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

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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.

MILK PRODUCTION, METHANE AND MEASUREMENT TECHNIQUE

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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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 $\times$ 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 (**SF₆**) 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$_6$ 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 Hamphsire,

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 × 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 ± 16.2 DIM at the start of the experiment and a

165   BW of 670 ± 4.0 kg throughout the experiment. In experiment 2, cows averaged ($\pm$ SEM) 114

166   ± 3.3 DIM at the start of the experiment and 678 ± 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, Bagenalstowm, 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 neglible. A gravimetrically measured amount of carbon

224   dioxide gas was relesed 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 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 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 12-25°C ± 2°C and a

254   relative humidity of 60 ± 10 %. The present experiment was conducted using six air changes

255   per h with environmental controls adjusted to give no more than ± 3°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 Abbotts, 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 65ºC 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 100ºC for DM determination (#930.15, AOAC, 2005). Total mixed ration

279   refusals and their corresponding DM (oven-dried at 100ºC 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 100ºC 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 -20ºC 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 × 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 × NDF treatment interaction for intakes of CP (P = 0.0.081), 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 × 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 x 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 ×

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 (± SEM) methane emission across dietary treatments for each technique

441   gave similar results for both methane production (GF, 448 ± 5.70 vs. RC, 458 ± 12.54 g/d) and

442   methane yield (GF, 20.9 ± 0.38 vs. RC, 23.8 ± 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 ± 1.53 and 12.10 ± 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**

592

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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[1]	8	8	8	8
Calf pellets[2]	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

[1]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. [2] 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.

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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	P 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[1]	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 [1]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[1] supplemented without or with additional (5% DM basis) NDF (MSNDF and GSNDF) in experiments 1 and 2

	Dietary Treatments				SEM	P values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
Experiment 1, wks 1 to 8								
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
Experiment 1, wks 10 to 12								
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				
Experiment 2, wk 4								
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   [1]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[1] supplemented without or with additional (5% DM basis) NDF (MSNDF and GSNDF) in experiments 1 and 2

	Dietary Treatments				SEM	P values		
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
Experiment 1, wks 1 to 8								
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[2], 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				
Experiment 2, wk 4								
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

	176	246	309	392	38.1	0.066	0.138	0.742
Urea, mg/L	176	246	309	392	38.1	0.066	0.138	0.742
n	4	4	4	4				

750 [1]Containing (DM basis) either 37.5 and 12.5 % (MS) or 12.5 and 37.5 % (GS) MS and GS, respectively.

751 [2] Energy-corrected milk.

Table 5. Methane emissions from lactating cows fed high maize (MS) or high grass silage (GS) total mixed rations[1] 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 | *P* values | | |
	MS	MSNDF	GS	GSNDF		Forage type	NDF	Forage type × NDF
Experiment 1, wks 10 to 12								
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[2] 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				
Experiment 2, wk 5								
DMI, kg/d[3]	21.7	20.5	18.4	17.0	0.95	0.011	0.205	0.950
Milk yield, kg/d[3]	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				

[1] Containing (DM basis) either 37.5 and 12.5 % (MS) or 12.5 and 37.5 % (GS) MS and GS, respectively.

[2] Energy-corrected milk.

[3]Measurements of DMI and milk yield were taken whilst animals were housed in respiration chambers and so were obtained alongside measurements of methane emission.
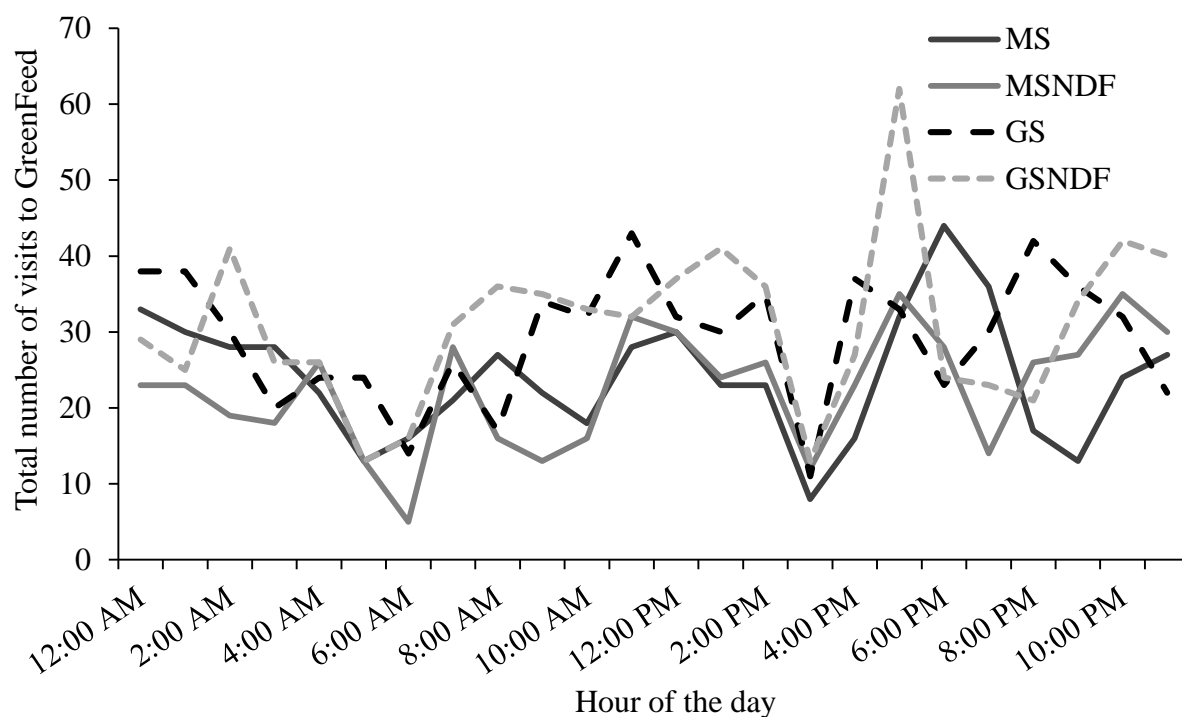
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Figure 1. Pattern of GreenFeed visitation, based on 3 wks of access to a single GreenFeed unit, cumulated over a 24 hour period, for 40 lactating dairy cows fed 4 dietary treatments of maize silage (MS), MS with added neutral detergent fiber (MSNDF), grass silage (GS) and GS with added NDF (GSNDF). Animals had unlimited access to GF during the 3 wks, except during milking (which occurred twice daily between 06:00 and 07:00 h and 15:00 and 14:00 h) and if another animal was occupying the unit.