration  
223 of the analysers, which was found to be negligible. A gravimetrically measured amount of carbon  
224 dioxide gas was released where the animals nose would be when feeding to check recovery of  
225 expired gases at the beginning and end of the measurement period. There was no recovery  
226 correction required in the current study. Data from GF was downloaded on a daily basis through  
227 a web-based data management system provided by C-Lock Inc.

228

229 Animals were adapted to GF use during the covariate period (wks -3 to -1) and again in wk 9,  
230 with methane measurements used for statistical analysis obtained during wks 10 to 12. During  
231 these periods, animals were able to access the GF unit at any time, except during milking and  
232 provided it was not in use by another animal. However, this did not necessarily generate a

233 measurement of methane. A 'visit' was defined as a successful methane measurement  
234 facilitated by feed delivery which could only occur when a specified time ( $> 240$  min) had  
235 elapsed since the previous visit. In this case, the enticement was provided and a 'visit' logged  
236 if the animal remained correctly positioned in the unit for a sufficient amount of time ( $> 3$  min)  
237 for a valid methane measurement. The unit was programmed to deliver feed in 50 g quantities  
238 at varying intervals over a 6 min period, so that up to 350 g pellet fresh weight was delivered  
239 during each complete visit, with up to a maximum of 6 visits per day (2 kg DM).

240

241 For experiment 2, measurement of gaseous exchange was obtained over 2 consecutive days in  
242 wk 5 using open-circuit respiration chambers and methods similar to those described by  
243 Cammell *et al.* (1981), but with the following exceptions. The chambers ( $22.3 \text{ m}^3$  capacity)  
244 were constructed from double-skin insulated steel panels and fitted with a profiled concrete  
245 floor with rubber mats, tubular steel sides to the standing, and neck yoke and food box  
246 arrangements similar to those in the main experimental unit. Glazed panels were fitted  
247 internally and externally to the chambers, so the animals had visual contact both between  
248 chambers and their local surroundings. An airlock of approximately  $5.2 \text{ m}^3$  was provided for  
249 service access to the faeces and urine balance equipment and for routine milking and animal  
250 inspection and was connected to the main chamber *via* double doors. Each chamber was fitted  
251 with a re-circulatory air conditioning system (Mueller; Caswell Refrigeration Ltd,  
252 Malmesbury, Wiltshire, SN16 9RH) to provide air movement of up to 20 times the chamber  
253 volume per h, environmental control across a temperature range of  $12\text{-}25^\circ\text{C} \pm 2^\circ\text{C}$  and a  
254 relative humidity of  $60 \pm 10 \%$ . The present experiment was conducted using six air changes  
255 per h with environmental controls adjusted to give no more than  $\pm 3^\circ\text{C}$  difference from the  
256 cowshed environment. The rate of air flow through the outlet ducting from the chambers was  
257 measured using factory calibrated turbine flow-meters (AOT Systems, Andover, Hampshire

258 SP10 5BY). Monitoring of temperature and relative humidity in the exhaust air flows was by  
259 sensors type RHA1 (Delta-T Devices Ltd, Burwell, Cambridge CB5 0EJ). The concentrations  
260 of oxygen in exhaust air flow was measured by a dual-channel paramagnetic oxygen analyzer  
261 (Servomex International Ltd, Crowborough, Sussex TN6 3DU) and carbon dioxide and  
262 methane concentrations were measured by dedicated dual-channel infra-red gas analyzers  
263 (ADC Manufacturing Ltd, Stanstead Abbots, Hertfordshire SG12 8HG). The gas analysis train  
264 was designed to allow automatic measurement at 4 min intervals from each chamber, giving  
265 15 values per chamber per h, with automatic zero and span calibration readings at 4 h intervals.  
266 Signal outputs from monitoring and gas analysis equipment were recorded by a data logger  
267 (type DL2e, Delta-T Devices Ltd). The data were automatically downloaded and compiled  
268 during each 24 h period using a desk top computer and associated software programs (7th Wave  
269 Software Ltd, Pangbourne, Berkshire RG8 7NB) based on specifically designed data logging  
270 programs. Heat production was estimated from gaseous exchange and urinary N output using  
271 the equation of Brouwer (1965).

272

### 273 *Sample Collection and Analyses*

274 For experiment 1, from wks 1 to 12 samples of TMR offered were taken 3 times per wk and  
275 frozen before a representative monthly bulk sample was created. This was oven-dried (Model  
276 ME/850/DIG/A, Genlab Ltd., Widnes, UK) at 65°C for 48 h (#930.15, AOAC, 2005), ground  
277 and stored for analyses of chemical composition. An additional sample of the bulked TMR was  
278 also oven-dried at 100°C for DM determination (#930.15, AOAC, 2005). Total mixed ration  
279 refusals and their corresponding DM (oven-dried at 100°C for 24 h) were measured thrice  
280 weekly and DMI calculated on a weekly basis. Milk production was determined daily  
281 throughout the experiment and milk samples (30 ml) were taken from 2 successive a.m. and  
282 p.m. milking's at weekly intervals and preserved with potassium dichromate (1 mg/ml;

283 Lactabs, Thomson and Capper, Runcorn, UK) for the determination of milk composition during  
284 wks 1 to 8.

285

286 For experiment 2, the TMR offered and refused was collected daily from individual cows  
287 during wks 4 and 5 for DMI determination by oven-drying at 100°C for 24 h. Additional daily  
288 samples were taken during wk 4 and pooled for individual cows to make a composite sample  
289 which was stored at -20°C for analyses of chemical composition. Milk production was  
290 determined daily throughout the experiment. Milk samples (30 ml) were taken at every milking  
291 in wk 4 and preserved with potassium dichromate for the determination of milk composition.

