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Incorporating white clover (*Trifolium repens* L.) into perennial ryegrass (*Lolium perenne* L.) swards receiving varying levels of nitrogen fertilizer: Effects on milk and herbage production

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ABSTRACT

White clover (*Trifolium repens* L.; clover) can offer a superior nutritional feed compared with perennial ryegrass (*Lolium perenne* L.; PRG) and offers an additional or alternative source (or both) of N for herbage production. The objective of this study was to investigate the effect of including clover into PRG swards receiving 150 (Cl150) or 250 kg of N/ha (Cl250) compared with a PRG-only sward receiving 250 kg of N/ha (Gr250) on herbage production, milk production, and herbage dry matter intake (DMI) in an intensive grass-based spring calving milk production system over 2 full lactations. A farm systems experiment was established in February 2013, and conducted over 2 grazing seasons [2013 (yr 1) and 2014 (yr 2)]. In February 2013 (yr 1), 42 Holstein-Friesian spring-calving dairy cows, and in February 2014 (yr 2), 57 Holstein-Friesian spring-calving dairy cows were allocated to graze the Cl150, Cl250, and Gr250 swards (n = 14 in yr 1 and n = 19 in yr 2) from February to November, at a stocking rate of 2.74 cows/ha. Herbage DMI was estimated twice in yr 1 (May and September) and 3 times in yr 2 (May, July, and September). Treatment did not have a significant effect on annual herbage production. Sward clover content was greater on the Cl150 treatment than the Cl250 treatment. The cows grazing both clover treatments (Cl250 and Cl150) produced more milk than the cows grazing Gr250 from June until the end of the grazing season. A significant treatment by measurement period interaction was observed on total DMI. In May, the cows on the Cl250 treatment had the greatest DMI. In July, the cows on the clover treatments had greater DMI than those on the Gr250 treatment, whereas in September, the cows on the Cl150 treatment had the lowest DMI. In conclusion, including clover in a PRG sward grazed by spring-calving dairy cows can result in increased animal performance, particularly in the second half of lactation. Reducing N fertilizer application to 150 kg of N/ha on grass-clover swards did not reduce herbage production compared with grass-only swards receiving 250 kg of N/ha. White clover can play an integral role in intensive grazing systems in terms of animal performance and herbage production.
Key words: white clover, perennial ryegrass, nitrogen, milk production, dry matter intake

INTRODUCTION

Pasture-based milk production systems in temperate regions are generally low cost because grazed grass is the primary feed source for dairy cows (Clark et al., 2007; Finneran et al., 2012). To maintain competitiveness, pasture-based dairy production systems must efficiently convert high-quality grazed pasture into milk (Dillon et al., 2008; Chapman et al., 2017). Increased milk production must occur through increased herbage production and utilization (Dillon et al., 2008) if grass-based systems are to remain profitable.

The objective of intensive pasture-based dairy production systems is to maximize milk production from grazed pasture grass (Penno, 2000; Dillon et al., 2008; Humphreys et al., 2008). Perennial ryegrass (*Lolium perenne* L.; **PRG**) is the predominant forage species used in grazing systems in temperate regions of the world (Fulkerson et al., 2007). Good grazing management practices (O'Donovan et al., 2011) can result in improved feed quality and to a certain extent negate some of the climatic and seasonal effects (Dillon et al., 2008) on pasture quality. The increasing stem proportion of the PRG plant in mid-summer can result in reduced herbage quality (Ulyatt et al., 1988; Hennessy et al., 2008; Wims et al., 2013) and subsequently reduced animal performance (Blaser, 1964; Wims et al., 2013). It has long been recognized that white clover

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(*Trifolium repens* L.; hereafter referred to as clover) provides a forage nutritionally superior to PRG (Thomson and Raymond, 1970) and offers an alternative to N fertilizer application or an additional source of N for the sward for herbage production (Ledgard and Steele, 1992; Phillips and James, 1998).

Intensive pasture-based systems usually rely on high N fertilizer input to provide an adequate supply of good-quality herbage for grazing animals. In mixed PRG-clover swards, however, clover can offer an alternative source of N. Ledgard and Steele (1992) and Schils et al. (1999) found that increasing the N fertilizer application can result in a reduction in sward clover content. Recently, Nyfeler et al. (2011) under cutting and Enriquez-Hidalgo et al. (2014a, 2016) and Egan et al. (2017) under grazing have succeeded in maintaining a high sward clover content (>20%) in swards receiving high levels of N fertilizer (>200 kg of N/ha). Sward management practices such as frequent (Harris and Clark, 1996; McKenzie et al., 2003) and tight (≤4 cm; Yu et al., 2008; Phelan et al., 2013) grazing have been shown to maintain or increase sward clover content in high N fertilizer application systems.

The benefits of clover for milk production have been reported in short-term component and indoor feeding experiments (Thomson et al., 1985; Harris et al., 1998) and full-season grazing studies (Egan et al., 2017). In grazed PRG-clover swards, an increase in milk production can occur due to a combination of both feed quality and intake factors (Clark and Harris, 1996; Harris et al., 1998). In high-stocking-rate (>2.5 cows/ha) pasture-based systems, clover is usually omitted due to high N application rates and the likely reduction in sward clover content due to high N input (Reid, 1970; Frame and Newbould, 1986; Davies, 1992), as well as the likely damage to clover stolons from treading and burial (Davies, 1992). Usually, in grass-clover systems N application rate is reduced, and stocking rate is lower than on grass-only high N systems [e.g., Humphreys et al. (2009) and Schils et al. (2000)].

Enriquez-Hidalgo et al. (2014a) and Egan et al. (2017) found similar herbage production on intensively grazed (8–9 times per year) grass-clover swards receiving 250 kg of N/ha compared with grass-only swards receiving 250 kg of N/ha. Consequently, it is likely that some reduction in N application to grass-clover systems at high stocking rate (>2.5 cows/ha) may be possible. Therefore, the objective of this study was to investigate the effect of including clover in PRG swards receiving 150 or 250 kg of N/ha compared with a PRG-only sward receiving 250 kg of N/ha on herbage production, milk production, and herbage DMI in an intensive grass-based spring-calving dairy production system.

