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

Interpretive Summary

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

Hammond

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

K.J. Hammond[1], A.K. Jones, D.J. Humphries, L.A. Crompton, C.K. Reynolds[2]

Sustainable Agriculture and Food Systems Research Division, Centre for Dairy Research, School of Agriculture, Policy and Development, University of Reading, PO Box 237, Earley Gate, Reading, RG6 6AR, UK.

[1]Present address: AgResearch Grasslands, Palmerston North, New Zealand.

[2]Corresponding author: c.k.reynolds@reading.ac.uk

ABSTRACT

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

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

Keywords: forage, fiber, milk production, methane emission

INTRODUCTION

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

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

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

With increasing use of GF, more studies have compared GF estimates with methane measurements using other techniques, however comparisons with RC are difficult as measurements are not simultaneous. In a summary of GreenFeed publications by Hammond et al. (2016), under a variety of conditions, GF, RC and sulphur hexafluoride (**SF_6**) techniques are shown to give a similar estimate of daily enteric methane emission for cattle on most occasions. However, it was concluded that suitability of the GF system will be affected by the experimental objectives and design. An example is Hammond et al. (2015a) who used dairy cattle to compare RC, GF and SF_6 measurement techniques. Although techniques were comparable for measurement of methane emission, it was concluded that further work was needed to determine how to best deploy the GF system to detect significant changes in methane emission attributable to individual animals and treatments, and that future studies should include a greater number of animals per treatment than is required for RC studies.

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

MATERIALS AND METHODS

Experimental Design

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

Experiment 2 used 4 lactating Holstein cows surgically fitted with rumen cannulae (type #1 C, 100 mm centre diameter, Bar Diamond Inc., Parma, Idaho, USA) in a previous lactation. Experiment 2 was a 4 × 4 Latin square design balanced for carry-over effects with 5-wk treatment periods. From wks 1 to 3 animals were group-housed with access to cubicles bedded with rubber mats and wood shavings, fed TMR diets ad libitum, and milked twice daily. During this time, animals were adapted to dietary treatments with feed intake measured using a roughage intake control feeding system (Insentec B.V., Marknesse, The Netherlands). During wk 4, animals were moved to individual tie stalls and in wk 5 animals were staggered in pairs to 2 individual RC for 2 consecutive days of methane measurements. Measurements included diet composition, feed intake and milk yield and composition during wk 4, and DMI, milk yield and methane emission whilst in RC during wk 5. Cows were weighed weekly and before and after measurements in RC.

Animals and Dietary Treatments

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

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

Dietary treatments fed in both experiments were either a high MS (375 g/kg DM) and low GS (125 g/kg) TMR (MS), or the reverse proportions (GS), without or with additional chopped barley straw and soy hulls incorporated to increase concentration of NDF (+47 g/kg DM; MSNDF and GSNDF). For experiment 1 the TMR was prepared with a Mix Max 10 Paddle Feeder (Hi Spec Engineering Ltd, Bagenalstowm, Republic of Ireland). For experiment 2 each TMR was prepared with a Dataranger (American Calan, Northwood, New Hampshire, USA). For both experiments ingredients were added in the order straw, concentrate mix, calf pellets, limestone, grass silage, maize silage. Dietary treatments were formulated to be isonitrogenous and meet or exceed the recommendation for MP, minerals and vitamins based on Feed into Milk (Thomas 2007) recommendations (Table 1). The GF used for estimating methane emission from individual animals in experiment 1 required calf pellets as a form of enticement to encourage animals to enter the sampling hood. Therefore, dietary treatments were formulated for both experiments to include a commercial calf pellet (chemical composition [g/kg DM] of ash, 85.1; oil, 46.5; ADF, 174; NDF, 289; starch, 259; water soluble carbohydrate [**WSCHO**], 91.3; nitrogen [**N**], 27.3; CP, 171; and gross energy [**GE**; MJ/kg], 18.1) that was incorporated into the TMR to form 8.7% of the formulated TMR (DM basis). When the GF was used in experiment 1 (wks 9 to 12), pellets were excluded from the TMR and fed in the GF. Pellets were included in the TMR throughout experiment 2.

