The effect of high and low levels of supplementation on milk production, nitrogen utilization efficiency, and milk protein fractions in late-lactation dairy cows

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ABSTRACT

To fill the feed deficit in the autumn/late lactation period in a seasonal grazing system, supplementation is required. This study aimed to investigate the use of baled grass silage or concentrate as supplementation to grazing dairy cows in late lactation. Eighty-four grass-based spring-calving dairy cows, averaging 212 d in milk, were allocated to 1 of 6 treatments [high grass allowance (HG), low grass allowance (LG), grass with a low concentrate allocation (GCL), grass with a low grass silage allocation (GSL), grass with a high concentrate allocation (GCH), and grass with a high grass silage allocation (GSH)] to measure the effects of using baled grass silage or concentrate as supplements to grazed grass. Effects on intake, milk yield, milk composition and N fractions, and N utilization efficiency were measured. Treatments HG and LG received 17 and 14 kg of dry matter (DM) grass/cow per d, respectively. Treatments GCL and GSL were offered 14 kg of DM grass/cow per d and 3 kg of DM of supplementation/cow per d. Treatments GCH and GSH were offered 11 kg of DM grass/cow per d and 6 kg of DM of supplementation/cow per d. Milk yield was greatest in the GCH treatment and milk solids yield was greatest in both concentrate-supplemented treatments. The HG and LG treatments excreted a greater quantity of N as a proportion of N intake than the supplemented treatments. The HG treatment also excreted the greatest total quantity of N. This indicates an improvement in N utilization efficiency when supplementation is offered compared with grazing only. Offering 6 kg of DM of either grass silage or concentrate as supplementation decreased milk true protein concentration compared with offering a grass-only diet. This suggests that increasing the proportion of supplementation relative to grass may negatively affect milk processability, which is associated with milk true protein concentration.

Key words: nutrition, grass silage, grass, concentrate, nitrogen, milk protein

INTRODUCTION

The ability to grow between 10 and 16 t grass DM/ha gives a competitive advantage because, in countries such as Ireland, grass is the cheapest feed source for milk production (Finneran et al., 2010). Seasonal grass growth patterns lead to deficits in the spring and autumn. When grass supply is limited, the grass-based diet of the dairy cow must be supplemented to ensure that cow nutritional requirements are met to extend the lactation at a low cost. Supplements such as grass silage and concentrates are commonly used to fill the feed deficit that arises when grass supply is limited (Kennedy et al., 2005).

Grass silage is the most important feed source available to Irish dairy cows after grazed grass (Finneran et al., 2010). Recent innovations in grassland management involve the preservation of grass as baled silage during times of grass surplus in the main grazing season. Baled grass silage can therefore be used as a tool to manage surplus grass and grass utilization. Grass silage bales are often made from higher quality grass than the conventional method of ensiling grass in a pit (McEniry et al., 2011). The effect on milk yield and milk composition of offering grass silage as a supplementary feed to grazing dairy cows in late lactation has been investigated with varying results. A review by Phillips (1988) concluded that offering grass silage to cows offered a grass-based diet in late lactation increased milk yield, decreased milk fat concentration, and had variable effects on milk protein concentration compared with grass-only diets. The experiments considered in the review all used grass silage, which was ensiled in a pit. O'Brien et al. (1996) investigated the effect of varying levels of grass silage and concentrates as supplementation to spring-calving grass-based dairy cows in late lactation; they concluded that supplementation with silage caused a linear decline in milk solids yield. Milk yield and milk solids yield were increased linearly

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with increasing concentrate supplementation. Grass was grazed to a target postgrazing sward height of 6 to 7 cm, which is substantially greater than the current recommendation of 4 to 4.5 cm. The grass silage used in the study was also from a pit.

Concentrate is often used as a supplement in late lactation. Although concentrate is more expensive than grass silage, the milk response (**MR**) to concentrate is greater than the MR to grass silage (Peyraud et al., 2004). Feeding supplementary concentrate to late lactation cows in autumn is also recommended to maintain milk lactose concentration above the target of 42.0 g/kg (O'Brien, 2008).

Researchers are interested in improving the nitrogen utilization efficiency (**NUE**) of dairy cows to reduce the N excreted in urine (Peyraud et al., 2004). Urinary N is the primary route for urea excretion (Spek et al., 2013). Olmos Colmenero and Broderick (2006) showed that the concentration of N in urine is directly related to the diet. Urine N excretion can be high in grass-based diets due to the high dietary protein concentration (Dijkstra et al., 2013).

Milk processability, which is the suitability of milk for processing into dairy products, can be improved by increasing particular N components in the milk, specifically CN (Hermansen et al., 1999). Casein concentration in milk is closely related to the diet of the dairy cow and can be increased with increases in both dietary CP and energy (Sutton et al., 1996).

Few data are available in the literature on the effect of including high-quality baled grass silage, in comparison with concentrate, in the diet of grazing dairy cows in late lactation in autumn. The effects on dairy cow N excretion and on the protein parameters in milk that effect milk processability are lacking. The objective of the current study was to measure the effects of offering different supplementary feeds to grazing dairy cows in late lactation on milk yield and composition, **NUE**, and milk N fractions. We hypothesized that when supplementary feeds were offered with a low allowance, grass-only diet (14 kg of DM >4 cm), the milk and milk solids yield response would be positive. We also hypothesized that using concentrate as the supplement would result in a greater milk yield response than grass silage. Finally, we hypothesized that using a larger allocation of grass, compared with grass silage supplementation, would give a greater milk and milk solids yield response.

MATERIALS AND METHODS

The experiment was conducted at Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, County Cork, Ireland (50°09'N,

8°16'W, 35 m above sea level). The area used was under permanent pasture with a predominantly perennial ryegrass (*Lolium perenne* L.) sward. The grass sward was composed of 50% Astonenergy and 50% Tyrella cultivars. The average age of the pasture was 4 yr. Prior approval for animal use was attained from the Teagasc Animal Ethics Committee (Ref-TAEC 14/2013) and the Irish Medicines Board (Ref-AE19132/P001).

The experiment was a complete randomized block design and was conducted between September 30 and November 16, 2013. Eighty-four lactating dairy cows (66 multiparous and 18 primiparous) at, on average, 212 DIM (SD = 23 d) were selected from the Teagasc Moorepark spring-calving dairy herd. The average calving date of the cows was March 2, 2013 (SD = 23 d). Prior to the beginning of the study, cows were offered 16 kg of DM grass/cow per d and no concentrate.