MATERIALS AND METHODS

Experimental Site and Meteorological Data

The experiment was undertaken at Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (52°16′N; 8°25′W; 49 m above sea level) in 2013 and 2014. The soil type was a free-draining acid brown earth of sandy loam-to-loam texture. Soils had a pH of 6.4 and were index 3 and 4 (scale 1 to 4, 1 = deficient, 4 = no response to application of nutrient; Alexander et al., 2008) for phosphorus and potassium.

Meteorological data, daily rainfall (mm), air temperatures (°C), and soil temperature at a depth of 100 mm (°C), for the experimental period were collected at the experimental site.

Experimental Design

A farm systems experiment was established in February 2013 and conducted over 2 grazing seasons [February 16 to November 18, 2013 (yr 1) and February 17 to November 21, 2014 (yr 2)]. A total of 15.33 ha in yr 1 and 21.89 ha in yr 2 of permanent grassland were used for the experiment. In July 2012, 7.8 ha was reseeded, 2.6 ha with a 50:50 mixture of Astonenergy (tetraploid) and Tyrella (diploid) PRG cultivars sown at a rate of 27.2 kg/ha, and 5.2 ha with the same PRG mixture plus a 50:50 mixture of Chieftan and Crusader medium leaf clover cultivars sown at a rate of 5 kg/ha. In June 2013, a further 6.14 ha was reseeded, 2.04 ha with the PRG grass mixture, and 4.09 ha with the PRG and clover mixture. The additional area (7.53 ha in yr 1 and 7.94 ha in yr 2) used in the study consisted of existing PRG swards, cv. Tyrella, sown in 2010 at a rate of 29 kg/ha and PRG (cv. Tyrella, 29 kg/ha)/clover (cv. Chieftain and Crusader, 50:50 mix at 5 kg/ha) swards established in 2010. All paddocks, both newly reseeded and existing paddocks, were blocked and evenly distributed among each sward type. A separate farmlet of 7 paddocks in yr 1 and 11 paddocks in yr 2 was created and permanently fenced for each of the 3 sward types (treatments), a PRG/clover sward receiving 150 kg of N/ha per yr (**Cl150**), PRG/clover sward receiving 250 kg of N/ha per yr (**Cl250**) and a PRG-only sward receiving 250 kg of N/ha per yr (**Gr250**). The total area of each treatment was 5.11 ha in yr 1 and 7.3 ha in yr 2. Within each farmlet, there were detailed measurement paddocks for each treatment (approximately 2 ha per treatment, reseeded in 2012). These paddocks were used to measure tiller density, stolon mass, and DMI. All other sward measurements, as outlined later, were conducted on all paddocks within each farmlet.

Table 1. Annual N fertilizer application regimen[1]

Application	Date	Cl150[2] (kg of N/ha)	Cl250[3] (kg of N/ha)	Gr250[4] (kg of N/ha)
1	Mid-January	28	28	28
2	March	28	28	28
3	April	28	33	33
4	Early May	9	33	33
5	Late May	9	27	27
6	June	9	17	17
7	Early July	9	17	17
8	Late July	9	17	17
9	August	9	17	17
10	September	12	33	33
Total		150	250	250

[1]Nitrogen applied in mid-January and March was applied as urea (46% N), and the remainder was applied as calcium ammonium nitrate (27% N).

[2]Cl150 = grass and white clover swards receiving 150 kg of N/ha per yr.

[3]Cl250 = grass and white clover swards receiving 250 kg of N/ha per yr.

[4]Gr250 = grass-only swards receiving 250 kg of N/ha per yr.

Swards received either 250 kg of N/ha or 150 kg of N/ha per yr, depending on treatment. Nitrogen was applied to all treatments as urea (46% N) until the end of April and as calcium ammonium nitrate (27% N) from early May to mid-September (Table 1). Nitrogen fertilizer was broadcast using an Abbey fertilizer applicator (Abbey Machinery, Nenagh, Co. Tipperary, Ireland).

In February of yr 1, 42 Holstein-Friesian (9 primiparous and 33 multiparous) spring-calving dairy cows were selected and blocked according to calving date, lactation number, 2-wk pre-experimental daily milk yield, milk fat, milk protein, and milk solids yield, and then randomly assigned to 1 of the 3 treatments (n = 14; Table 2). In February of yr 2, 57 Holstein-Friesian (18 primiparous and 39 multiparous) spring-calving dairy cows were selected and blocked according to calving date, lactation number, 2-wk pre-experimental daily milk yield, milk fat, milk protein, and milk solids yield, and then randomly assigned to 1 of the 3 treatments (n = 19; Table 2). Additionally, in yr 2, one additional cow was included in each treatment to maintain a stocking rate of 2.74 cows/ha, and that cow's data were not included in the statistical analysis. All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorized by the Health Products Regulatory Authority, which is the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2010/63/EU; European Council, 2010) for the protection of animals used for scientific purposes.

Animal and Grazing Management

Nine grazing rotations were used on each treatment (February to November) in both years. Herbage mass (**HM**) and sward clover content were measured in each paddock before each grazing, and data presented are mean values of each rotation. Target pregrazing HM (4 cm above ground level; >4 cm) was 1,300 to 1,600 kg of DM/ha for the duration of the experiment. Cows were turned out to grass by day and night as they calved from early February in both years. During the first grazing rotation, all treatments were allocated pasture according to the spring rotation planner (Teagasc,

Table 2. Initial herd characteristics for the animals used in the experiment in 2013 and 2014

Item	2013	SD	2014	SD
Cows/treatment	14		19	
Mean calving date	February 1	6.5	February 7	14.9
Mean lactation number	2.90	1.47	2.75	1.55
Mean pre-experimental daily milk yield (kg/cow)	23.8	3.54	27.9	5.51
Mean pre-experimental daily milk fat (%)	4.48	0.62	4.76	0.53
Mean pre-experimental daily milk protein (%)	3.38	0.25	3.24	0.20
Mean pre-experimental daily milk solids (kg/cow)	1.88	0.38	2.23	0.45
Mean BW (kg)	505	48.1	519	54.3
Mean BCS	3.13	0.20	3.20	0.19
Herd EBI (€)[1]	155	34.7	165	37.1

[1]Herd EBI (€) = EBI is a single figure profit index aimed at identifying the most profitable bulls and cows for breeding dairy herd replacements. It comprises information on 7 sub-indexes related to profitable milk production. More information is available at www.icbf.com.