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

Methane Emission

For experiment 1, a single GF unit was used to estimate individual cow methane emission during wks 10 to 12. Details of the GF operation and use are given by Zimmerman and Zimmerman (2012), Hammond et al. (2015a), Hristov et al. (2015) and Huhtanen et al. (2015). Briefly, GF operation was initiated when an animal placed its head inside the GF hood. A radio frequency identification (RFID) reader identified the animal's ear tag and GF sampling was activated when the animals head (located by an infrared sensor) was located close to the sampling inlet within the hood (muzzle within 30 cm of the sampling inlet as detailed by Huhtanen et al., 2015), and it was deemed that sufficient time had elapsed since the previous methane measurement for that animal. Position of the animals head within the hood was monitored using sensors to ensure complete breath collection. The GF unit was set up outside the end of a cubicle yard within a polytunnel that minimized the effect of wind on measurements. Gates were positioned to allow access to the GF by only 1 animal at a time.

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

Animals were adapted to GF use during the covariate period (wks -3 to -1) and again in wk 9, with methane measurements used for statistical analysis obtained during wks 10 to 12. During these periods, animals were able to access the GF unit at any time, except during milking and provided it was not in use by another animal. However, this did not necessarily generate a measurement of methane. A 'visit' was defined as a successful methane measurement facilitated by feed delivery which could only occur when a specified time (> 240 min) had elapsed since the previous visit. In this case, the enticement was provided and a 'visit' logged if the animal remained correctly positioned in the unit for a sufficient amount of time (> 3 min) for a valid methane measurement. The unit was programmed to deliver feed in 50 g quantities at varying intervals over a 6 min period, so that up to 350 g pellet fresh weight was delivered during each complete visit, with up to a maximum of 6 visits per day (2 kg DM).

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

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

Sample Collection and Analyses

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

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

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

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

Statistical Analyses

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

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

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

RESULTS

Diet Composition

Differences in diet composition observed for bulk samples taken during the production and methane measurement periods of experiment 1 (Table 1) were similar to differences observed for experiment 2 (Table 2). For experiment 2, high MS diets had greater DM ($P < 0.001$) and OM ($P = 0.002$) contents, a greater concentration of starch ($P < 0.001$), and lower concentrations of CP ($P = 0.077$), NDF ($P = 0.025$), ADF ($P = 0.005$) and oil ($P = 0.002$), compared to high GS diets (Table 2). There was no forage type effect on WSCHO. Added NDF increased concentrations of NDF (*P* = 0.006) and ADF (*P* = 0.002), and decreased starch (*P* < 0.001). There were forage type × NDF treatment interactions for concentrations of OM (*P* = 0.074), CP (*P* = 0.096), starch (*P* = 0.094) and oil (*P* = 0.036). The starch:NDF ratio was higher for high MS diets (*P* = 0.004), and decreased with added NDF (*P* = 0.002).

Animal Performance

Intakes of individual dietary components are given in Table 3 for both experiments 1 and 2. Cows fed high MS diets in experiment 1 during wks 1 to 8 had greater intakes of DM (*P* < 0.001), OM (*P* = 0.001), CP (*P* = 0.001), NDF (*P* = 0.006) and starch (*P* < 0.001), compared to high GS, with no effect on intakes of ADF and oil. Added NDF treatment increased intakes of NDF (*P* = 0.021) and ADF (*P* = 0.005), and decreased intakes of starch (*P* < 0.001) and oil (*P* = 0.079), with no effects on intakes of DM, OM or CP. During wks 10 to 12 in experiment 1, cows fed high MS diets had higher (*P* < 0.01) intakes of all individual dietary components, compared to cows fed high GS diets. Adding NDF decreased intakes of OM (*P* = 0.030) and starch (*P* < 0.001), and increased intakes of NDF (*P* = 0.002) and ADF (*P* = 0.002). There was a forage type × NDF treatment interaction for intakes of CP (*P* = 0.0.081), starch (*P* = 0.007) and oil (*P* = 0.026).

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

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

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

Methane Emission

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

During methane measurements in experiment 2 (Table 5), DMI was higher (P = 0.011) for high MS compared to high GS diets, and there was no effect of NDF treatment. Cows fed high MS during methane measurements had a higher milk yield (P = 0.004) and ECM yield (P = 0.034) than cows fed high GS, and added NDF decreased milk yield (P = 0.024). Methane production (g/d) was higher (P = 0.097) for cows fed high MS compared to high GS, with no effect of NDF treatment. Methane yield (g/kg DMI) was 8% lower (P < 0.018) for cows fed high MS compared high GS diets. Although there was no effect of NDF treatment, there was a significant forage type × NDF treatment interaction (P = 0.015), with added NDF increasing methane yield for high MS, but not for high GS diets, as observed in experiment 1. There was no effect of forage type when methane was expressed per unit of milk yield (g/kg milk), but methane per kg ECM yield tended to be lower for high MS diets (*P* = 0.063). Methane per kg BW tended to be lower when diets included additional NDF (*P* = 0.099).