292

293 Samples of the TMR offered and refused for both experiments were analyzed by wet chemistry  
294 as detailed by Hammond et al. (2014). Samples were analyzed for N (macro Kjeldahl method),  
295 GE (combustion using an adiabatic bomb calorimeter), NDF and ADF (procedures of  
296 Robertson and Van Soest, 1981, and Mertens 2002), starch (enzymatic conversion to glucose  
297 and glucose measured using amyloglucosidase), oil (acid extraction) and ash (combustion)  
298 concentrations. Milk samples were analyzed using mid-infrared spectroscopy (Foss Electric  
299 Ltd, York, UK) to determine fat, protein, casein, lactose and MUN concentrations, and 4%  
300 FCM and energy-corrected milk (ECM) yield was calculated as detailed by Gaines (1928) and  
301 Gaillard et al. (2016), respectively.

302

### 303 *Statistical Analyses*

304 For experiment 1, weekly means of variables measured for each cow were statistically analyzed  
305 from wks 1 to 8 (production period) and wks 10 to 12 (methane measurement period)  
306 separately. The methane emission data statistically analyzed were the daily averages for  
307 individual animals for the 3-wk measurement period. Data were analyzed using the MIXED

308 Procedure of SAS Version 9.2 (2011) and a model testing for fixed effects of forage type (1  
309 df), added NDF treatment (1 df) and their interaction (1 df), their two-way and three-way  
310 interactions, random effects of cow, and repeated effects of wk within cow using the covariance  
311 structure (compound symmetry, heterogeneous compound symmetry, autoregressive,  
312 heterogeneous autoregressive or unstructured) giving the best fit based on the lowest BIC value  
313 for each variable of interest. In addition, averages of weekly measurements during the 3 wk  
314 covariate period were used as a covariate in the statistical analysis.

315

316 For experiment 2, means of variables measured for each cow and period were used in the  
317 statistical analysis. Data were analyzed using the MIXED Procedure of SAS (as for experiment  
318 1) and a model tested the fixed effects of forage type (1 df), added NDF treatment (1 df) and  
319 their interaction (1 df), and random effects of cow (3 df) and the repeated effect of period (3  
320 df) using the covariance structure (compound symmetry, heterogeneous compound symmetry,  
321 autoregressive, heterogeneous autoregressive or unstructured) giving the best fit based on  
322 lowest BIC value for each variable of interest. Methane emission data from both experiments  
323 were analysed for homogenous distribution and outliers using the Univariate Procedure of SAS  
324 and residual analysis using the Mixed Procedure. Least square means are reported.

325

326

## RESULTS

### 327 *Diet Composition*

328 Differences in diet composition observed for bulk samples taken during the production and  
329 methane measurement periods of experiment 1 (Table 1) were similar to differences observed  
330 for experiment 2 (Table 2). For experiment 2, high MS diets had greater DM ( $P < 0.001$ ) and  
331 OM ( $P = 0.002$ ) contents, a greater concentration of starch ( $P < 0.001$ ), and lower  
332 concentrations of CP ( $P = 0.077$ ), NDF ( $P = 0.025$ ), ADF ( $P = 0.005$ ) and oil ( $P = 0.002$ ),



333 compared to high GS diets (Table 2). There was no forage type effect on WSCHO. Added NDF  
334 increased concentrations of NDF ( $P = 0.006$ ) and ADF ( $P = 0.002$ ), and decreased starch ( $P <$   
335  $0.001$ ). There were forage type  $\times$  NDF treatment interactions for concentrations of OM ( $P =$   
336  $0.074$ ), CP ( $P = 0.096$ ), starch ( $P = 0.094$ ) and oil ( $P = 0.036$ ). The starch:NDF ratio was higher  
337 for high MS diets ( $P = 0.004$ ), and decreased with added NDF ( $P = 0.002$ ).

338

### 339 *Animal Performance*

340 Intakes of individual dietary components are given in Table 3 for both experiments 1 and 2.  
341 Cows fed high MS diets in experiment 1 during wks 1 to 8 had greater intakes of DM ( $P <$   
342  $0.001$ ), OM ( $P = 0.001$ ), CP ( $P = 0.001$ ), NDF ( $P = 0.006$ ) and starch ( $P < 0.001$ ), compared  
343 to high GS, with no effect on intakes of ADF and oil. Added NDF treatment increased intakes  
344 of NDF ( $P = 0.021$ ) and ADF ( $P = 0.005$ ), and decreased intakes of starch ( $P < 0.001$ ) and oil  
345 ( $P = 0.079$ ), with no effects on intakes of DM, OM or CP. During wks 10 to 12 in experiment  
346 1, cows fed high MS diets had higher ( $P < 0.01$ ) intakes of all individual dietary components,  
347 compared to cows fed high GS diets. Adding NDF decreased intakes of OM ( $P = 0.030$ ) and  
348 starch ( $P < 0.001$ ), and increased intakes of NDF ( $P = 0.002$ ) and ADF ( $P = 0.002$ ). There was  
349 a forage type  $\times$  NDF treatment interaction for intakes of CP ( $P = 0.0081$ ), starch ( $P = 0.007$ )  
350 and oil ( $P = 0.026$ ).

351

352 Cows fed high MS diets in experiment 2 had greater intakes of DM ( $P = 0.011$ ), OM ( $P =$   
353  $0.024$ ), and starch ( $P = 0.001$ ), compared to high GS diets. Forage type had no effect on intakes  
354 of CP, NDF, ADF or oil. Added NDF increased ADF intake ( $P = 0.089$ ), and decreased intake  
355 of starch ( $P = 0.002$ ). There was a forage type  $\times$  NDF treatment interaction for intake of CP ( $P$   
356  $= 0.033$ ).