2009). Swards were rotationally grazed and on-off grazing (Kennedy et al., 2009) was used as a management tool to facilitate grazing during periods of inclement weather. Break fences allowed a fresh allocation of pasture to be offered after each morning and evening milking during the first rotation (February to early April). Subsequently fresh herbage was allocated every 24 to 36 h for the remainder of the experiment following morning or evening milking. The area allocation was based on herbage available >4 cm. Target postgrazing sward height (**PostGSH**) was <4 cm in the first rotation, and 4 cm for the remainder of the year. Fresh water was continuously available to the cows. Grass supply for each treatment was managed independently using the farm cover technique (O'Donovan et al., 2002). A visual assessment (O'Donovan et al., 2002) of pasture cover was undertaken weekly and recorded on PastureBase Ireland (Hanrahan et al., 2017), and grazing management decisions were made as described by Wims et al. (2014). Pasture surplus to grazing requirements was conserved as bale silage. Additionally, 33% of the land area in each treatment was closed for first-cut silage in early April for approximately 42 d and 25% of the land area in each treatment was closed for second-cut silage in early June for approximately 49 d. All silage was conserved as bales.

All cows on each treatment received similar concentrate supplementation. Concentrate supplementation was used in the spring period (February to April) on all treatments and in the main grazing season (May to September) only when a herbage deficit occurred on all 3 treatments. If a deficit occurred on an individual treatment during the main grazing season, conserved silage from that treatment was fed to the cows. In yr 1, concentrates were fed, from February 16 to April 23, July 24 to July 31, and September 25 to November 18, a total of 496 kg of DM of concentrate/cow. In yr 2, concentrates were fed, from February 17 to April 9 and November 1 to November 21, a total of 272 kg of DM of concentrate/cow. The concentrate ingredients were as follows: maize (13%), beet pulp/ molasses (15.5%), soybean meal (30%), maize distillers (12%), acid buff (0.7%), maize/beet (2.5%) salt (0.5%), barley (15%), rapeseed meal (7.5%), and Megalac (3.3%; McDonnell Brothers Limited, Fermoy, Co. Cork, Ireland). The concentrate composition was 154.1 g/kg of DM of CP (40.9 g/kg of DM NDF and 102.8 g/kg of DM ash. Swards were not topped (mechanically conditioned) during the experiment.

Sward Measurements

Herbage Mass. Pregrazing HM (>4 cm) was measured in each paddock of each treatment before grazing by cutting 2 strips 1.2 m wide and of a known length (approximately 8 m) in the area due to be grazed next with an Etesia lawn mower (Etesia UK. Ltd., Warwick, UK). The harvested material was collected and weighed, and a 100-g sub-sample was dried at 95°C for 16 h to determine the DM content. Compressed sward height was measured before and after harvesting on each cut strip by taking 10 measurements per strip using a rising plate meter with a steel plate (diameter 355 mm and 3.2 kg/m^2; Jenquip, Feilding, New Zealand) to determine sward density (kg of DM/cm per ha) using the following equation:

$$\text{sward density (kg of DM/cm per ha)} = \frac{\text{herbage mass}\left(\text{kg of DM/ha}\right)}{\text{precutting sward height} - \text{postcutting sward height}}.$$

The average paddock pregrazing HM >4 cm was then calculated according to the following equation:

$$\text{pregrazing HM} > 4\text{ cm (kg of DM/ha)} = [\text{pregrazing sward height (cm)} - 4\text{ cm}] \times \text{sward density (kg of DM/cm per ha)}.$$

Pre- and Postgrazing Compressed Sward Heights. Pregrazing sward height (**PreGSH**) was recorded for each treatment by taking a minimum of 50 measurements in the area about to be grazed following a W shape across the area using the rising plate meter described previously. After grazing, a similar procedure was used to determine PostGSH above ground level.

Sward Clover Content. The sward clover was determined once in each Cl150 and Cl250 paddock in every rotation. Herbage was sampled >4 cm using Gardena hand shears (Accu 90, Gardena International GmbH, Ulm, Germany). Grab samples of herbage were collected at random locations along a W-shaped transect in the area due to be grazed next. The sample was then mixed, and two 70-g sub-samples were removed and separated into grass (sown PRG and weed grasses) and clover fractions, which were dried at 60°C for 48 h in a forced convection oven (Parsons Lane, Hope Valley, UK) to determine the DM proportions.

Herbage Chemical Composition. Once per paddock in each rotation, herbage representative of that selected by the cows (i.e., defoliating at the previous day's PostGSH) was manually collected with Gardena hand shears (as previously described) as described by Ganche et al. (2013). Samples were stored at −18°C. The frozen herbage sample was bowl-chopped (Muller, Type MKT 204 Special, Saabrücken, Germany), freeze-dried at −50°C for 120 h, and milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss, DK-3400 Hillerød, Denmark), and stored for analyses. The chemical composition of the harvested herbage was analyzed by wet chemistry for organic matter digestibility (**OMD**), CP, NDF, ADF, and ash concentrations. The OMD was estimated using the in vitro neutral detergent cellulase method (Fibertec Systems, Foss, Ballymount, Dublin, Ireland) described by Morgan et al. (1989). Crude protein concentration was determined using a N analyzer (FP-428, Leco Australia Pty Ltd., Castle Hill, New South Wales, Australia) based on the AOAC method 990–03 (AOAC, 1990). The NDF and ADF concentrations were determined using a fiber analyzer (Ankom Technology, Macedon, NY) based on the method described by Van Soest et al. (1991). Amylase and a sodium sulfite solution were used in the NDF concentration determination process. Ash concentration was estimated by burning a subsample in a muffle furnace at 500°C for 12 h.