Methane Measurement Techniques

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

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

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

For methane production (g/d), a tendency for a forage type effect was observed using RC in experiment 2 (*P* = 0.097), but not with GF in experiment 1. Methane yield (g/kg DMI) was measured to be lower from lactating cows fed high MS diets compared to high GS diets, using both GF ($P < 0.001$) and RC ($P = 0.018$) techniques, but the magnitude of the difference varied between techniques (24% vs. 8% lower for high MS vs. high GS diets for GF and RC techniques, respectively). For both experiments, there was (or tended to be) a forage type × NDF treatment interaction ($P = 0.093$ for GF and $P = 0.015$ for RC) when methane emission was expressed per unit of DMI, with methane yield increasing with NDF addition for high MS diets, but not high GS diets. When methane was expressed per unit of milk yield (g/kg milk), there were forage type ($P < 0.001$) and NDF treatment ($P = 0.016$) effects measured with GF, but not RC. Averaging (± SEM) methane emission across dietary treatments for each technique gave similar results for both methane production (GF, 448 ± 5.70 vs. RC, 458 ± 12.54 g/d) and methane yield (GF, 20.9 ± 0.38 vs. RC, 23.8 ± 0.73 g/kg DMI).

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

DISCUSSION

Effect of Forage Type and Added NDF on Dairy Cow Performance

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

As observed in previous studies (as reviewed by Kahn et al., 2015), the higher starch and lower fiber contents of high MS diets were likely to be responsible for increased DMI and milk yield for cows in both experiments, compared to high GS diets. Khan et al. (2015), summarized data from 13 published studies with 37 direct comparisons which showed inclusion of MS in a GS-based diets fed to dairy cows improved DMI by 2 kg/d, milk yield by 1.9 kg/d, and milk protein concentration by 1.2 g/kg, with significant increases in yields of milk protein, fat, and lactose. A similar trend was found in this study whereby, compared with high GS diets, high MS improved DMI by 5.4 kg/d, milk yield by 5.4 kg/d and milk protein concentration by 0.35 g/kg for cows fed over 8 wks in experiment 1, and respective improvements of 3.4 kg/d, 6 kg/d and 1.6 g/kg for cows fed over 4 wks in experiment 2. The high feed intake of MS is the main driver of greater milk yields, with multiple mechanisms regulating DMI such as NDF and starch content, rate of degradability and rate of rumen passage (Khan et al., 2015). The higher feed intakes for lactating cows of experiment 1 compared to cows of experiment 2 was likely due to a number of factors including milk yield, DIM, ties stalls and experimental design. Dietary treatments were crossed over for cows in experiment 2 and had shorter periods of adaptation (3 wks), compared the continuous design of experiment 1 where animals were maintained on the same diet for the entire experimental duration.

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

Effect of Forage Type and NDF Concentration on Methane Emissions

The positive relationship between DMI (kg/d) and methane emission (g/d) is thoroughly documented in the literature (e.g. Mills et al., 2001) and also observed in the present study, with a slope of 4.19 ± 1.53 and 12.10 ± 4.3 for experiments 1 and 2, respectively ($P < 0.01$ and $P < 0.02$, respectively). Previous comparisons have found replacing GS with MS decreased methane emission and yield to varying extents (Reynolds et al., 2010). McCourt et al. (2007), Brask et al. (2013), and van Gastelen et al. (2015) all reported higher feed intakes and lower methane yields for lactating cows offered MS compared to GS, but no subsequent effect on milk production. However, Staerfl et al. (2012), Livingstone et al. (2015), and Hammond et al. (2015b) have reported inconsistent effects of high MS vs. high GS diets on cattle methane emission. In our study, cows fed high MS and high GS diets had similar methane production (g/d) in experiment 1 (with a significant forage type × NDF treatment interaction), but greater methane production on a high MS diet in experiment 2. For both experiments, cows fed high MS had a lower methane yield compared to high GS diets (24% lower in experiment 1 using GF and 8% lower in experiment 2 using RC). Cows fed high MS diets had greater milk yields than cows fed high GS diets, however, when expressing methane per unit of milk yield, only in experiment 1 did cows fed high MS have a lower methane output per unit milk produced compared to high GS. The lower methane yield for the high MS diets is likely attributed to the source of starch and NDF affecting rates of fermentation in the rumen. High starch diets are known to be an effective method for lowering enteric methane emission (Beauchemin et al., 2008). Increased intake of starch enhances fermentation pathways that decrease methane production. With increasing dietary starch concentration there is lowered rumen pH which can decrease fiber digestion and cause an inhibition of methanogen activity and therefore methane production (Janssen, 2010). Livingstone et al. (2015) found no effect on methane yield when replacing GS with MS in a TMR for lactating dairy cows and concluded higher concentrations of NDF in their high MS diets may have counteracted negative effects of a higher starch concentration and MS composition per se on methane yield compared to high GS diets. This observation is partly supported in this study where adding NDF to the diet increased methane yield from cows fed high MS, but not high GS.