357

358 Production data (including BW, milk yield and composition) were collected from cows of  
359 experiment 1 during wks 1 to 8 (Table 4). During this period, cows fed high MS diets had a  
360 greater BW ( $P = 0.002$ ; which was due to a greater average daily live weight gain [data not  
361 shown]) and milk yield ( $P = 0.001$ ) than cows fed high GS, with no effect of NDF treatment.  
362 There were no forage type or NDF treatment effects on FCM, but cows fed high MS diets  
363 produced more ECM ( $P = 0.031$ ). Yields (g/d) of milk protein ( $P = 0.001$ ), lactose ( $P = 0.001$ )  
364 and casein ( $P = 0.001$ ) were greater for cows fed high MS than high GS diets, and added NDF  
365 decreased milk protein ( $P = 0.031$ ) and casein ( $P = 0.049$ ) yields. Forage type affected  
366 concentrations (g/kg) of milk fat (MS lower than GS;  $P = 0.018$ ), lactose (MS higher than GS;  
367  $P = 0.011$ ) and casein (MS higher than GS;  $P = 0.053$ ). Milk urea concentration was lower ( $P$   
368  $< 0.001$ ) for high MS compared to high GS diets. Added NDF increased milk fat concentration  
369 ( $P = 0.041$ ), with a significant forage type  $\times$  NDF treatment interaction ( $P = 0.049$ ) due to a  
370 greater increase for high MS than for high GS diets. Added NDF decreased milk protein ( $P =$   
371  $0.021$ ) and casein ( $P = 0.066$ ) concentrations, whilst milk urea concentration increased ( $P =$   
372  $0.001$ ).

373

374 In experiment 2, cows fed high MS had a greater BW ( $P = 0.015$ ) and milk yield ( $P = 0.076$ ),  
375 than cows fed high GS diets, with no effect of NDF treatment (Table 4). There was no effect  
376 of forage type or NDF treatments on FCM or ECM yields. Cows fed high MS had greater yields  
377 of milk protein ( $P = 0.043$ ), lactose ( $P = 0.060$ ) and casein ( $P = 0.048$ ), compared to high GS  
378 diets, with no effect of NDF treatment. There was no effect of forage type or NDF treatments  
379 on milk component concentrations, except for milk urea, which tended to be lower ( $P = 0.066$ )  
380 for MS than GS.

381

382 ***Methane Emission***

383 During methane measurements in experiment 1 (Table 5), DMI was higher ( $P < 0.001$ ) for the  
384 high MS compared to high GS diets, and not affected by NDF addition. Similarly, milk ( $P =$   
385 0.003) and ECM ( $P = 0.017$ ) yields were higher for the high MS compared to the high GS diets,  
386 and there was no effect of NDF addition. Methane production was not affected by forage type  
387 or NDF treatments (averaging 448 g/d), but there was a forage type  $\times$  NDF treatment  
388 interaction trend ( $P = 0.096$ ), with methane emission being lowest for high MS diets without  
389 additional NDF. Methane yield (g/kg DMI) was 24% lower ( $P < 0.001$ ) for cows fed high MS  
390 compared to high GS, and added NDF increased ( $P = 0.064$ ) methane yield for high MS diets,  
391 but not high GS diets (forage type  $\times$  NDF treatment interaction,  $P = 0.093$ ). Methane expressed  
392 per unit of milk and ECM yields (g/kg milk) were lower ( $P < 0.001$ ) for cows fed high MS  
393 compared to high GS, and increased with added NDF ( $P < 0.016$ ). Methane per kg BW tended  
394 to be greater for high GS diets without additional NDF, but not when NDF was added (forage  
395 type  $\times$  NDF treatment interaction,  $P = 0.052$ ).

396

397 During methane measurements in experiment 2 (Table 5), DMI was higher ( $P = 0.011$ ) for high  
398 MS compared to high GS diets, and there was no effect of NDF treatment. Cows fed high MS  
399 during methane measurements had a higher milk yield ( $P = 0.004$ ) and ECM yield ( $P = 0.034$ )  
400 than cows fed high GS, and added NDF decreased milk yield ( $P = 0.024$ ). Methane production  
401 (g/d) was higher ( $P = 0.097$ ) for cows fed high MS compared to high GS, with no effect of  
402 NDF treatment. Methane yield (g/kg DMI) was 8% lower ( $P < 0.018$ ) for cows fed high MS  
403 compared high GS diets. Although there was no effect of NDF treatment, there was a  
404 significant forage type  $\times$  NDF treatment interaction ( $P = 0.015$ ), with added NDF increasing  
405 methane yield for high MS, but not for high GS diets, as observed in experiment 1. There was  
406 no effect of forage type when methane was expressed per unit of milk yield (g/kg milk), but

407 methane per kg ECM yield tended to be lower for high MS diets ( $P = 0.063$ ). Methane per kg  
408 BW tended to be lower when diets included additional NDF ( $P = 0.099$ ).

409

#### 410 *Methane Measurement Techniques*

411 The present experiments were conducted simultaneously using lactating dairy cows of a similar  
412 BW fed the same dietary treatments. Although methane emission measurements obtained using  
413 GF and RC were not statistically comparable, an objective was to determine if dietary treatment  
414 effects on methane emission would be detected using both techniques.

415

416 Using the GF technique, there were 2,567 visits made to the GF by 40 cows over 3 wks. The  
417 average time that methane was sampled from each animal during each GF visit was 4.8 min  
418 (Table 5), with an average of 3.0 visits/animal/d, with 94% of cows visiting the GF every day  
419 during the 3 wks of GF access. This resulted in approximately 5 h of methane measurements  
420 for each cow in experiment 1. Cows housed in RC had 2 consecutive days of approximately 23  
421 h methane measurements, which was equivalent to 184 h of methane measurements for each  
422 cow in experiment 2.