Animal Measurements

Milk Production. Cows were milked twice daily, at 0630 and 1530 h, with an average of 3 h per day spent off the paddocks in the collecting yard and the milking parlor. Individual daily milk yield (kg) was recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat and protein concentrations were determined weekly from one successive evening and morning milking. The concentrations of these constituents were determined using Milkoscan 203 (Foss Electric DK–3400, Hillerød, Denmark). Milk solids yield (kg) was calculated as the yield of milk fat plus the yield of milk protein. Milk yield and milk solids yield per hectare were calculated by multiplying total lactation yields by the annual stocking rate for each treatment for each year as described by Patton et al. (2012).

BW and BCS. Body weight and BCS were recorded fortnightly throughout the experiment. An electronic portable weighing scale with the Winweigh software package (Tru-test Limited, Auckland, New Zealand) was used to record BW. The BCS was scored by an experienced independent observer on a scale from 1 to 5 (where 1 = emaciated, 5 = extremely fat) with 0.25 increments (Lowman et al., 1973).

Herbage Intake Estimations. Individual DMI was estimated using the *n*-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum, 1989) twice in 2013 (mid-May and mid-September), and 3 times in 2014 (mid-May, mid-July, and mid-September). All cows were dosed twice daily, after milking, for 12 consecutive days with a paper bullet (Carl Roth GmbH, Karlsruhe, Germany) containing 500 mg of dotriacontane (C_{32} − alkane). From d 7 to 12 of dosing fecal samples were collected from each cow twice daily, before morning and evening milking, either in the paddock during the hour immediately before milking by observing the cows and collecting the sample when voided, or by rectal grab sampling after milking. Fecal samples were stored at −18°C until the end of the collection period. Fecal samples from each cow were thawed and bulked together (12 g from each sample), dried at 60°C for 48 h, milled through a 1-mm screen, and analyzed for alkane content.

In conjunction with the fecal collection, 2 herbage samples of approximately 15 individual grass snips were manually collected using Gardena hand shears (as described previously) mimicking the grazing defoliation pattern observed on previously grazed swards on d 6 to 11. The daily samples were stored at −18°C. Additionally, on the Cl250 and Cl150 treatments, a sub-sample of the herbage was manually separated into 100 g of grass and 100 g of clover and also frozen. The frozen herbage samples were bowl-chopped (Muller, Type MKT 204 Special, Saabrücken, Germany), freeze-dried at −50°C for 72 h, milled through a 1-mm screen, and analyzed for alkane content.

The DMI was calculated as described by Hameleers and Mayes (1998). The PRG/clover ratio consumed was estimated from the concentrations of the odd-chain *n*-alkanes by using an iterative routine (Microsoft Excel Solver; Microsoft Corp., Redmond, WA) that minimizes the sum of squares of the discrepancy between the observed *n*-alkane fecal levels (expressed as a proportion of total alkane concentration and corrected for their recoveries; Dillon et al., 1995) and expected fecal *n*-alkane concentration calculated from the *n*-alkane concentration of the individual forage components.

$$\text{Minimize } \Sigma\ [(\text{calculated alkane proportion} - \text{actual proportion})^2]\ i \ldots \text{n};$$

$$\text{calculated proportion alkane}_i = \frac{p \times A_i + (1 - p) \times B_i}{p \times A_i + (1 - p) \times B_t};$$

$$\text{actual proportion alkane}_i = \frac{F_i}{F_t};$$

where A_i, B_i, F_i = concentration of individual *n*-alkane$_i$ in herbage A and B and feces F; p = proportion of the diet of herbage A (set to be $0 < p < 1$); B_t, F_t = sum of *n*-alkane concentrations used in the calculation (C_{27}, C_{29}, C_{31}, C_{33}, C_{35}) of herbage B and feces F; and i = odd chain *n*-alkanes involved in calculation (C_{27}, C_{29}, C_{31}, C_{33}, C_{35}).

Daily herbage intake (kg of DM/d) $I =$

$$\frac{\left[\frac{F_i}{F_j} - 1 \times \left(D_j + I_c\right) - I_c\right]}{\left\{\left[p \times A_i + \left(1-p\right) \times B_i\right] - \frac{F_i}{F_j} \times \left[p \times A_j + \left(1-p\right) \times B_j\right]\right\}},$$

where p = proportion of the diet of herbage A; I_c = intake concentration (kg of DM/d); D_j = dosed amount of C_{32}; A_i, B_i, $F_i = i$ is the concentration of C_{31} in herbage A and B and feces F; and A_j, B_j, $F_j = j$ is the concentration of C_{32} in herbages A and B and feces F.

Statistical Analyses

All data were analyzed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC). Pregrazing HM, sward clover content, PreGSH, and PostGSH were analyzed using PROC MIXED, with year, treatment, rotation, and the associated interactions included in the model. Paddock was the experimental unit, with paddock introduced as the random factor and rotation the repeated measure. Data are presented as least squares means ± standard error. Annual HM was analyzed using PROC GLM in SAS.

Daily milk yield, daily fat content, daily protein content, and daily milk solids yield were analyzed using PROC MIXED in SAS, with treatment, week, and the associated interactions included in the model. The individual cow was the experimental unit, and week was the repeated measure. Cumulative milk yield and milk solids yield were analyzed using PROC Mixed in SAS. Days in milk and block were included as covariates in the model.