Methane Measurement Techniques to Detect Dietary Treatment Effects

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

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

Unlike experiment 2, which used RC and found methane production (g/d) to vary between dietary treatments, methane production estimated using GF was not significantly affected by dietary treatment for experiment 1. This difference between experiments could be due to a number of factors, including the animals themselves and their gut microbes, their level of intake, the timing of measurements, and other environmental factors. The difference in the results could also be due to differences in the timing of methane sampling measurements relative to diurnal patterns of methane production and feeding. Respiration chambers take a continuous measurement of methane over 24 h, thus capturing varying methane emission patterns, whereas methane measurements using GF rely on animal visitation, which is mostly dictated by the behaviour of the animal.

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

CONCLUSIONS

This study examined the effects of variations in forage proportions of MS and GS, with or without additional NDF concentration on feed intake, milk production and composition, and methane emission in lactating dairy cattle, and used GF as an alternative method to RC to measure dietary effects on methane emission. As hypothesized, cows fed high MS diets had a greater DMI, milk production, and lower methane yield (g/kg DMI), compared to cows fed high GS diets. Added NDF to both high MS and GS diets decreased DMI and milk yield, and increased methane yield for high MS but not high GS diets. Both the GF and RC methods detected similar dietary treatment effects on methane yield, although the magnitude of the difference varied between experiments (and techniques) for dietary treatments. Overall average methane production and yield were similar for the 2 experiments using different cows, experimental conditions, and measurement techniques.

ACKNOWLEDGEMENTS

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

REFERENCES

AOAC. 2005. Official Method of Analysis, 18th ed. 17th Edition ed., Gaithersburg, Maryland, USA.

Beauchemin, K. A., M. Kreuzer, F. P. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. of Exp. Agric. 48:21-27.

Brask, M., P. Lund, A. L. F. Hellwing, M. Poulsen, and M. R. Weisbjerg. 2013. Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. Anim. Feed Sci. Technol. 184:67-69.

Brouwer, E. 1965. Report of sub-committee on constants and factors. In *Energy metabolism* (ed. K. L. Blaxter), *European Association for Animal Production publication no 11*, pp. 441-443. Academic Press, London.

Cammell, S. B., D. J. Thomson, D. E. Beever, M. J. Haines, M. S. Dhanoa, and M. C. Spooner. 1986. The efficiency of energy utilization in growing cattle consuming fresh perennial ryegrass (*Lolium perenne* cv. Melle) or white clover (*Trifolium repens* cv. Blanca). Br. J. Nutr. 55:669-680.

Dorich, C. D., R. K. Varner, A. B. D. Pereira, R. Martineau, K. J. Soder, and A. F. Brito. 2015. Short communication: Use of a portable, automated, open-circuit gas quantification system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions in Holstein cows fed ad libitum or restricted. J. Dairy Sci. 98: 2676-2681.

Gaines, W. L. 1928. The energy basis of measuring milk yield in dairy cows. Univ. Illinois Agric. Exp. Station Bull. 308.

Gaillard, C., N. C. Friggens, M. Taghipoor, M. R. Weisbjerg, J. O. Lehmann, and J. Sehested. 2016. Effects of an individual weight-adjusted feeding strategy in early lactation on milk production of Holstein cows during extended lactation. J. Dairy Sci. 99:2221-2236.

Hammond, K. J., D. J. Humphries, D. B. Westbury, A. Thompson, L. A. Crompton, P. Kirton, C. Green, and C. K. Reynolds. 2014. The inclusion of forage mixtures in the diet of growing dairy heifers: Impacts on digestion, energy utilisation, and methane emissions. Agric. Eco. Environ. 197:88-95.

Hammond, K. J., D. J. Humphries, L. A. Crompton, C. Green, and C. K. Reynolds. 2015a. Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. Anim. Feed Sci. Technol. 203:41-52.