423

424 The number of visits to the GF was affected by dietary treatment, whereby cows fed MS diets  
425 visited the GF less frequently on a daily basis than cows fed GS diets ( $P = 0.023$ ) (Table 5).  
426 Cows fed added NDF tended to have a longer GF visit duration than diets without added NDF  
427 ( $P = 0.016$ ). The pattern of cow visitation to the GF, based on all cow visits during the 3-wk  
428 measurement period and cumulated over 24 h, is given in Figure 1.

429

430 For methane production (g/d), a tendency for a forage type effect was observed using RC in  
431 experiment 2 ( $P = 0.097$ ), but not with GF in experiment 1. Methane yield (g/kg DMI) was

432 measured to be lower from lactating cows fed high MS diets compared to high GS diets, using  
433 both GF ( $P < 0.001$ ) and RC ( $P = 0.018$ ) techniques, but the magnitude of the difference varied  
434 between techniques (24% vs. 8% lower for high MS vs. high GS diets for GF and RC  
435 techniques, respectively). For both experiments, there was (or tended to be) a forage type  $\times$   
436 NDF treatment interaction ( $P = 0.093$  for GF and  $P = 0.015$  for RC) when methane emission  
437 was expressed per unit of DMI, with methane yield increasing with NDF addition for high MS  
438 diets, but not high GS diets. When methane was expressed per unit of milk yield (g/kg milk),  
439 there were forage type ( $P < 0.001$ ) and NDF treatment ( $P = 0.016$ ) effects measured with GF,  
440 but not RC. Averaging ( $\pm$  SEM) methane emission across dietary treatments for each technique  
441 gave similar results for both methane production (GF,  $448 \pm 5.70$  vs. RC,  $458 \pm 12.54$  g/d) and  
442 methane yield (GF,  $20.9 \pm 0.38$  vs. RC,  $23.8 \pm 0.73$  g/kg DMI).

443  
444 For GF measurements of methane, the range in methane production and yield (lowest to highest  
445 value) was 256 to 567 g/d and 14 to 29 g/kg DMI, respectively. The between-animal CV for  
446 GF methane production and yield was 5.7% and 5.2%, respectively, and the within-animal CV  
447 for methane production and yield was 10.5% and 14.4%, respectively. For RC measurements,  
448 the range in methane production and yield was 387 to 566 g/d and 19 to 29 g/kg DMI,  
449 respectively. The between-animal CV for methane production and yield using RC was 8.2%  
450 and 7.3%, respectively, and the within-animal CV for methane production and yield was 6.7%  
451 and 6.4%, respectively. Repeatability for measurements of methane production and yield  
452 (calculated as described by Herskin et al., 2003) for experiment 1 was 0.772 and 0.745,  
453 respectively and for experiment 2 was 0.761 and 0.764, respectively.

454

455

## DISCUSSION

456 *Effect of Forage Type and Added NDF on Dairy Cow Performance*

457 Overall, in this study, high MS dietary chemical composition was higher in starch and lower in  
458 NDF concentrations compared to high GS diets. The addition of straw and soyhulls decreased  
459 starch and increased fiber concentrations for both MS- and GS-based diets. There were  
460 differences in dietary treatment composition between wks 1 to 8 and 10 to 12 in experiment 1.  
461 The high MS diets had higher fiber and lower starch concentrations in wks 10 to 12, and the  
462 high GS diets had higher starch concentration, compared to the same respective diets fed in  
463 wks 1 to 8. This was largely due to the influence of variable amounts of pellets dispensed by  
464 the GF unit during wks 9 to 12. The GF unit was only available during wks 9 to 12 and during  
465 this period concentrate pellets were removed from the TMR and instead provided via the GF  
466 as enticement to generate a measure of methane, with an allowance of up to 2 kg DM/cow/d.  
467 The amount of pellet animals received was dependant on actual visits to the unit, and although  
468 up to 6 visits/d were possible for each dietary treatment, the number of visits achieved fell  
469 below this target. There were more visits to the GF unit by animals fed high GS diets than by  
470 animals fed high MS diets (Table 5, 3.4 vs. 2.7 visits/animal/d).

471

472 As observed in previous studies (as reviewed by Kahn et al., 2015), the higher starch and lower  
473 fiber contents of high MS diets were likely to be responsible for increased DMI and milk yield  
474 for cows in both experiments, compared to high GS diets. Khan et al. (2015), summarized data  
475 from 13 published studies with 37 direct comparisons which showed inclusion of MS in a GS-  
476 based diets fed to dairy cows improved DMI by 2 kg/d, milk yield by 1.9 kg/d, and milk protein  
477 concentration by 1.2 g/kg, with significant increases in yields of milk protein, fat, and lactose.  
478 A similar trend was found in this study whereby, compared with high GS diets, high MS  
479 improved DMI by 5.4 kg/d, milk yield by 5.4 kg/d and milk protein concentration by 0.35 g/kg  
480 for cows fed over 8 wks in experiment 1, and respective improvements of 3.4 kg/d, 6 kg/d and  
481 1.6 g/kg for cows fed over 4 wks in experiment 2. The high feed intake of MS is the main driver

482 of greater milk yields, with multiple mechanisms regulating DMI such as NDF and starch  
483 content, rate of degradability and rate of rumen passage (Khan et al., 2015). The higher feed  
484 intakes for lactating cows of experiment 1 compared to cows of experiment 2 was likely due  
485 to a number of factors including milk yield, DIM, ties stalls and experimental design. Dietary  
486 treatments were crossed over for cows in experiment 2 and had shorter periods of adaptation  
487 (3 wks), compared the continuous design of experiment 1 where animals were maintained on  
488 the same diet for the entire experimental duration.