Treatments and Grazing Management

The cows were blocked based on DIM, lactation number (3 ± 1.3 ; mean $\pm$ SD), milk yield (19 ± 3.1 kg of milk/cow per day), milk protein concentration (37.0 ± 2.7 g/kg), milk solids yield (1.6 ± 0.21 kg), BW (525 ± 54 kg), and BCS (3.2 ± 0.28 ; 1–5 scale of Edmonson et al., 1989). From within block, cows were randomly assigned to 1 of 6 feeding treatments: high grass allowance only (**HG**), low grass allowance only (**LG**), grass with a low concentrate allocation (**GCL**), grass with a low grass silage allocation (**GSL**), grass with a high concentrate allocation (**GCH**), and grass with a high grass silage allocation (**GSH**). Treatments HG and LG received 17 and 14 kg of DM grass/cow per d, respectively. Treatments GCL and GSL were offered 14 kg of DM grass/cow per d and 3 kg of DM of supplementation/cow per d. Treatments GCH and GSH were offered 11 kg of DM grass/cow per d and 6 kg of DM of supplementation/cow per d. Fresh grass was offered daily. The GCL and GCH treatments received their concentrate allocation in 2 equal measures during morning and afternoon milkings via the Dairymaster feed delivery system (Dairymaster, Causeway, Co. Kerry, Ireland). The grass silage was offered on an outdoor feeding pad. A self-locking stall swing (O'Donovan Engineering, Coachford, Co. Cork, Ireland) was used to secure cows within the GSL and GSH treatments and ensure that they received the correct grass silage allocation. Treatment GSL had approximately 1 h to consume their supplementary feed. Treatment GSH had approximately 1 h 30 min to consume their supplementary feed. The DM of the grass silage was assessed 6 times per week to calculate the daily fresh weight grass silage allocation per cow per day. Samples of the grass silage bale were taken the day before feeding,

and a subsample of 0.1 kg was dried at 90°C for 16 h for DM estimation. During each day of the study, any grass silage not eaten by the cows within the GSL and GSH treatments was weighed back and a sample taken for DM estimation to assess individual cow daily grass silage intake. Feed troughs in the milking parlor were checked daily for any concentrate not eaten, and the refusals were collected and weighed.

The experimental grazing area consisted of 30.39 ha divided into 15 individual paddocks with permanent fences. Treatment groups grazed adjacent to one another in the same paddocks and were separated using a temporary electric strip-wire fence. The size of the area allocated to each treatment was determined by calculating the pregrazing herbage mass. The pregrazing herbage mass (>4 cm) was determined twice weekly by cutting 4 strips of grass per paddock (1.2 m × 10 m) using an Etesia mower (Etesia UK Ltd., Warwick, UK). Ten measurements of compressed sward height were taken pre- and postcutting using a rising plate meter (diameter 355 mm; Jenquip, Feilding, New Zealand). All mown grass from each cut was weighed and then a sample was collected (~0.3 kg). A subsample of 0.1 kg was dried at 90°C for 16 h for DM estimation. Pre- and postgrazing sward height was assessed daily using a Jenquip rising plate meter. Pre- and postgrazing sward heights were measured by taking 50 measurements/treatment per d.

Once weekly, 24 h before grazing, grass samples were collected from each treatment grazing area to determine the quality of the grass offered. Following close observation of the treatment groups, grass was cut using a Gardena hand shears (Husqvarna AB, S-561 82 Huskvarna, Sweden) to heights similar to the postgrazing heights of the individual treatments. Cuts were taken ($n = 20$) at 5-m intervals across the diagonals of each treatment paddock. Samples were immediately frozen once collected. Samples were freeze-dried, milled, and passed through a 1-mm sieve (Cyclotech 1093 sample mill, Foss Electric, Hillerød, Denmark) in preparation for chemical analysis. Once weekly, grass silage samples were taken for analysis of chemical composition. Samples were collected with a silage core. Once every 2 wk, samples of concentrate were collected for analysis of chemical composition.

Chemical Analysis

The CP concentration of the grass samples was determined using a Leco N Analyzer (Leco FP-528, Leco Corporation, St. Joseph, MI). Ash was calculated by placing the samples in a Gallenkamp muffle furnace size 3 (Thermo Fisher Scientific Inc., Waltham, MA) for 16 h at 500°C. Neutral detergent fiber concentration

was analyzed using the Ankom Fiber Analyzer (Ankom Technology Corporation, NY) using procedures determined by Van Soest et al. (1991). The samples were analyzed for OM digestibility (Fibertec Systems, Foss, Ballymount, Dublin, Ireland) using the neutral detergent cellulase in vitro method of Morgan and Stakelum (1987). Silage samples were analyzed immediately after collection for CP, NDF, ash, DM digestibility, and OM digestibility by near infrared reflectance spectroscopy (NIR; Foss-NIR System DK). Concentrate samples were analyzed for CP, NDF, ash, and starch by NIR.

Animal Measurements

Milking took place daily at 0700 and 1500 h. Individual milk yields were recorded during milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, milk protein, milk lactose, MUN, and milk CN concentrations were determined weekly from 1300 and 0100 h sample taken on successive days. Milk composition was analyzed using a Milkoscan 203 (Foss Electric DK). Milk solids yield (kg) was calculated by summing milk fat and protein yields. Solids-corrected milk yield was calculated using the equation of Tyrrell and Reid (1965).

During wk 3, 5, and 7, milk samples were collected from a successive p.m. and a.m. milking. Milk samples collected were proportional to the milk yield of each cow. The individual milk samples from each of the 2 milkings were composited per treatment, giving composite samples for each treatment.

From the composite milk samples, approximately 500 mL per treatment was used for detailed wet chemistry of N fractions. This included total milk protein percentage, nonprotein N, and noncasein N. These were measured using the Kjeldahl method (IDF 20-3: 2004, IDF 20-4: 2001, and IDF 29-1: 2004, respectively) using a Tecator Digester Auto and Kjeltac 8400 distiller (Foss Electric).

A further 1,000 mL per treatment was skimmed using a Disc Bowl Centrifuge (Armfield Ltd., Ringwood, UK). The milk was heated to 40°C in a waterbath and then poured into the bowl section of the centrifuge. After approximately 20 s, the top valve was opened to allow the milk into the separating discs. The skim milk was collected and the cream discarded. The centrifuge was dismantled and all of the components cleaned. Once reassembled, the process was repeated for the other treatment milk samples.

One hundred milliliters of the skim milk per treatment was used for protein composition analysis. Separation of the milk protein fractions was achieved in reverse phase mode using an Agilent Poroshell 300SB C18 column (2.1 mm × 75 mm; Agilent Technologies

UK Ltd., Cheshire, UK). The HPLC equipment consisted of an Agilent 1200s with quaternary pump and multi-wavelength detector. Gradient elution and peak detection were performed according to Mounsey and O'Kennedy (2009). The skim milk samples and standards were prepared according to Guyomarc'h et al. (2007) and diluted volumetrically. All CN and whey standards were supplied by Sigma-Aldrich (Sigma-Aldrich, Vale Road, Arklow, Co. Wicklow, Ireland).