Milk production variables were analyzed using the following model:

$$Y_{ijkl} = \mu + C_i(S_k) + P_j + S_k + \mathrm{Wk}_l + \mathrm{P}_j \times S_k + \mathrm{Wk}_l \times S_k + \mathrm{b1}_{Xijk} + e_{ijkl},$$

where Y_{ijkl} is the response of the i cow in the k sward at l week, μ is the mean, $C_i(S_k)$ the random effect of the cow within treatment, P_j is the lactation (j = 1 to 5), S_k is the treatment (k = grass or clover), Wk_l is the week of the experiment (1 to 39), $P_j \times S_k$ is the interaction of P × S, $\mathrm{Wk}_l \times S_k$ is the interaction of Wk × S, $\mathrm{b1}_{Xijk}$ is the respective pre-experimental variable, and e_{ijkl} is the residual error term.

Herbage DMI estimations were analyzed using PROC MIXED in SAS. Treatment, measurement period, and the associated interactions (treatment × measurement period) were included in the model. Individual cow was the experimental unit and measurement period (May, July, or September) was the repeated measure.

RESULTS

Metrological Data

Meteorological data for the February to November period are shown in Table 3. Mean daily temperature for the experimental period in yr 1 and 2 were similar to the previous 10-yr average (10.6, 11.2, and 10.9°C, respectively). The 10-yr average rainfall was 827 mm; in yr 1 rainfall was 74% (656 mm) of the 10-yr average, and in yr 2 rainfall was 120% (999 mm) of the year average. Average soil temperature at 100-mm soil depth in yr 1 and 2 (11.8 and 12.8°C, respectively) experimental period was similar to the previous 10-yr average (12.5°C).

Sward Measurements

Sward pregrazing HM, PreGSH, and PostGSH are shown in Table 4 (yr 1 and 2). Rotation had a sig-

Table 3. Metrological data during the experimental period (February to November) in yr 1 (2013) and yr 2 (2014) compared with the previous 10-yr average (2003–2012)

Month	February	March	April	May	June	July	August	September	October	November	Average[1]
Temperature (°C)											
2013	4.9	4.4	7.7	10.3	13.3	17.7	15.8	13.9	12.0	6.0	10.6
2014	5.5	7.0	9.6	11.8	14.6	16.4	14.0	14.0	11.4	7.5	11.2
2003 to 2012	5.8	7.0	9.0	11.3	14.0	15.2	15.3	13.5	10.5	7.8	10.9
Rainfall (mm/mo)											
2013	46	85	71	64	35	61	52	38	150	54	656
2014	223	88	76	72	92	59	65	23	154	148	999
2003 to 2012	56	77	59	72	87	91	83	83	103	116	827
Soil temperature (°C >100 mm)											
2013	4.9	5.0	8.2	11.3	13.8	21.1	18.0	15.7	13.3	7.0	11.8
2014	5.1	7.3	10.9	14.1	18.2	19.0	17.0	15.8	12.4	8.1	12.8
2003 to 2012	5.4	7.4	9.6	13.1	16.9	17.2	17.4	15.0	10.7	12.5	12.5

[1]Average = mean value February to November.

Table 4. The effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, Cl150; PRG and clover, receiving 250 kg of N/ha per yr, Cl250; and PRG only, receiving 250 kg of N/ha per yr, Gr250] and rotation number on pre- and postgrazing sward height, pregrazing herbage mass, and sward density in yr 1 and 2

			Rotation									Level of significance		
Item	Treatment	Mean[1]	1	2	3	4	5	6	7	8	9	Treatment	Rotation	Treatment × rotation
Yr 1														
Pregrazing sward height (cm)	Cl150	8.9	7.6	7.5	9.1	11.6	8.4	8.0	9.9	8.0	10.3	0.064	0.001	0.001
	Cl250	9.6	8.1	8.1	10.0	11.6	9.8	9.0	10.3	8.8	10.8			
	Gr250	9.7	8.6	8.3	9.8	12.7	9.7	9.4	9.5	7.5	12.0			
	SED[2]	0.4	0.32	0.39	0.41	0.42	0.41	0.35	0.39	0.34	0.36			
Postgrazing sward height (cm)	Cl150	3.9	3.6	3.8	4.0	4.0	4.0	4.1	4.1	4.0	4.0	0.562	0.001	0.137
	Cl250	3.9	3.5	3.7	4.0	4.0	4.0	4.1	4.2	4.1	3.9			
	Gr250	4.0	3.7	3.8	4.0	4.0	4.0	4.1	4.1	4.0	4.1			
	SED	0.04	0.03	0.05	0.05	0.05	0.05	0.04	0.05	0.04	0.04			
Pregrazing herbage mass (>4 cm; kg of DM/ha)	Cl150	1,347	1,259	1,068	1,059	1,784	1,180	1,603	1,621	1,312	1,537	0.2724	0.001	0.05
	Cl250	1,406	1,240	1,028	1,381	1,941	1,458	1,776	1,670	1,265	1,387			
	Gr250	1,577	1,619	1,120	1,074	1,866	1,727	2,030	1,429	1,205	1,690			
	SED	98.7	132.0	181.7	180.9	181.2	186.1	151.1	170.6	140.0	159.4			
Sward density (>4 cm; kg of DM/cm per ha)	Cl150	226.2	248.4	240.6	198.4	205.6	218.0	289.2	233.2	241.5	190.1	0.4334	0.001	0.2258
	Cl250	230.5	238.2	208.4	191.3	218.6	236.1	303.2	239.3	259.9	179.4			
	Gr250	242.9	286.9	244.5	177.6	245.2	240.6	264.2	223.2	263.7	194.1			
	SED	9.1	13.04	18.57	18.49	18.56	19.06	15.16	17.37	14.05	16.12			
Yr 2														
Pregrazing sward height (cm)	Cl150	9.2	8.6	6.7	6.7	7.8	7.1	9.8	12.3	10.7	12.8	0.7465	0.001	0.001
	Cl250	9.7	9.9	9.0	7.2	8.4	7.2	9.8	12.4	11.7	12.2			
	Gr250	8.7	6.7	8.5	7.4	8.1	6.5	9.5	12.2	11.2	8.1			
	SED[2]	0.9	0.99	1.03	1.07	1.02	1.08	1.15	1.08	0.99	1.02			
Postgrazing sward height (cm)	Cl150	4.1	4.1	3.9	4.0	4.3	4.5	4.2	4.2	4.4	3.9	0.9101	0.001	0.101
	Cl250	4.1	4.1	3.8	4.1	4.0	4.2	4.1	4.1	4.0	4.4			
	Gr250	4.2	4.3	4.2	4.4	4.4	4.5	3.9	3.6	4.6	4.2			
	SED	0.05	0.04	0.06	0.06	0.09	0.05	0.04	0.05	0.04	0.06			
Pregrazing herbage mass (>4 cm; kg of DM/ha)	Cl150	1,538	929	1,440	1,534	1,452	1,294	1,538	1,897	2,307	1,526	0.8598	0.001	0.2479
	Cl250	1,588	1,055	1,441	1,358	1,507	1,420	1,662	1,800	2,294	1,934			
	Gr250	1,574	1,224	1,390	1,383	1,608	1,569	1,882	1,712	2,027	2,102			
	SED	67.1	127.87	165.27	168.70	155.88	171.25	193.52	145.67	122.52	120.43			
Sward density (>4 cm; kg of DM/cm per ha)	Cl150	237.1	202.9	200.7	188.1	196.1	260.2	288.4	260.5	263.5	264.5	0.4125	0.001	0.1426
	Cl250	235.3	204.9	194.3	181.2	214.6	211.8	304.2	247.6	267.5	286.4			
	Gr250	245.2	209.9	222.7	178.3	211.1	245.5	281.5	253.9	323.9	308.4			
	SED	5.4	12.82	16.90	17.26	15.80	17.57	19.95	14.77	12.22	12.00			