489

490 In this study, adding NDF to the diet had no significant effects on DMI or milk yield for cows  
491 of either experiments 1 or 2, except it decreased milk yield in wk 5 of experiment 2. For cows  
492 of experiment 1, added NDF decreased milk protein yield and concentration, reflecting a  
493 decrease in diet ME concentration and rate and extent of digestible carbohydrate supply. In a  
494 study by Kendall et al. (2009), early lactation cows fed 28% NDF and highly digestible NDF  
495 diets produced more milk, fat and protein than those consuming 32% NDF and low digestible  
496 NDF diets. Dry matter intake was also greater for cows consuming 28% NDF diets but this  
497 was not affected by NDF digestibility.

498

#### 499 ***Effect of Forage Type and NDF Concentration on Methane Emissions***

500 The positive relationship between DMI (kg/d) and methane emission (g/d) is thoroughly  
501 documented in the literature (e.g. Mills et al., 2001) and also observed in the present study,  
502 with a slope of  $4.19 \pm 1.53$  and  $12.10 \pm 4.3$  for experiments 1 and 2, respectively ( $P < 0.01$  and  
503  $P < 0.02$ , respectively). Previous comparisons have found replacing GS with MS decreased  
504 methane emission and yield to varying extents (Reynolds et al., 2010). McCourt et al. (2007),  
505 Brask et al. (2013), and van Gastelen et al. (2015) all reported higher feed intakes and lower  
506 methane yields for lactating cows offered MS compared to GS, but no subsequent effect on

507 milk production. However, Staerfl et al. (2012), Livingstone et al. (2015), and Hammond et al.  
508 (2015b) have reported inconsistent effects of high MS vs. high GS diets on cattle methane  
509 emission. In our study, cows fed high MS and high GS diets had similar methane production  
510 (g/d) in experiment 1 (with a significant forage type × NDF treatment interaction), but greater  
511 methane production on a high MS diet in experiment 2. For both experiments, cows fed high  
512 MS had a lower methane yield compared to high GS diets (24% lower in experiment 1 using  
513 GF and 8% lower in experiment 2 using RC). Cows fed high MS diets had greater milk yields  
514 than cows fed high GS diets, however, when expressing methane per unit of milk yield, only  
515 in experiment 1 did cows fed high MS have a lower methane output per unit milk produced  
516 compared to high GS. The lower methane yield for the high MS diets is likely attributed to the  
517 source of starch and NDF affecting rates of fermentation in the rumen. High starch diets are  
518 known to be an effective method for lowering enteric methane emission (Beauchemin et al.,  
519 2008). Increased intake of starch enhances fermentation pathways that decrease methane  
520 production. With increasing dietary starch concentration there is lowered rumen pH which can  
521 decrease fiber digestion and cause an inhibition of methanogen activity and therefore methane  
522 production (Janssen, 2010). Livingstone et al. (2015) found no effect on methane yield when  
523 replacing GS with MS in a TMR for lactating dairy cows and concluded higher concentrations  
524 of NDF in their high MS diets may have counteracted negative effects of a higher starch  
525 concentration and MS composition per se on methane yield compared to high GS diets. This  
526 observation is partly supported in this study where adding NDF to the diet increased methane  
527 yield from cows fed high MS, but not high GS.

528

### 529 *Methane Measurement Techniques to Detect Dietary Treatment Effects*

530 The GF system has capability to estimate methane emission from greater numbers of animals,  
531 is less restrictive to animal behaviour for measurement of methane emission, and does not



532 require extensive laboratory equipment or labour. In our study, although the magnitude of the  
533 difference in methane yield varied between techniques for dietary treatments (24% lower in  
534 experiment 1 using GF and 8% lower in experiment 2 using RC), overall the techniques were  
535 able to detect similar dietary treatment effects for methane emission from lactating dairy cows  
536 (448 g/d and 20.9 g/kg DMI for GF vs. 458 and 23.8 for RC, respectively). This was similar to  
537 Hammond et al. (2015a), who found that despite concordance analyses finding no agreement  
538 between GF and RC, overall the GF system provide an average (grand mean) estimate of  
539 methane emission by growing dairy cattle that was not different to RC measurements.

540

541 Both techniques detected a significant interaction between forage type and NDF treatment, and  
542 measured a lower methane yield from cows fed high MS compared to high GS diets. This is in  
543 contrast to Hammond et al. (2015a) who used 4 growing dairy cattle in a 4 × 4 Latin square  
544 design and found GF unable to detect changes in methane emission due to treatment or animal  
545 effects that were detected using RC. In that study, cattle had GF access for only 7 d of each  
546 treatment period, and entered RC for 72 h at the end of the treatment period, whereas in the  
547 present study, a greater number of GF measurements were obtained daily from more animals  
548 over a longer period (3 wks) in an attempt to increase the sample size and better represent the  
549 daily pattern of methane emission.

550

551 Unlike experiment 2, which used RC and found methane production (g/d) to vary between  
552 dietary treatments, methane production estimated using GF was not significantly affected by  
553 dietary treatment for experiment 1. This difference between experiments could be due to a  
554 number of factors, including the animals themselves and their gut microbes, their level of  
555 intake, the timing of measurements, and other environmental factors. The difference in the  
556 results could also be due to differences in the timing of methane sampling measurements

557 relative to diurnal patterns of methane production and feeding. Respiration chambers take a  
558 continuous measurement of methane over 24 h, thus capturing varying methane emission  
559 patterns, whereas methane measurements using GF rely on animal visitation, which is mostly  
560 dictated by the behaviour of the animal.

561

562 The reliance of a feed enticement in order to generate a measure of methane is a limitation of  
563 the GF technique, as observed with a varying diet composition within experiment 1 (wks 1 to  
564 8 vs. wks 10 to 12) and compared to experiment 2. This is a concern in both pastoral grazing  
565 systems and animal nutrition studies where there is the possibility of excessive or variable  
566 contribution of attractant to the animals diet, even if restrictions are imposed (Dorich et al.,  
567 2015, Hammond et al., 2015a, Waghorn et al., 2013). Animals on a high GS diet visited the  
568 GF more regularly than on a high MS diet and this influenced the overall composition and  
569 intakes of starch and NDF, despite the attempt to accommodate this in the TMR formulation.  
570 A similar observation was found in Hammond et al. (2015a) where more visits to the GF were  
571 made when heifers were grazing a multi-species sward compared to ryegrass and clover.