Body weight and BCS were recorded weekly. Body weight was assessed using a portable weighing scale and the Winweigh Software Package (Tru-Test Ltd., Auckland, New Zealand). During weighing, BCS was assessed by an experienced trained observer using the method of Edmonson et al. (1989), where 1 is emaciated and 5 is obese.

Individual cow grass DMI was estimated during wk 4 of the study using the n-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989). All cows were dosed twice daily, before milking, for 12 consecutive days with a filter paper bung (Carl Roth, GmbH and Co. KG, Karlsruhe, Germany) containing 500 mg of dotriacontane (C₃₂). From d 7 of dosing fecal grab samples were collected from each cow twice daily for the remaining 6 d. The fecal grab samples were then bulked (12 g of each sample collected) and dried for 48 h in a 40°C oven in preparation for chemical analysis.

In conjunction with the fecal collection, the diet of animals was also sampled. Grass representative of that grazed (taking cognizance of the previous day's defoliation height recorded for each treatment) was collected from each treatment grazing area before a.m. grazing on d 6 to 11 (inclusive) of the intake measurement period. Two samples of approximately 250 g were taken from each treatment grazing area with a Gardena hand shears. Grass silage samples were collected daily from the feed face immediately after the silage was dispensed. Concentrate samples were collected daily from the in-parlor feeders.

The n-alkane concentrations (C₂₅–C₃₆) of the feces, grass, grass silage, and concentrate were analyzed using a modification of the method of Mayes et al. (1986), which used direct saponification (Dillon, 1993). The DMI of grass, grass silage, and concentrate were estimated from the concentrations of the n-alkanes (C₂₇–C₃₅) in the DM of the feces, grass, grass silage and concentrate, and the daily dosage rate of C₃₂. A least-squares optimization procedure was used (Microsoft Excel SOLVER, Microsoft Corp., Redmond, WA). This was a modification of the "Eatwhat" method of Dove and Moore (1995). The method minimized the sum of the squared discrepancies for each alkane between the actual fecal n-alkane concentration (corrected for

incomplete recovery) and calculated fecal n-alkane concentration in DM as follows:

$$\text{calculated } F_i = \text{grass intake } G_i + \text{grass silage intake } S_i + \text{concentrate intake } C_i + \text{dose}_i / \text{fecal DM output},$$

where F_i , G_i , S_i , and C_i were the respective concentrations of n-alkane i in the feces, grass, grass silage, and concentrate DM, respectively, and Dose_i was the amount of n-alkane i in the daily dose. In the above equation, the DMI of grass and grass silage, and the estimation of fecal DM output, were the unknown variables determined using the optimization method. All other parameters were known variables. Dose_i was zero for all alkanes except for C₃₂-alkane. The recovery correction factors used to adjust the actual n-alkane concentrations in the DM of the feces were those reported by Dillon (1993). They were 0.618, 0.686, 0.722, 0.769, 0.777, 0.844, and 0.891 for heptacosane (C₂₇-alkane), octacosane (C₂₈-alkane), nonacosane (C₂₉-alkane), triacontane (C₃₀-alkane), hentriacontane (C₃₁-alkane), tritriacontane (C₃₃-alkane), and pentatriacontane (C₃₅-alkane), respectively. The recovery factor for C₃₂-alkane was assumed to be the same as that of C₃₃-alkane. The DMI of grass silage by GSH and GSL was estimated using the same least-squares procedure as described above.

Fecal samples were also analyzed for N concentration using a Leco N Analyzer (Leco FP-528, Leco Corporation, St. Joseph, MI). Fecal N excretion was calculated using the estimated fecal output and the analyzed fecal N concentration.

Urine N excretion was determined by subtracting the N excreted in feces and milk from the total N intake (van Vuuren et al., 1993).

The efficiency of N utilization was calculated as N excreted in milk (protein yield/6.25) divided by total N intake {[N concentration in grass (n = CP/6.25) × grass DMI] + supplementary feed N intake (where applicable)}, assuming a zero N accretion in the body.

Blood samples were collected from each cow via coccygeal venipuncture in the week before the beginning of the study, during wk 3 and 5, and in the final week of the study. Blood samples were collected immediately after morning milking (approximately 0830 h). Samples were collected into a Vacutainer (BD, Dun Laoghaire, Ireland) containing lithium heparin as an anticoagulant. Samples were immediately centrifuged at 3,000 × g (4°C) for 10 min. The plasma was decanted and immediately frozen at –20°C before analysis. The blood metabolites analyzed were glucose, NEFA, BHBA, BUN, total protein, and albumin. Analysis was carried out using appropriate kits (glucose, total protein,

and BUN: ABX Mira, Cedex 4, France; NEFA: Wako Chemicals GmbH, Neuss, Germany; BHBA: Randox Laboratories Ltd., Crumlin, County Antrim, UK; Albumin, Horiba ABX, Montpellier, France) on an ABX Pentra 400 autoanalyzer (ABX Mira).

Statistical Analysis

All statistical analyses were carried out using mixed models in SAS v9.3 (SAS Institute Inc., Cary, NC). The data for milk yield, solids corrected milk yield, milk solids yield, milk protein concentration, milk fat concentration, milk lactose concentration, milk CN concentration, MUN concentration, BW, BCS, and blood metabolites were analyzed. The respective pre-experimental variables were used as a covariate in the analysis. The fixed factors in the model were experimental week, treatment, and the interaction of treatment and week. Week was the repeated measure. Cow was included as the random effect. The covariance structure with the lowest Akaike information criterion was retained in the final model (compound symmetry).

Composite milk N fractions, composite milk protein composition, N intake, fecal N excretion, urine N excretion, total N excretion, and DMI were analyzed using mixed models in SAS v9.3. The model included treatment. No repeated statement or covariance structure was used in the model.

In all cases, statistically significant differences between least squared means were tested using the Tukey test for pairwise comparison of treatment means.

RESULTS

We found no significant difference in pregrazing herbage mass and pregrazing sward height among treatments. The mean pregrazing herbage mass during the study was (mean $\pm$ SD) $2,032 \pm 281$ kg of DM/ha (>4 cm) and the pregrazing sward height was (mean $\pm$ SE) 12.2 ± 0.23 cm. Treatment HG (mean $\pm$ SE; 4.53 ± 0.048 cm) had a greater postgrazing sward height than all other treatments ($P < 0.001$). Treatments GSL (4.11 ± 0.048 cm), GCL (4.03 ± 0.048 cm), and GSH (4.02 ± 0.048 cm) had a significantly greater postgrazing sward height than LG (3.77 ± 0.048 cm). Treatment GSL also had a greater postgrazing sward height than GCH (3.89 ± 0.048 cm).