[1]Mean = average for each treatment across the 9 rotations.

[2]SED = standard error of the difference between means.

nificant ($P < 0.05$) effect on all herbage parameters measured in both years of the study (Table 4).

No effect of treatment ($P > 0.07$) was observed on PreGSH (10.1 cm ± 0.06). A significant treatment by rotation interaction ($P < 0.001$) was observed on PreGSH in yr 1 and 2 (Table 4). In yr 1, the Gr250 treatment had the greatest PreGSH in rotations 2 and 9 compared with both clover treatments. The Cl250 treatment had the lowest PreGSH in yr 1 in rotation 6 and had the greatest PreGSH in rotations 5 and 8. Average PostGSH was 3.94 and 4.13 cm ± 0.045 in yr 1 and 2, respectively ($P < 0.001$). There was no effect of treatment ($P = 0.11$) on PostGSH (4.0 cm ± 0.02).

A significant treatment by rotation interaction effect ($P < 0.05$) was observed on pregrazing HM in yr 1. The Gr250 treatment had the greatest pregrazing HM in rotation 1 compared with the Cl250 and Cl150 treatments. In rotation 5 and 6, the Gr250 treatment had a greater pregrazing HM than the Cl150 treatment with the Cl250 treatment intermediate to both. All swards had a similar pregrazing HM in rotations 2, 3, 4, 7, 8, and 9.

Increasing the level of N fertilizer had a significant effect ($P < 0.001$) on sward clover content (Table 5). The Cl150 treatment had a greater clover content compared with the Cl250 treatment (266 and 225 g/kg of DM ± 7.3, respectively). Sward clover content was greater in yr 2 compared with yr 1 ($P < 0.05$; data not shown; 259 and 233 g/kg of DM ± 7.2, respectively). Rotation had an effect on sward clover content ($P < 0.001$; Figure 1). Sward clover content was least in rotation 1 (February and March), 84.9 and 72.3 g/kg of DM ± 21.8 for both the Cl150 and Cl250 treatments, respectively, and greatest in rotation 7 (July/August) (431 g/kg of DM ± 20.4) on Cl150 and in rotation 8 (348 g/kg of DM ± 20.4) on Cl250.

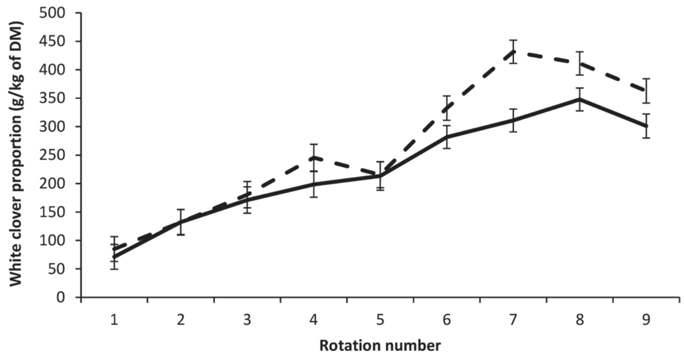

Figure 1. Least squares means of white clover proportion for a *Lolium perenne* L. (PRG) and clover sward, receiving 150 kg of N/ha per yr (broken black line); and a PRG and clover sward, receiving 250 kg of N/ha per yr (solid black line) in the pregrazing herbage mass (>4 cm). Data are means for 2013 and 2014. Error bars represent standard error of the difference between means.

Treatment did not have a significant effect ($P > 0.05$) on total cumulative herbage production (13,039, 14,175, and 13,288 kg of DM/ha ± 420.1, on Cl150, Cl250, and Gr250, respectively). Cumulative herbage production was, however, lower ($P < 0.01$) in yr 1 (12,150 kg of DM/ha ± 390.4) than in yr 2 (14,865 kg of DM/ha ± 304.2; data not shown).