Hammond, K. J., D. J. Humphries, L. A. Crompton, P. Kirton, and C. K. Reynolds. 2015b. Effects of forage source and extruded linseed supplementation on methane emissions from growing dairy cattle of differing body weights. J. Dairy Sci. 98: 8066-8077.

Hammond, K. J., G. C. Waghorn, and R. S. Hegarty. 2016. The GreenFeed system for measurement of enteric methane emissions from cattle. Anim. Prod. Sci. 56: 181-189.

Herskin, M. S., R. Müller, L. Schrader and J. Ladewig. 2003. A laser-based method to measure thermal nociception in dairy cows: Short-term repeatability and effects of power output and skin condition. J. Anim. Sci. 81: 945-954.

Hristov, A. N., J. Oh, F. Giallongo, T. Frederick, H. Weeks, P. R. Zimmerman, R. A. Hristova, S. R. Zimmerman, and A. F. Branco. 2015. The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. J. Vis. Exp. 103:e52904; doi:10.3791/52904.

Huhtanen, P., E. H. Cabezas-Garcia, S. Utsumi, and S. Zimmerman. 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J. Dairy Sci. 98: 3394-3409.

IPCC. 2007. Climate change 2007: The Phsyical Science Basis. In Contribution of Working Group 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., Tignor, M., Miller, H., (Eds). Cambridge University Press, UK and New York, USA.

Janssen, P. H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160:1-22.

Kendall, C., C. Leonardi, P. C. Hoffman, and D. K. Combs. 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. J. Dairy Sci. 92:313-323.

Khan, N. A., P. Yu, M. Ali, J. W. Cone, and W. H. Hendriks. 2015. Nutritive value of maize silage in relation to dairy cow performance and milk quality. J. Sci. Food Agric. 95:238-252.

Kliem, K. E., R. Morgan, D. J. Humphries, K. J. Shingfield, and D. I. Givens. 2008. Effect of replacing grass silage with maize silage in the diet on bovine milk fatty acid composition. Anim. 2:1850-1858.

Livingstone, K. M., D. J. Humphries, P. Kirton, K. E. Kliem, D. I. Givens, and C. K. Reynolds. 2015. Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. J. Dairy Sci. 98:4000-4011.

Mc Court, A. R., T. Yan, and C. S. Mayne. 2007. Effect of forage type on methane production from dairy cows. Proc. Brit. Soc. Anim. Sci.: p 48. Southport, UK.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral deterent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Intern. 85:1217-1240.

Mills, J. A., J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab, and J. France. 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. J. Anim. Sci. 79:1584-1597.

O'Mara, F. P., J. J. Fitzgerald, J. J. Murphy, and M. Rath. 1998. The effect on milk production of replacing grass silage with maize silage in the diet of dairy cows. Livest. Prod. Sci. 55:79-87.

Phipps, R. H., R. F. Weller, R. J. Elliott, and J. D. Sutton. 1988. The effect of level and type of concentrate and type of conserved forage on dry matter intake and milk production of lactating dairy cows. J. Agric. Sci. 111:179-186.

Phipps, R. H., J. D. Sutton, and B. A. Jones. 1995. Forage mixtures for dairy cows: the effect on dry-matter intake and milk production of incorporating either fermented or urea-treated whole-crop wheat, brewers' grains, fodder beet or maize silage into diets based on grass silage. Anim. Sci. 61:491-496.

Phipps, R. H., R. F. Weller, and A. J. Rook. 1992. Forage mixtures for dairy cows: The effect on dry matter intake and milk production of incorporating different proportions of maize silage into diets based on grass silages of differing energy value. J. Agric. Sci. 118:379-382.

Reynolds, C. K., S. B. Cammell, D. J. Humphries, D. E. Beever, J. D. Sutton, and J. R. Newbold. 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. J. Dairy Sci. 84:2250-2259.

Reynolds, C. K., L. A. Crompton, J. A. N. Mills, D. J. Humphries, P. Kirton, A. E. Relling, T. H. Misselbrook, D. R. Chadwick, and D. I. Givens. 2010. Effects of diet protein level and forage source on energy and nitrogen balance in methane and nitrogen excretion in lactating dairy cows. G. M. Crovetto, ed. Energy and Protein Metabolism and Nutrition Symposium, 127:736. Wageningen Academic Publishers, The Netherlands.

Reynolds, C. K., D. J. Humphries, P. Kirton, M. Kindermann, S. Duval, and W. Steinberg. 2014. Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J. Dairy Sci. 97:3777-3789.