The chemical composition of the grass throughout the study is shown in Table 1 and was similar among treatments. The chemical composition of the silage and concentrate offered throughout the study is shown in Table 2.

Grass DMI, supplement DMI, and total DMI are presented in Table 3. A treatment effect on grass DMI

Table 1. Chemical composition of the grass throughout the experiment

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM silage (GSH)	SEM	P-value
DM (g/kg)	141	147	150	142	151	145	1.8	0.5755
Ash (g/kg of DM)	78	71	80	81	78	79	2.0	0.7458
CP (g/kg of DM)	274	278	272	273	268	278	4.9	0.9937
NDF (g/kg of DM)	377	385	385	375	404	374	6.9	0.7633
OM digestibility (g/kg)	796	814	812	789	783	795	14.9	0.6833

Table 2. Chemical composition of the supplementation offered throughout the experiment

Item	Concentrate	Grass silage
DM (g/kg)	900	310
CP (g/kg of DM)	159	156
NDF (g/kg of DM)	83	498
Ash (g/kg of DM)	57	101
Starch (g/kg of DM)	267	—
DM digestibility (g/kg)	—	705
OM digestibility (g/kg)	—	727

($P < 0.001$) was observed. Treatment GSH had a lower grass DMI than all treatments. The grass DMI of GCH and GSL was lower than that of HG, LG, and GCL. A significant effect of treatment on supplement DMI ($P < 0.001$) was observed. Treatment GCL and GSL had a similar supplement DMI, which was significantly lower than GCH and GSH. Treatment GCH had a greater supplement DMI than GSH. A significant treatment effect was observed on total DMI. The total DMI of GCH was greater than GSL and GSH. Total DMI in the HG, LG, and GCL treatments were similar and greater than GSH. The total DMI of GSL was similar to that of HG, LG, GCL, and GSH.

Milk yield, SCM yield, milk solids yield, milk fat concentration, milk protein concentration, milk lactose concentration, MUN concentration, and milk CN concentration are presented in Figure 1. We detected significant treatment by week interactions in all parameters ($P < 0.01$).

During every week of the experiment, GCH milk yield was significantly greater than LG and HG. During wk 1 to 6, GCH milk yield was greater than GSL. During wk 4 to 6, GCH milk yield was greater than GSH. During wk 7, GCH milk yield was greater than GCL. During every week of the experiment, GCL milk yield was greater than LG. During wk 1 to 6, GCL milk yield was greater than HG and GSL. During wk 5 and 6, GCL milk yield was greater than GSH. During wk 1 to 5 of the experiment, GSH milk yield was greater than LG. During wk 1 to 3, GSH milk yield was greater than HG. During wk 3, GSH milk yield was greater than GSL. No significant differences among the HG, LG and GSL treatments were observed. In summary, a treatment effect on milk yield and SCM was present. Treatment GCH and GCL had a significantly higher milk yield than HG, LG, and GSL. Treatment GSH was greater than LG, but similar to the other 4 treatments. Treatments HG, LG, and GSL were similar to one another. Treatment GCL and GCH had a greater SCM than all other treatments.

During every week of the experiment GCH milk solids yield was greater than LG, HG, and GSH. During

Table 3. The effect of offering different supplementation to grazing late lactation dairy cows on postgrazing sward height and DMI (wk 4 of study)

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM grass silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM grass silage (GSH)	SEM	P-value
Postgrazing sward height (cm)	4.53 ^a	3.77 ^c	4.03 ^{bc}	4.11 ^b	3.89 ^c	4.02 ^{bc}	0.048	<0.001
Grass DMI (kg/d)	17.0 ^a	16.5 ^{ab}	14.9 ^b	12.3 ^c	12.3 ^c	8.9 ^d	0.22	<0.001
Supplement DMI (kg/d)	—	—	3.0 ^a	2.9 ^a	6.0 ^c	5.1 ^b	0.02	<0.001
Total DMI (kg/d)	17.0 ^{ab}	16.5 ^{ab}	17.9 ^{ab}	15.2 ^{bc}	18.3 ^a	14.0 ^c	0.22	<0.001

^{a-d}Within a row, means with different superscripts differ significantly.

Figure 1. The effect of offering high grass allowance (HG; —◆—), low grass allowance (LG; -◇-), grass with low concentrate allowance (GCL; —■—), grass with low grass silage allowance (GSL; ···●···), grass with high concentrate allowance (GCH; —□—), or grass with high silage allowance (GSH; ···○···) to grazing late lactation dairy cows on milk yield and composition. *Significant differences between treatments during this observation week ($P < 0.05$); error bars indicate standard errors.

wk 1 to 6, GCH milk solids yield was greater than GSL. During wk 2, 3, 4, 6, and 7 GCH milk solids yield was greater than GCL. During wk 1 to 7 of the experiment, GCL milk solids yield was greater than LG and GSH. During wk 1 to 6, GCL milk solids yield was greater than HG and GSL. During wk 3 to 7, GSL milk solids yield was greater than LG. During wk 6 and 7, GSL milk solids yield was greater than GSH. During wk 7, GSL milk solids yield was greater than GCL and HG. During wk 7, HG milk solids yield was greater than GSH. During wk 3, 5, and 7, LG milk solids yield was less than HG. During wk 3 and 4, LG milk solids yield was less than GSH. In summary, a treatment effect on milk solids yield was observed. Treatment GCH had a significantly higher milk solids yield than all other treatments. Treatment GCL had a significantly higher milk solids yield than HG, LG, GSL, and GSH. Treatments GSL and HG had similar milk solids yield, which was greater than LG. Treatment GSH had a milk solids yield that was intermediate, and not significantly different, compared with HG, GSL, and LG.

During wk 5 and 6, the milk protein % of HG was greater than all other treatments. During wk 2, LG milk protein % was greater than the milk protein % of GCH, GCL, GSH, and GSL. During wk 4 and 5, LG milk protein % was greater than the milk protein % of GCH, GCL, and GSH. During wk 7, LG and HG milk protein % was greater than the milk protein % of GSH. No differences were found among GCH, GCL, GSH, and GSL treatments. In summary, an effect of treatment on milk protein concentration was present. Treatment HG had a greater milk protein concentration than GCH, GSH, GCL, and GSL. Treatment LG had a greater milk protein concentration than GCH, GSH, and GCL (which were similar to each other). Treatment GSL had a milk protein concentration which was intermediate, and not significantly different, compared with LG, GCH, GSH, and GCL.

The treatment by week interaction had a significant effect on milk fat, milk lactose, MUN, and milk CN concentrations, but no consistent differences among treatments were observed.