Treatment did not affect ($P > 0.05$) herbage OMD, CP, or ADF content (Table 6). Treatment had a significant effect ($P < 0.001$) on NDF concentration. The Cl250 and Cl150 treatments had a lower NDF concentration than the Gr250 treatment (338.4 and 358.3 g/

Table 5. The effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, Cl150; PRG and clover, receiving 250 kg of N/ha per yr, Cl250; and PRG only, receiving 250 kg of N/ha per yr, Gr250] on milk production and milk composition, BW and BCS, silage fed, and sward clover content during the experimental period (February to November in yr 1 and 2)

	Treatment				Level of significance		
Item	Cl150	Cl250	Gr250	SED[1]	Treatment	Week	Treatment × week
Clover content (g/kg of DM)	266[a]	225[b]	—	7.3	0.001	0.001	—
Cumulative herbage production (kg of DM/ha)	13,039	14,175	13,288	420.1	0.13	—	—
Milk yield (kg/cow per d)	21.8[ab]	22.1[a]	20.9[b]	0.33	0.05	0.001	0.001
Milk fat (g/kg)	46.2[a]	45.0[b]	44.6[b]	0.37	0.01	0.001	0.001
Milk protein (g/kg)	36.5	35.9	36.9	0.10	0.55	0.001	0.77
Milk solids yield (kg/cow per d)	1.75[a]	1.76[a]	1.66[b]	0.020	0.001	0.001	0.05
Cumulative[2] milk yield (kg/cow)	6,166	6,229	5,984	136.5	0.06	—	—
Cumulative milk solids yield (kg/cow)	499	497	464	12.3	0.05	—	—
BW (kg/cow)	548	549	538	5.0	0.23	0.001	0.001
BCS (1–5)	3.13[a]	3.16[a]	3.05[b]	0.015	0.001	0.001	0.001
Cumulative milk yield (kg/ha)	16,896	17,070	16,152	374.2	0.06	—	—
Cumulative milk solids yield (kg/ha)	1,367	1,361	1,276	31.4	0.05	—	—
Silage fed (kg of DM/cow)	664[a]	584[b]	571[b]	13.22	0.05	—	—

[a,b]Values in the same row not sharing a common superscript are significantly different.

[1]SED = standard error of the difference between means.

[2]Cumulative = total for experimental period (February to November 2013 and 2014).

Table 6. The effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, Cl150; PRG and clover, receiving 250 kg of N/ha per yr, Cl250; and PRG only, receiving 250 kg of N/ha per yr, Gr250] and rotation on herbage chemical composition during the experiment (February to November in yr 1 and 2)

			Rotation number									Level of significance		
Item	Treatment	Mean[1]	1	2	3	4	5	6	7	8	9	Treatment	Year	Rotation
OMD[2]	Cl150	838.9	817.0	859.9	847.3	844.4	844.7	830.3	836.7	843.1	826.4			
(g/kg of DM)	Cl250	842.9	823.3	865.5	856.1	858.7	849.7	825.2	835.3	843.2	828.8			
	Gr250	845.7	827.7	865.9	863.7	855.3	849.3	831.6	837.5	840.1	840.5			
	SED[3]	1.9	6.07	7.26	7.23	6.59	6.91	6.70	6.59	5.75	6.17	0.06	0.001	0.001
CP	Cl150	232.4	225.3	227.8	242.8	207.9	214.9	217.0	246.2	244.9	264.7			
(g/kg of DM)	Cl250	236.3	227.7	224.0	240.2	238.0	224.5	216.2	236.0	246.5	274.2			
	Gr250	227.0	232.0	225.6	231.8	219.3	217.0	201.3	222.9	232.3	260.9			
	SED	4.0	7.18	8.37	8.41	7.80	8.07	7.84	7.74	6.85	7.28	0.29	0.47	0.001
NDF	Cl150	338.3[a]	363.8	326.0	330.8	321.5	329.5	359.5	344.9	316.5	352.4			
(g/kg of DM)	Cl250	338.5[a]	351.1	321.0	336.4	323.0	328.5	355.0	359.9	329.2	342.1			
	Gr250	358.3[b]	375.7	324.8	335.0	336.1	347.5	373.5	390.1	361.6	380.1			
	SED	3.8	9.20	10.99	10.96	10.05	10.47	10.15	10.00	8.72	9.36	0.001	0.001	0.001
ADF	Cl150	239.0	236.8	201.7	222.3	210.0	228.9	243.8	259.4	263.4	284.4			
(g/kg of DM)	Cl250	237.7	235.1	192.1	230.3	215.0	221.3	247.3	253.5	256.0	288.7			
	Gr250	240.3	238.0	200.6	232.7	219.5	213.7	241.0	256.3	264.8	296.1			
	SED	2.8	7.92	9.48	9.45	8.64	9.03	8.75	8.61	7.50	8.06	0.77	0.05	0.001
Ash	Cl150	90.4	89.3	82.1	96.6	87.9	86.2	89.9	96.5	91.2	94.0			
(g/kg of DM)	Cl250	86.6	82.6	83.9	73.4	90.6	82.8	87.4	93.8	87.9	97.1			
	Gr250	86.3	90.5	81.3	87.4	87.9	81.4	86.1	90.9	87.9	83.5			
	SED	1.21	3.54	4.24	4.23	3.86	4.04	3.91	3.85	3.36	3.60	0.05	0.001	0.01

[a,b]Values in the same column not sharing a common superscript are significantly different.

[1]Mean = average for each treatment across the 9 rotations.

[2]OMD = organic matter digestibility.

[3]SED = standard error of the difference between means.

kg of DM ± 3.8, respectively). Treatment had a significant effect ($P < 0.05$) on ash concentration, which was greater in the Cl150 treatment than the Cl250 and Gr250 treatments.