Robertson, J. B. and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123-158 in The Analysis of Dietary Fiber in Food. Vol. 3. W. James and O. Theander, ed. Marcel Dekker Inc, New York, USA.

S.A.S Institute Inc., 2011. SAS 9.3 Utilities. SAS Institute Inc., Cary, NC, USA.

Staerfl, S. M., J. O. Zeitz, M. Kreuzer, and C. R. Soliva. 2012. Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agric. Eco. Environ. 148:111-120.

Thomas, C. (2007). Feed into milk: A new applied feeding system for dairy cows. Nottingham University Press, UK.

van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, G. Klop, S. J. J. Alferink, W. H. Hendriks, and J. Dijkstra. 2015. Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets. J. Dairy Sci. 98:1915-1927.

Waghorn, G. C., E. J. Garnett, C. S. Pinares-Patino, and S. Zimmerman. 2013. Implementation of GreenFeed in a dairy herd grazing pasture. Advances in Animal Biosciences, Proceedings of the 5th Greenhouse Gases and Animal Agriculture Conference (GGAA2013), Dublin, Ireland, 4:436.

Zimmerman, P.R., and R. S. Zimmerman. 2012. Method and system for monitoring and reducing ruminant methane production. United States Patent number US2009028806 A1.

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.

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				

[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

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

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.

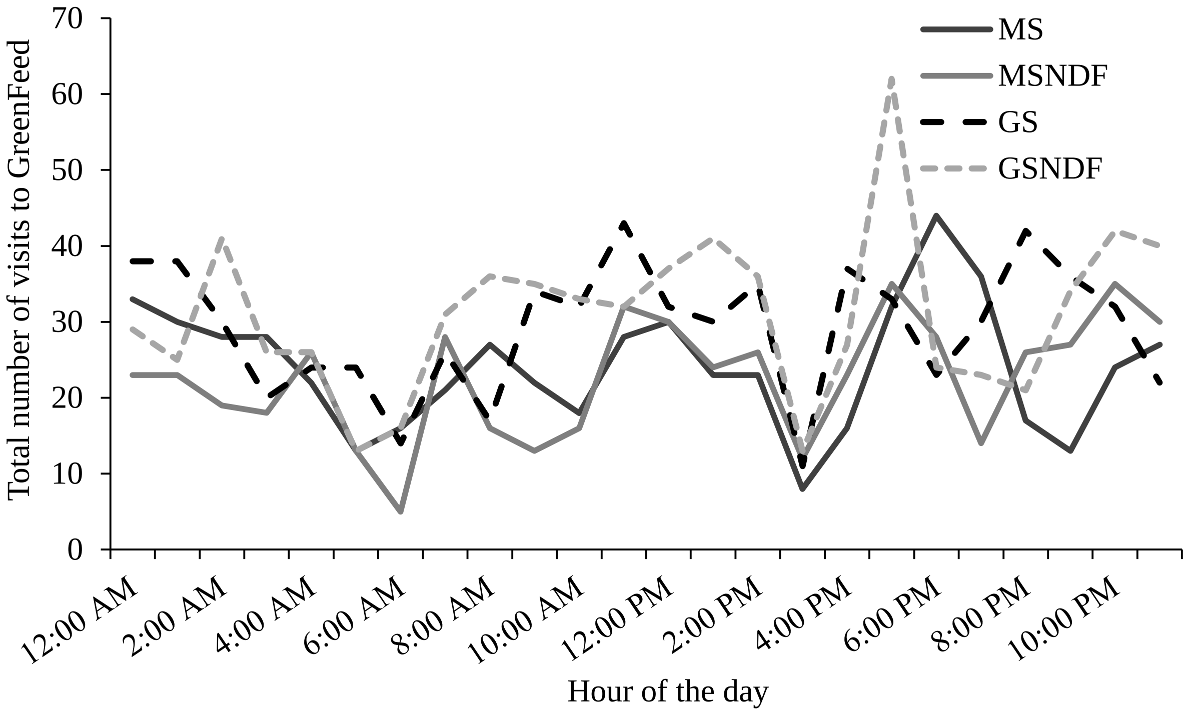

Figure 1. Pattern of GreenFeed visitation, based on 3 wks of access to a single GreenFeed unit, cumulated over a 24 hour period, for 40 lactating dairy cows fed 4 dietary treatments of maize silage (MS), MS with added neutral detergent fiber (MSNDF), grass silage (GS) and GS with added NDF (GSNDF). Animals 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.