Composite milk N fractions are presented in Table 4. We observed a treatment effect on the concentration of total protein ($P < 0.01$), true protein ($P < 0.01$), and CN protein ($P < 0.05$). Treatment LG had a greater concentration of total protein than GSL, GSH, and GCH. Treatments HG and GCL did not differ significantly compared with any other treatment. The concentration of true protein in the grass-only treatments was significantly greater than GCH and GSH. Treatment GCL and GSL did not differ significantly compared with any other treatment. Casein protein concentration was greater in HG and GSL than in

GCH. Treatments LG, GCL, and GSH were similar to each other, and did not differ compared with any other treatment.

The protein composition of the composite milk samples is presented in Table 5. We detected that the concentration of α_{S1} -CN differed significantly among treatments ($P < 0.01$). The HG treatment had a greater concentration of α_{S1} -CN than GSH. All other treatments were intermediate and did not differ significantly to HG and GSH. Treatment GSH also had a lower concentration of α_{S2} -CN than LG ($P < 0.05$). All other treatments were intermediate and did not differ significantly to LG and GSH. The concentration of β -CN was greater in HG and GSL than in LG and GCH ($P < 0.01$). The concentration of β -CN in GCL and GSH did not differ from any of the other treatments. We found a significant treatment effect on the concentration of κ -CN ($P < 0.01$). The concentration of κ -CN in HG was significantly greater than that of GSL. All other treatments were intermediate and did not differ significantly compared with HG and GSL. We observed that the whey proteins (β -LG and α -LA) were both significantly affected by treatment. The concentration of β -LG in GCL was significantly greater than GCH ($P < 0.05$). We found no other significant differences among treatments in the concentration of β -LG. Treatments HG and GCH had a greater concentration of α -LA than GCL ($P < 0.01$). All other treatments were intermediate and did not differ significantly compared with HG, GCH, and GCL.

Nitrogen utilization efficiency data are presented in Table 6. We observed a significant effect of treatment on N intake ($P < 0.001$). Treatments HG, LG, and GCL had a greater N intake than GSL and GSH. The N intake of GCH was greater than GSH. No difference in N intake between GSL and GCH or between GSL and GSH was observed. We found a significant treatment effect on fecal N excretion ($P < 0.01$). Fecal N excretion was greater in GCH and GCL than in LG, GSL, and GSH. The fecal N excretion of HG was intermediate and did not differ compared with any other treatment. We detected a significant treatment effect on the excretion of urine N ($P < 0.001$). The urine N excretion of HG, LG, and GCL was greater than GSL and GSH. Treatment GCH had a greater urine N excretion than GSH. No difference was found in urine N excretion between GSL and GCH or between GSL and GSH. The proportion of N intake that was excreted in the urine and feces was significantly lower in GCH and GSH compared with HG, LG, and GCL ($P < 0.001$). Treatment GSL excreted a similar proportion of N intake compared with GCL, GCH, and GSH. Treatments HG and LG excreted a greater proportion of N intake than all other treatments.

Table 4. The effect of offering different supplementation to grazing late lactation dairy cows on milk N fractions

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM grass silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM grass silage (GSH)	SEM	P-value
Total protein (g/kg)	41.5 ^{ab}	42.4 ^a	41.1 ^{ab}	40.1 ^b	39.6 ^b	39.4 ^b	0.20	0.0061
True protein (g/kg)	38.2 ^a	38.8 ^a	37.6 ^{ab}	37.5 ^{ab}	36.2 ^b	36.2 ^b	0.01	0.0007
Casein protein (g/kg)	28.9 ^a	28.6 ^{ab}	27.9 ^{ab}	29.0 ^a	26.5 ^b	27.0 ^{ab}	0.23	0.0449
Whey protein (g/kg)	10.3	9.2	9.8	8.5	9.7	9.3	0.24	0.4446
Nonprotein N (g/kg)	0.51	0.56	0.54	0.42	0.53	0.49	0.020	0.3992
True protein as a proportion of total protein	0.92	0.92	0.92	0.93	0.91	0.92	0.004	0.3888
Casein as a proportion of true protein	0.76	0.74	0.74	0.77	0.73	0.74	0.008	0.4532

^{a,b}Within a row, means with different superscripts differ significantly.

Table 5. The effect of offering different supplementation to grazing late lactation dairy cows on milk protein composition

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM grass silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM grass silage (GSH)	SEM	P-value
α_{s1} -CN (g/L)	15.88 ^a	14.93 ^{ab}	15.55 ^{ab}	15.51 ^{ab}	14.87 ^{ab}	14.53 ^b	0.102	0.0115
α_{s2} -CN (g/L)	3.17 ^{ab}	3.30 ^a	3.19 ^{ab}	3.15 ^{ab}	3.14 ^{ab}	2.96 ^b	0.022	0.0110
β -CN (g/L)	11.67 ^a	10.78 ^b	11.47 ^{ab}	11.57 ^a	10.71 ^b	10.90 ^{ab}	0.070	0.0017
κ -CN (g/L)	5.54 ^a	4.86 ^{ab}	5.33 ^{ab}	4.64 ^b	5.04 ^{ab}	5.13 ^{ab}	0.065	0.0003
β -LG (g/L)	5.44 ^{ab}	5.21 ^{ab}	5.75 ^a	5.42 ^{ab}	4.93 ^b	5.43 ^{ab}	0.061	0.0290
α -LA (g/L)	0.76 ^a	0.69 ^{ab}	0.58 ^b	0.74 ^{ab}	0.83 ^a	0.68 ^{ab}	0.016	0.0055

^{a,b}Within a row, means with different superscripts differ significantly.

Blood metabolites data are presented in Table 7. We found no significant effects of treatment on total protein concentration or BUN concentration. The concentration of glucose in HG cows was greater than that of LG and GSH ($P < 0.05$). Treatments GCL, GSL, and GCH were intermediate and did not differ compared with any other treatment. We observed a significant effect of treatment on NEFA concentration ($P < 0.05$). Cows within the GSL treatment had a significantly greater blood NEFA concentration than GCL, GCH, and GSH. Treatment HG and LG were intermediate and did not differ to any other treatment. We detected a significant treatment effect on blood BHBA concentration ($P < 0.01$). Treatments HG, LG, and GSH had a lower concentration of BHBA than GCH. The BHBA concentration of cows in GCL and GSL were intermediate and did not differ to any other treatment. We also found a significant effect of treatment on blood albumin concentration ($P < 0.05$). Cows within HG, LG, and GCH had a greater concentration of blood albumin than GSH. The GCL and GSL treatments were intermediate and did not differ significantly compared with any other treatment.

We observed no significant effect of treatment on BW and BCS during the experiment. The mean BW of the HG, LG, GCL, GSL, GCH, and GSH treatments was 523, 511, 520, 518, 528, and 512 ± 15.0 kg, respectively. The mean BW during each week of the experiment (1–7) was 524, 512, 517, 524, 530, 522, and 526 ± 7.1 kg, respectively. The mean BCS of HG, LG, GCL, GSL, GCH and GSH was 3.28, 3.17, 3.22, 3.24, 3.40, and 3.26 ± 0.053 , respectively. The mean BCS during each week of the experiment (1–7) was 3.40, 3.26, 3.26, 3.46, 3.55, 3.26, and 3.26 ± 0.029 , respectively.