572

573

## CONCLUSIONS

574 This study examined the effects of variations in forage proportions of MS and GS, with or  
575 without additional NDF concentration on feed intake, milk production and composition, and  
576 methane emission in lactating dairy cattle, and used GF as an alternative method to RC to  
577 measure dietary effects on methane emission. As hypothesized, cows fed high MS diets had a  
578 greater DMI, milk production, and lower methane yield (g/kg DMI), compared to cows fed  
579 high GS diets. Added NDF to both high MS and GS diets decreased DMI and milk yield, and  
580 increased methane yield for high MS but not high GS diets. Both the GF and RC methods  
581 detected similar dietary treatment effects on methane yield, although the magnitude of the

582 difference varied between experiments (and techniques) for dietary treatments. Overall  
583 average methane production and yield were similar for the 2 experiments using different cows,  
584 experimental conditions, and measurement techniques.

585

586

#### ACKNOWLEDGEMENTS

587 This study was funded by Defra, the Scottish Government, DARD, and the Welsh Government  
588 as part of the UK's Agricultural GHG Research Platform project ([www.ghgplatform.org.uk](http://www.ghgplatform.org.uk)).  
589 Contributions from the technical staff of CEDAR in the daily routine of experiments and care  
590 of cows, as well as assistance from C-Lock Inc., with regards to GreenFeed operation and use,  
591 is gratefully acknowledged.

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**Table 1** Diet formulations (g/kg DM) for total mixed rations with higher proportions of maize (MS) or grass silage (GS), without or with added NDF (MSNDF and GSNDF) and fed to lactating cows in experiments 1 and 2 and chemical composition (DM basis, g/kg) of diets for experiment 1.

	MS	MSNDF	GS	GSNDF
Grass silage	125	125	375	375
Maize silage	375	375	125	125
Barley straw	10	50	10	50
Cracked wheat	91	12	107	38
Maize meal	0	0	108	103
Molassed sugarbeet feed	50	50	0	0
Soy hulls	12	50	0	41
Wheat feed	97	84	70	50
Soybean meal	97	104	92	105
Rapeseed meal	30	38	0	0
Molasses	8	8	8	8
Dicalcium phosphate	5	5	5	5
Salt	5	5	5	5
Hi magnesium mineral <sup>1</sup>	8	8	8	8
Calf pellets <sup>2</sup>	87	87	87	87
Composition, g/kg DM				
Experiment 1, wks 1 to 8				
DM, g/kg fresh matter	431	430	410	407
OM	927	923	919	907
CP	154	159	159	170
NDF	340	391	366	395
ADF	192	220	219	239
Starch	216	179	193	140
Oil	39.5	37.6	42.6	43.0
Water soluble carbohydrate	45.8	38.7	40.6	40.1
Starch:NDF	0.64	0.46	0.53	0.35
Experiment 1, wks 10 to 12				
DM, g/kg fresh matter	431	435	383	378
OM	927	911	926	899
CP	163	178	168	161
NDF	344	411	366	401
ADF	199	242	223	243
Starch	219	144	212	141
Oil	37.7	37.7	41.7	45.1
Water soluble carbohydrate	40.2	35.7	36.0	25.1
Starch:NDF	0.64	0.35	0.58	0.35

<sup>1</sup>Containing (per kg): 220 g calcium, 40 g phosphorus, 50 g magnesium, 80 g sodium, 30 mg selenium, 120 mg cobalt, 400 mg iodine, 5000 mg manganese, 6000 mg zinc, 3000 mg copper, 400000 i.u. vitamin A, 75000 i.u. vitamin D, 2600 i.u. vitamin E, and 100 mg biotin. <sup>2</sup> Chemical composition of calf pellets was [g/kg DM] ash, 85.1; oil, 46.5; ADF, 174; NDF, 289; starch, 259; WSCHO, 91.3; nitrogen, 27.3; CP, 171; and gross energy [MJ/kg], 18.1.

**Table 2.** Chemical composition (DM basis, g/kg) of high maize (MS) or high grass silage (GS) forage diets without or with additional NDF (MSNDF and GSNDF) for experiment 2.

	Dietary Treatments				SEM	<i>P</i> values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
Experiment 2, wk 4								
DM, g/kg fresh matter	425	425	401	397	6.50	<0.001	0.341	0.326
OM	930	922	911	914	2.49	0.002	0.299	0.074
CP	164	140	169	181	7.74	0.077	0.265	0.096
NDF	307	369	354	385	10.5	0.025	0.006	0.172
ADF	172	214	201	239	7.06	0.005	0.002	0.747
Starch	247	196	193	137	5.51	<0.001	<0.001	0.094
Oil	35.9	36.3	44.7	42.2	0.42	0.002	0.135	0.036
WSCHO <sup>1</sup>	48.2	43.6	41.4	38.3	5.23	0.250	0.436	0.873
Starch:NDF	0.82	0.51	0.56	0.37	0.04	0.004	0.002	0.209

748 <sup>1</sup>Water soluble carbohydrate.