DISCUSSION

Grass silage is the most important feed source available to Irish dairy cows after grazed grass (Finneran et al., 2010). Recent grassland management recommendations in Ireland have resulted in more baled grass silage being used as supplementation. This has led to baled grass silage becoming an increasingly important feed source in Ireland. The objective of this study was to investigate the effects of offering different supplementary feeds to grazing spring-calving dairy cows in late lactation on milk yield and composition, NUE, and milk N fractions.

The grass grazed by the cows on the different treatments was similar, indicated by the similarity in chemical composition. The grazing management was in accordance with that recommended by O'Donovan (2000). Differences in grazing pressure applied by the different treatments were reflected in differences in

Table 6. The effect of offering different supplementation to grazing late lactation dairy cows on nitrogen utilization efficiency

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM grass silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM grass silage (GSH)	SEM	P-value
Total N intake (g/d)	691 ^a	680 ^a	713 ^a	591 ^{bc}	676 ^{ab}	514 ^c	11.2	<0.001
Excretion								
Feces (g/d)	111 ^{ab}	107 ^b	124 ^a	105 ^b	127 ^a	103 ^b	2.0	0.001
Urine (g/d)	510 ^a	498 ^a	492 ^a	404 ^{bc}	448 ^{ab}	336 ^c	8.3	<0.001
Total (g/d)	620 ^a	605 ^{ab}	617 ^a	509 ^{bc}	575 ^{ab}	439 ^c	10.0	<0.001
Proportion of N excreted	0.89 ^a	0.89 ^a	0.87 ^b	0.86 ^{bc}	0.85 ^c	0.85 ^c	0.001	<0.001

^{a-c}Within a row, means with different superscripts differ significantly.

Table 7. The effect of offering different supplementation to grazing late lactation dairy cows on blood metabolites

Item	17 kg of DM grass (HG)	14 kg of DM grass (LG)	14 kg of DM grass + 3 kg of DM concentrate (GCL)	14 kg of DM grass + 3 kg of DM grass silage (GSL)	11 kg of DM grass + 6 kg of DM concentrate (GCH)	11 kg of DM grass + 6 kg of DM grass silage (GSH)	SEM	P-value
Glucose (mmol/L)	3.6 ^a	3.1 ^b	3.2 ^{ab}	3.4 ^{ab}	3.2 ^{ab}	3.1 ^b	0.05	0.0263
NEFA (mmol/L)	0.14 ^{ab}	0.14 ^{ab}	0.12 ^b	0.15 ^a	0.12 ^b	0.12 ^b	0.003	0.0545
BHBA (mmol/L)	0.36 ^a	0.37 ^a	0.49 ^{ab}	0.40 ^{ab}	0.55 ^b	0.33 ^a	0.016	0.0002
Total protein (g/L)	73.1	77.2	51.7	72.6	73.1	75.3	2.80	0.2070
Blood urea N (mmol/L)	7.6	5.9	8.1	6.4	6.7	6.2	0.71	0.8744
Albumin (g/L)	31.0 ^a	30.7 ^a	29.0 ^{ab}	29.5 ^{ab}	30.7 ^a	26.6 ^b	0.41	0.0122

^{a,b}Within a row, means with different superscripts differ significantly.

postgrazing sward height. The lower postgrazing sward height in the LG treatment indicated that the grazing pressure was greater than in HG, which had a higher postgrazing sward height. The lower postgrazing sward height was indicative of the lower grass allowance offered to the LG treatment compared with the HG treatment. Offering supplementation such as silage or concentrate reduced the grazing pressure, and therefore increased postgrazing sward height compared with the LG treatment.

Dry Matter Intake and Milk Production

Grass DMI can be reduced when cows are offered supplementary feeds along with their grass allowance, which is known as substitution rate (**SR**; Kellaway and Harrington, 2004). Substitution rate is calculated as $SR (kg/kg) = (grass\ DMI\ in\ unsupplemented\ treatment - grass\ DMI\ in\ supplemented\ treatment) / supplement\ DMI$. It is therefore possible to discuss SR on the GCL and GSL treatments compared with the LG treatment (all offered 14 kg of DM >4 cm). It is not possible for HG to be included in this comparison because the cows on this treatment were offered a different herbage allowance (17 kg of DM >4 cm). Grass, supplement, and animal factors have been identified as being important factors influencing SR and MR (Stockdale, 2000). The factors affecting SR include the amount and type of base feed, the amount and type of supplement offered, and the stage of lactation and production potential of the cow (Baudracco et al., 2010). The type of supplement offered affects SR because different feeds have different fill values (**FV**; filling effect of a feed in the rumen; Faverdin et al., 2011). The FV of grass silage is greater than that of concentrate. The SR therefore increases when grass silage is used as the supplementary feed compared with using concentrate at the same supplementation rate or compared with an unsupplemented grass diet (Bargo et al., 2003). In the current study, offering 3 kg of DM grass silage as supplementation to 14 kg of DM grass caused a SR of 1.45 kg of DM grass per kg of grass silage DMI. We found no SR when 3 kg of DM concentrate was offered as supplementation to 14 kg of DM grass. This demonstrates that, with a similar grass allowance, the FV of the supplementary feed influences the SR. Stockdale and Trigg (1985) found that when 14.9 kg of DM grass and 3.6 kg of DM concentrate was consumed, no substitution of grass by concentrate occurred, which agrees with the current study.

Cows within the HG treatment were offered 17 kg of DM >4 cm and had a postgrazing sward height of 4.5 cm. This resulted in a lower total DMI than offered. Cows within the LG treatment were offered 14

kg of DM >4 cm and had a postgrazing sward height of 3.8 cm, which resulted in a greater total DMI than offered. The result of this was a similar total DMI in the 2 grass-only treatments. Total DMI was reduced by 10.6 and 17.6% when 3 or 6 kg of DM grass silage was offered, respectively, compared with when 17 kg of DM grass was offered. In comparison, total DMI was increased by 5.3 and 7.6% when 3 or 6 kg of DM concentrate was offered, respectively, compared with when 17 kg of DM grass was offered. The reduction in total DMI with grass silage is due to the greater FV of grass silage compared with concentrate. Bargo et al. (2003) observed that total DMI can be increased by up to 24% when up to 10 kg of DM concentrate are included in the diet of grazing dairy cows compared with grass-only diets. A target of supplementary feeding is to increase total DMI. In the case of the current study, total DMI was increased when concentrate was offered as supplementation. Offering 6 kg of DM of grass silage reduced total DMI. Offering 3 kg of DM of grass silage also statistically reduced total DMI compared with GCH, but not compared with the grass-only treatments or the GCL treatment.

Energy intake is associated with milk yield and milk solids production (Broderick, 2003). This agrees with the current study because milk yield, milk solids yield, and SCM were greatest on GCH and GCL, which had the highest DMI and likely the highest energy intake due to the energy-dense nature of concentrate.

The MR of the HG treatment was 0.2 kg of milk/kg of extra grass offered (compared with the LG treatment). This is similar to the finding of Delaby et al. (2001) who reported that for grass allowances between 12 and 22 kg of DM, 5 kg of DM extra grass consumed would result in a MR of approximately 1 kg of milk, which is a MR of 0.2 kg of milk/kg of extra grass. As grass allowance increases further, the MR would be expected to decrease (Bargo et al., 2003). The MR of the GSL treatment in the current study was 0.2 kg of milk/kg of grass silage offered. This is lower than that reported by Crosse and Gleeson (1987; 0.39 kg of milk/kg of grass silage offered) who offered spring-calving dairy cows in late lactation grazed grass supplemented with grass silage. They offered grass silage *ad libitum*. The grass silage intake was 8.6 kg of DM. The grass allowance was more restricted than in the current study. The MR of the GCL cows in the current study was 1.4 kg of milk/kg concentrate offered. This is higher than that observed by Delaby et al. (2001) and Burke et al. (2008). Burke et al. (2008) observed a MR of 0.9 kg of milk/kg concentrate consumed when concentrate was used as a supplement for grazing dairy cows. It was reported that a MR of 1 kg of milk/kg of concentrate can be expected provided that the level of concentrate does not exceed

6 kg per cow/d (Peyraud et al., 2004). The cause of the increased MR in the current study may, again, be related to the quality of the grass consumed by the cows. A poorer quality base feed would be expected to result in a larger MR (Bargo et al. 2003). These results suggest that further research should be carried out to explore the nutritive value of autumn grass.

During wk 3, 5, and 7, the milk solids yield of the LG cows was significantly lower than the HG cows. No significant difference was observed in grass DMI between the treatments in wk 4, but the increased grazing pressure of the LG cows, demonstrated by a lower postgrazing sward height, may offer an explanation for the reduction in milk solids yield.

A treatment by week effect on milk fat concentration was present, but a consistent treatment effect was not observed. A reduction in milk fat concentration can be caused by changes in rumen fermentation patterns such as a decrease in rumen pH (Plaizier et al., 2008). The significant differences in the current study were predominantly that, during some weeks, the milk fat concentration of HG and LG was greater than GSH and GSL. The use of forage-based supplements would be expected to prevent milk fat depression due to the buffering effect of the forages on rumen pH. The buffering effect is a result of increased saliva production caused by chewing (Andersen et al., 1999). This did not occur. A reduction in milk fat concentration when grass silage was fed has previously been reported, but with no potential explanation offered (Phillips, 1988; O'Brien et al., 1996) and therefore requires further investigation. The rapid degradation in the rumen of rapidly degradable feeds, such as starch-based concentrates, leads to increased concentrations of VFA and a consequent reduction in rumen pH (Krause and Oetzel, 2006). It was anticipated that the GCH would have the lowest milk fat concentration due to the high proportion of concentrate in the diet and low proportion of fibrous feed in the form of grass. This was only observed in wk 8 when GCH had a lower milk fat concentration than HG. On the GCH diet, two-thirds of DMI was grass, which clearly maintained healthy rumen conditions for the duration of the study.

Milk protein concentrations in GCL, GSL, GCH, and GSH were lower than in HG. Broderick (2003) reported an increase in milk protein concentration when dietary CP increases from 150 to 167 g/kg. They showed that further increases in dietary CP to 187 g/kg had no further positive effect on milk protein concentration. Dietary CP intake in the current study exceeded requirements on all treatments due to the greater grass CP concentration than anticipated (average 274 g of CP/kg of DM). The dietary CP concentrations of the HG and LG treatments in the current study were 274 and

278 g/kg of DM, respectively. The GCL, GSL, GCH, and GSH treatments had a dietary CP concentration of 253, 251, 232, and 234 g/kg of DM, respectively. The milk yield of GCL, GSL, and GCH was greater than HG, which may have diluted the milk protein, reducing the concentration compared with the HG treatment.

Milk Nitrogen Fractions and Milk Protein Composition

Although significant differences were observed among the treatments in the concentration of total and true protein in the composite milk samples, true protein as a proportion of total protein was similar among treatments. The lower true protein concentration in the GCH and GSH treatments can therefore be attributed to the lower concentration of total protein in these treatments. An increased concentration of CN in milk is important from a processing perspective because it is associated with increased cheese yield (Lucey and Kelly, 1994). Energy intake has been positively associated with both milk total protein concentration (Phillips, 1988) and CN concentration (Kefford et al., 1995). The reduced total and true protein concentration of GSH is therefore unsurprising considering its total DMI was low. The low concentration of true protein and CN protein in GCH was unexpected because it had the greatest total DMI and thus total energy intake.

The concentration of the different proteins within milk largely determines the milk technological properties and nutritional value (Dalgleish, 1993). The 6 major proteins found in bovine milk are α_{S1} -CN, α_{S2} -CN, β -CN, and κ -CN, together with the whey proteins α -LA and β -LG. The concentrations of all proteins were within the typical range found in dairy cows as specified by Farrell et al. (2004). Differences existed in the concentration of the different proteins among the treatments. α_{S1} -Casein is the principal component of the CN micelle, which is dispersed as a colloid in milk (Fox and McSweeney, 2003). In general, the treatments that had the highest CN concentration had the highest α_{S1} -CN concentration (HG and GSL) and those that had the lowest CN concentration had the lowest α_{S1} -CN concentration (GCH and GSH). Hermansen et al. (1999) reported that the concentration of α_{S1} -CN can be altered due to the supply of urea to the small intestine. The supply of amino acids and urea to the small intestine is largely dependent on the diet, with an increase in dietary CP and energy among the factors that can increase supply (Hristov et al., 2004). The decreased concentration of α_{S1} -CN in GSH compared with HG is likely due to the large proportion of a low energy supplementary feed included in the diet of GSH, resulting in GSH having the lowest energy intake. This

may have reduced the availability of amino acids for CN synthesis. α_{S2} -Casein occurs at a lower concentration in bovine milk than α_{S1} -CN, but the 2 are closely linked because when one is reduced, the other is also reduced (Dalgleish and Corredig, 2012). Thus the low total DMI and energy intake exhibited by GSH cows is likely to account for their lower milk α_{S2} -CN concentration. The concentration of β -CN and κ -CN were highest in HG, but they were not the lowest in GSH. The sample numbers in the analysis of the protein composition and the milk N fractions of the composite samples were low ($n = 3$ per treatment). Continued research is needed to fully establish the effects of nutrition on milk N fractions and protein composition.