**Table 3.** Feed component intake (kg/d) from lactating cows fed high maize (MS) or high grass silage (GS) total mixed rations<sup>1</sup> supplemented without or with additional (5% DM basis) NDF (MSNDF and GSNDF) in experiments 1 and 2

	Dietary Treatments				SEM	<i>P</i> values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
<b>Experiment 1, wks 1 to 8</b>								
DM	26.4	25.9	21.8	22.0	0.35	<0.001	0.591	0.311
OM	22.7	21.4	19.3	18.9	0.70	0.001	0.292	0.333
CP	3.80	3.82	3.37	3.54	0.11	0.001	0.378	0.538
NDF	8.34	9.07	7.73	8.22	0.26	0.006	0.021	0.650
ADF	4.71	5.18	4.58	4.99	0.15	0.273	0.005	0.854
Starch	5.41	4.09	4.14	2.94	0.13	<0.001	<0.001	0.646
Oil	0.98	0.88	0.91	0.90	0.03	0.367	0.079	0.103
<b>Experiment 1, wks 10 to 12</b>								
DM	25.2	24.1	19.5	19.0	0.67	<0.001	0.277	0.631
OM	22.9	21.5	17.5	16.6	0.59	<0.001	0.030	0.455
CP	4.04	4.20	3.18	2.97	0.10	<0.001	0.795	0.081
NDF	8.50	9.50	6.82	7.42	0.23	<0.001	0.002	0.414
ADF	4.94	5.59	4.19	4.51	0.14	<0.001	0.002	0.255
Starch	5.45	3.32	4.08	2.66	0.12	<0.001	<0.001	0.007
Oil	0.93	0.87	0.78	0.84	0.03	0.002	0.925	0.026
n	10	10	10	10				
<b>Experiment 2, wk 4</b>								
DM	21.4	21.0	18.2	18.0	1.02	0.011	0.733	0.855
OM	19.9	20.2	15.2	16.3	0.78	0.024	0.150	0.234
CP	3.29	3.28	3.03	3.36	0.13	0.152	0.047	0.033
NDF	6.50	7.69	6.48	6.75	0.50	0.383	0.210	0.429
ADF	3.65	4.60	3.76	4.10	0.31	0.545	0.089	0.382
Starch	5.52	4.16	3.55	2.40	0.20	0.001	0.002	0.183
Oil	0.81	0.77	0.75	0.75	0.06	0.558	0.671	0.757
n	4	4	4	4				

749 <sup>1</sup>Containing (DM basis) either 37.5 and 12.5 % (MS) or 12.5 and 37.5 % (GS) MS and GS, respectively.

**Table 4.** Body weight, milk yield and composition from lactating cows fed high maize (MS) or high grass silage (GS) total mixed rations<sup>1</sup> supplemented without or with additional (5% DM basis) NDF (MSNDF and GSNDF) in experiments 1 and 2 35

	Dietary Treatments				SEM	<i>P</i> values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
<b>Experiment 1, wks 1 to 8</b>								
Body weight, kg	677	677	665	661	3.87	0.002	0.686	0.673
Yield								
Milk, kg/d	38.4	37.1	35.4	34.5	0.74	0.001	0.155	0.311
FCM, kg/d	37.4	37.4	38.6	37.1	0.93	0.133	0.971	0.332
ECM <sup>2</sup> , kg/d	34.2	34.3	33.1	32.1	0.76	0.031	0.598	0.457
Fat, g/d	1302	1386	1343	1311	42.6	0.703	0.537	0.158
Protein, g/d	1211	1144	1099	1057	24.4	0.001	0.031	0.586
Lactose, g/d	1723	1673	1576	1532	36.7	0.001	0.204	0.925
Casein, g/d	883	838	801	769	18.6	0.001	0.049	0.718
Concentration								
Fat, g/kg	34.0	37.7	38.2	38.3	0.91	0.018	0.041	0.049
Protein, g/kg	31.7	31.0	31.2	30.8	0.21	0.111	0.021	0.519
Lactose, g/kg	44.8	45.1	44.5	44.3	0.20	0.011	0.999	0.271
Casein, g/kg	23.1	22.8	22.7	22.4	0.17	0.053	0.066	0.975
Urea, mg/L	288	314	324	434	6.29	<0.001	<0.001	<0.001
n	10	10	10	10				
<b>Experiment 2, wk 4</b>								
Body weight, kg	693	688	664	676	21.5	0.015	0.587	0.172
Yield								
Milk, kg/d	31.6	33.6	27.4	25.8	2.05	0.076	0.807	0.243
FCM, kg/d	29.6	30.8	29.6	25.5	2.39	0.256	0.583	0.296
ECM, kg/d	29.2	29.7	28.3	24.3	2.55	0.174	0.492	0.343
Fat, g/d	1135	1211	1118	1017	103	0.313	0.908	0.392
Protein, g/d	1035	977	917	779	69.4	0.043	0.217	0.534
Lactose, g/d	1451	1445	1369	1141	6.70	0.060	0.290	0.253
Casein, g/d	765	718	667	568	54.9	0.048	0.247	0.616
Concentration								
Fat, g/kg	32.0	39.6	37.8	40.8	3.37	0.467	0.410	0.640
Protein, g/kg	32.7	31.2	30.4	30.3	1.12	0.108	0.402	0.380
Lactose, g/kg	45.4	45.6	44.7	44.8	0.40	0.153	0.767	0.996
Casein, g/kg	24.2	23.0	22.2	22.1	1.01	0.109	0.499	0.438

	Urea, mg/L	176	246	309	392	38.1	0.066	0.138	0.742
	n	4	4	4	4				
750	<sup>1</sup> Containing (DM basis) either 37.5 and 12.5 % (MS) or 12.5 and 37.5 % (GS) MS and GS, respectively.								
751	<sup>2</sup> Energy-corrected milk.								

**Table 5.** Methane emissions from lactating cows fed high maize (MS) or high grass silage (GS) total mixed rations<sup>1</sup> supplemented without or with additional (5 % DM basis) NDF (MSNDF and GSNDF) and obtained using a GreenFeed unit (experiment 1) or respiration chambers (experiment 2).