The concentration of β -LG was lowest in GCH. β -Lactoglobulin is the most abundant whey protein, accounting for approximately 58% of total whey protein (Hogarth et al., 2004). Aleandri et al. (1990) reported a positive association between the concentration of β -LG and milk fat concentration. Milk fat concentration was significantly reduced in the GCH treatment in wk 8, but no consistent significant differences among treatments were found. Differences in β -LG concentration have also been associated with blood albumin concentration (Gray and Mackenzie, 1987). The concentration of blood albumin in GCH was intermediate to the other treatments. Because of the lack of corroborating evidence, the differences in β -LG among treatments may not be of biological significance. It is desirable to increase the concentration of α -LA in bovine milk so that the milk will more closely mimic the protein composition of human milk. This would make it more suitable for processing into infant milk formula (Lien, 2003). During lactation α -LA complexes with galactosyltransferase to produce lactose synthase. This is responsible for the production of lactose (Bauman et al., 2006). Milk volume is regulated by lactose (Mirzaei-Aghsaghalil and Fathi, 2012). In the current study, the concentration of α -LA was greatest in HG and GCH. Treatment GCH had the highest milk yield of the 6 treatments in the study offering an explanation for the increase in α -LA concentration. It is unclear why GCL, which had the second highest milk yield, had the lowest α -LA concentration.

Blood Metabolites and Nitrogen Utilization Efficiency

Blood metabolites such as glucose, NEFA, BHBA, total protein, and albumin concentrations can be used as indicators of nutritional stress (LeBlanc, 2010). The concentration of glucose is associated with carbohydrate metabolism (McDonald et al., 2001). Blood glucose concentration in the current study, although significantly different among treatments, was within the

normal range proposed by Mee and Nolan (1994) and was comparable with the blood glucose concentrations reported by Law et al. (2009). During times of BW and BCS loss, FA may not be completely oxidized in the liver, resulting in an increase in the concentration of BHBA in the blood (LeBlanc, 2010). An increase in BHBA is more likely to be observed in early lactation due to BW and BCS loss postcalving (McNamara et al., 2003). In late lactation, the desired concentration of NEFA is below 0.4 mmol/L (Whitaker et al., 1983). The concentration of NEFA in the current study was below this target. The NEFA and BHBA concentrations in all treatments in the current study were within the normal ranges proposed by Mee and Nolan (1994) and were similar to those reported by Law et al. (2009).

The N intake in the current study was similar to that measured by Burke et al. (2008). In that study grass allowances in the HG treatment (20 kg of DM grass/cow), LG treatment (15 kg of DM grass/cow), and supplemented treatments (15 kg of DM grass/cow) were greater than in the current study. Grass CP concentration in the current study was greater than in the study by Burke et al. (2008). This accounts for the similarity in N intake across the 2 studies. According to Tamminga (1992), a large proportion of N intake in dairy cows is not recovered in milk, with proportionally 0.75 to 0.85 excreted in the feces and urine. As stage of lactation increases, NUE decreases (Castillo, 2001). This means that N excreted in feces and urine increases. The proportion of N excreted in the feces and urine in the current study ranged from 0.85 to 0.89. Given that the current study was conducted using cows in late lactation, with an average DIM of 233 d when NUE was measured, NUE was expected to be poorer than when NUE was measured on similar animals in early lactation (Reid et al., 2015). Urine is the primary route for N excretion and can be increased with increasing N intake (Spek et al., 2013). The N intake in HG, LG, GCL, and GCH were greatest in the current study, with GSH having the lowest N intake. This was reflected in the urine excretion because urine N excretion in HG, LG, GCL, and GCH was the greatest, and GSH had the lowest urine N excretion. Olmos Colmenero and Broderick (2006) observed that as N intake increases urine N excretion also increases. Fecal N excretion is poorly related to dietary N intake (Whelan et al., 2012). Fecal N consists largely of endogenous N and undigested feed N (Whelan et al., 2012). The increased fecal N in the GCL and GCH treatments may be due to an increase in rumen undegradable protein (RUP) as a result of the inclusion of concentrate in the diet. The concentration of RUP was estimated to be greater in the concentrate (64 g/kg of DM) than in the grass (47 g/kg of DM; figures from Jarrige, 1989). Nitrogen

excreted as a proportion of N intake was lowest in GCH and GSH. The milk protein yield of the GCH treatment was greatest, offering an explanation for the increased NUE compared with the grass-only treatments, which had a similar N intake. The increased NUE in GSH may be attributed to its reduced total N intake, which reduced the quantity of excess N to be excreted in urine and feces, compared with the grass-only treatments. The grass-only treatments had the highest proportion of N intake excreted. The N intake of the grass-only treatments was high. The milk yield and milk protein yield of the grass-only treatments was low, which may explain why the NUE was lower than GCL, which had a similar N intake, but increased NUE. Higgs et al. (2013) reported that N intake is the main factor influencing NUE, with an increase in N intake reducing NUE. The NUE of HG and LG was poorer than the NUE of GSL and GSH. The N intake of HG and LG was greater than GSL and GSH, resulting in an increase in the proportion of N intake being excreted. The concentration of BUN is closely related to the concentration of MUN and urine N (Spek et al., 2013). In the current study, BUN and MUN were not significantly different among treatments. This indicates that although differences were present among treatments in NUE, differences in N excretion were not great enough to affect BUN or MUN concentrations.

CONCLUSIONS

When grass quality, quantity, or both, are deficient, total DMI can be increased with the use of supplementation. We found that a high MR can be achieved by reducing the SR, resulting in an increase in milk yield and milk solids yield. Offering concentrate as supplementation to late lactation spring-calving dairy cows increased milk yield and milk solids yield compared with cows offered grass silage as supplementation or cows on a grass-only diet. We detected no difference in milk solids yield when supplementing 14 kg of DM or 11 kg of DM grass with 3 kg of DM or 6 kg of DM concentrate, respectively. We also observed no difference in DMI, milk yield, or milk composition in offering 11 kg of DM grass and 6 kg of DM grass silage compared with 14 kg of DM grass and 3 kg of DM grass silage. Nitrogen utilization efficiency at the animal level was improved with the addition of supplementary feeds into the grass-based diet. The full life-cycle assessment implications of grass-based dairy cow supplementation strategies on N excretion should, however, be investigated. Offering supplementation reduced the milk total protein concentration, true protein concentration, and CN concentration compared with the grass-only diets. α_{S1} -Casein was also highest in the HG treatment. This

suggests that milk from a grass-only diet may be more suitable for processing, even if milk production is not as high as from supplemented diets.

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