	Dietary Treatments				SEM	<i>P</i> values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
<b>Experiment 1, wks 10 to 12</b>								
DMI, kg/d	25.2	24.1	19.5	19.0	0.67	<0.001	0.277	0.631
Milk yield, kg/d	35.6	33.3	30.0	28.0	1.67	0.003	0.207	0.943
ECM <sup>2</sup> yield, kg/d	31.7	30.6	29.1	27.9	1.06	0.017	0.287	0.904
Methane emissions								
g/d	410	461	460	460	15.1	0.110	0.109	0.096
g/kg DMI	16.5	18.9	24.0	24.1	0.68	<0.001	0.064	0.093
g/kg milk yield	11.7	14.2	15.6	16.4	0.64	<0.001	0.016	0.200
g/kg ECM	13.1	15.2	15.9	16.6	0.51	0.001	0.011	0.168
g/kg BWT	0.591	0.697	0.696	0.686	0.029	0.118	0.111	0.052
GreenFeed visits								
Average daily per cow	2.76	2.58	3.35	3.54	0.33	0.023	0.983	0.576
Visit duration (min)	4.58	5.10	4.70	4.88	0.14	0.716	0.016	0.225
n	10	10	10	10				
<b>Experiment 2, wk 5</b>								
DMI, kg/d <sup>3</sup>	21.7	20.5	18.4	17.0	0.95	0.011	0.205	0.950
Milk yield, kg/d <sup>3</sup>	32.9	30.7	29.5	27.1	1.83	0.004	0.024	0.820
ECM yield, kg/d	31.3	30.6	25.6	24.2	1.47	0.034	0.138	0.282
Methane emissions								
g/d	495	472	462	418	26.5	0.097	0.176	0.627
g/kg DMI	21.8	23.7	25.5	24.2	0.82	0.018	0.412	0.015
g/kg milk yield	15.6	15.8	15.4	16.3	0.97	0.711	0.211	0.325
g/kg ECM	16.1	16.3	16.8	17.0	0.81	0.063	0.64	0.992
g/kg BWT	0.711	0.687	0.701	0.617	0.034	0.198	0.099	0.314
n	4	4	4	4				

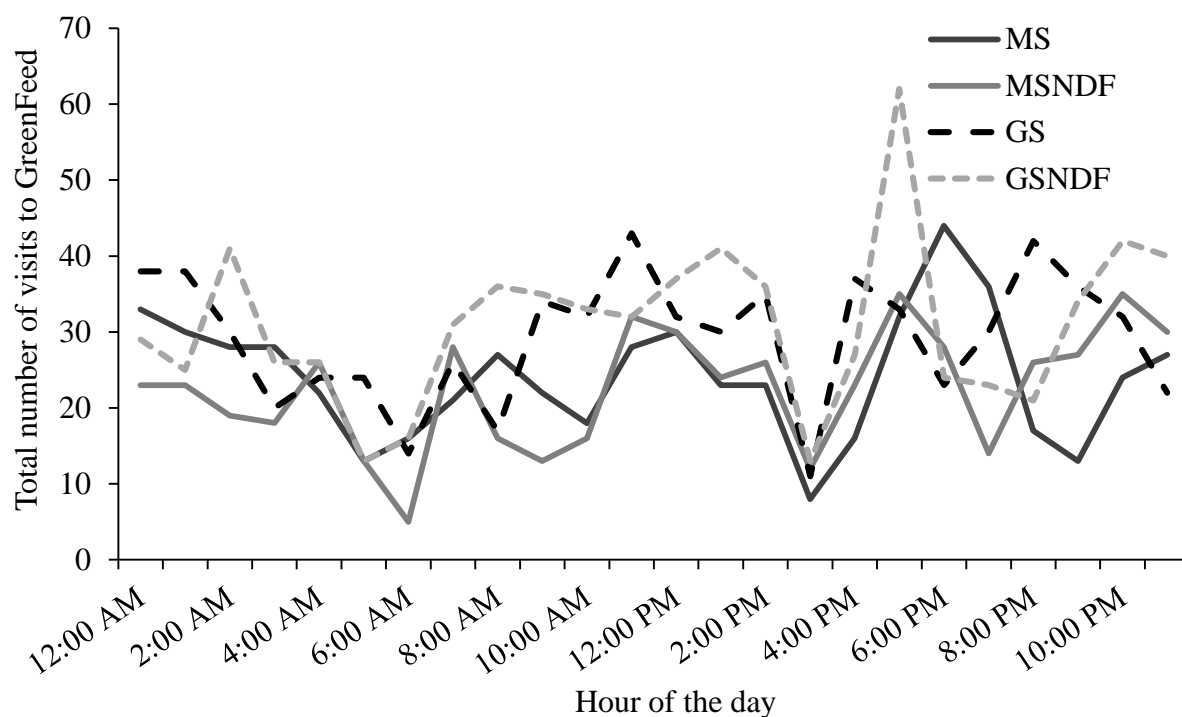
<sup>1</sup> Containing (DM basis) either 37.5 and 12.5 % (MS) or 12.5 and 37.5 % (GS) MS and GS, respectively.

<sup>2</sup> Energy-corrected milk.

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<sup>3</sup>Measurements of DMI and milk yield were taken whilst animals were housed in respiration chambers and so were obtained alongside measurements of methane emission.





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754 **Figure 1.** Pattern of GreenFeed visitation, based on 3 wks of access to a single GreenFeed unit,  
 755 cumulated over a 24 hour period, for 40 lactating dairy cows fed 4 dietary treatments of maize  
 756 silage (MS), MS with added neutral detergent fiber (MSNDF), grass silage (GS) and GS with  
 757 added NDF (GSNDF). Animals had unlimited access to GF during the 3 wks, except during  
 758 milking (which occurred twice daily between 06:00 and 07:00 h and 15:00 and 14:00 h) and if  
 759 another animal was occupying the unit.