Animal Production

Milk Yield, Milk Solids Yield, BW, and BCS. A significant treatment by week interaction ($P < 0.05$) was observed on daily milk solids yield (Figure 2). All treatments had similar daily milk solids yield for experimental wk 1 to 14, and in wk 16, 17, 30, and 41. In wk 19, 23, 25, 37, and 40, cows grazing the Cl250 treatment had greater daily milk solids yield compared with cows grazing the Gr250 treatment. In wk 34, the cows grazing the Cl150 treatment had greater daily milk solids yield compared with the cows grazing the Gr250 treatment. In all other weeks, the cows grazing the Cl250 and Cl150 treatments had greater daily milk solids yield than the cows grazing the Gr250 treatment. This resulted in a significant difference ($P < 0.05$) in cumulative milk solids yield per cow and per hectare. Cows grazing the Cl150 and Cl250 treatments had greater milk solids yield per cow (499 and 497 kg/cow ± 12.8, respectively) and milk solids yield per hectare (1,367 and 1,361 kg/ha ± 31.4, respectively) compared with the Gr250 treatment (466 kg/cow ± 11.5 and 1,276 kg/ha ± 31.4, respectively).

A significant treatment by week interaction ($P < 0.001$) was observed on daily milk yield (Figure 3). All 3 treatments had similar milk yield from wk 1 to 8 and in wk 30, 40, and 41 of the experimental period. In wk 9, 13, 15, 18, 19 20, 23, and 37, daily milk yield was greater on the Cl250 treatment than on the Gr250 treatment. In all other weeks milk yield was similar on Cl250 and Cl150, and greater than Gr250. Although not significantly different, the Cl150 and Cl250 treatments tended ($P = 0.06$) to have greater cumulative milk yield per cow and per hectare compared with the Gr250 treatment.

A significant treatment by week of experiment interaction ($P < 0.001$) was observed on daily milk fat concentration (Table 5). Milk fat concentration was higher for cows on the Cl150 treatment than for cows on the Cl250 and Gr250 treatments in wk 28 and 32 to 35 (data not shown).

Daily milk yield, milk solids yield, fat concentration, and protein concentration were greater ($P < 0.001$) in yr 2 than in yr 1 (data not shown). Because different cows were used in each year, and there were differences in weather conditions and pregrazing HM, the year effect was expected and is therefore not discussed any further in this paper.

No significant effect of treatment was observed on BW (Table 5). However, a significant effect of treatment was observed on BCS; the Gr250 cows had a significantly lower BCS compared with both the Cl250 and Cl150 treatments.

Silage Fed During the Experiment. The cows in the Cl150 treatment were fed a greater quantity of silage than the cows in the Cl250 and Gr250 treatments ($P < 0.05$; Table 5). Year also had a significant effect (data not shown; $P < 0.001$) with more silage fed per cow in yr 1 compared with yr 2 (917 and 295 kg of DM/cow ± 10.8, respectively).

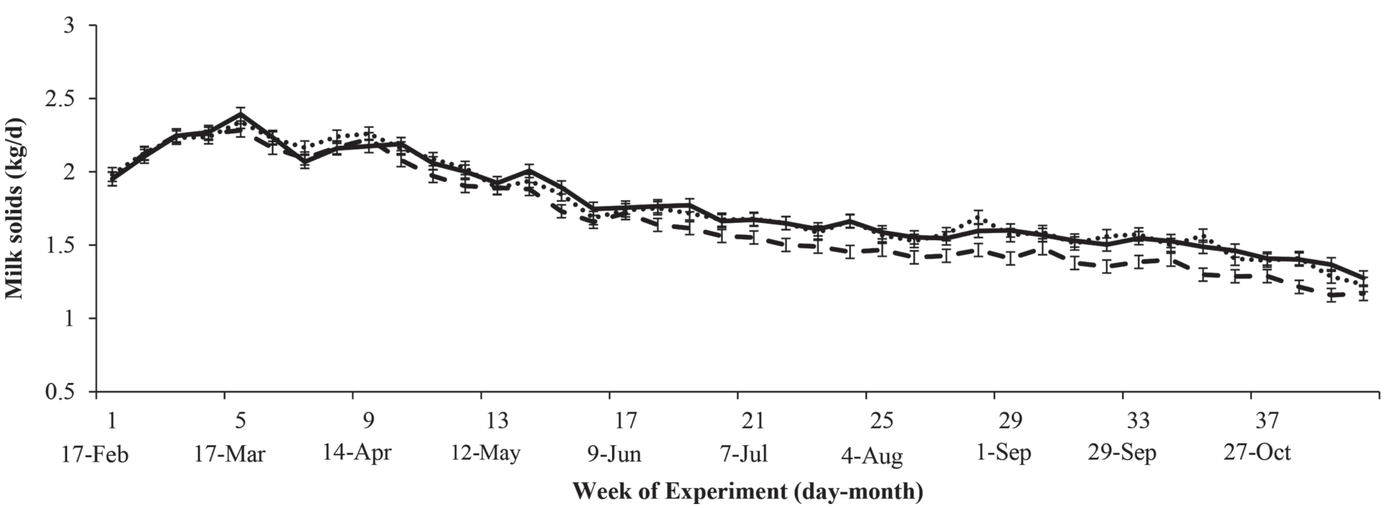

Figure 2. Least squares means of the effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, dotted black line; PRG and clover, receiving 250 kg of N/ha per yr, solid black line; and PRG-only sward, receiving 250 kg of N/ha per yr, broken black line] on dairy cow daily milk solids. Data are presented as a mean of 2 yr (February to November 2013 and 2014). Error bars represent standard error of the difference between means.

Table 7. The effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, Cl150; PRG and clover, receiving 250 kg of N/ha per yr, Cl250; and PRG only, receiving 250 kg of N/ha per yr, Gr250] and measurement period (May, July, and September) on total DMI, perennial ryegrass DMI, and white clover DMI (LSM) in yr 1 (2013) and yr 2 (2014)

			Measurement period			Level of significance		
Item	Treatment	Mean	May	July[1]	September	Treatment	Measurement period	Treatment × measurement period
Total DMI	Cl150	16.6[b]	16.7[b]	18.4[a]	14.7[b]	0.01	0.001	0.001
(kg of DM/cow)	Cl250	17.3[a]	17.5[a]	18.5[a]	15.8[a]			
	Gr250	16.5[b]	16.9[b]	17.1[b]	15.6[a]			
	SEM	0.17	0.23	0.29	0.23			
Grass DMI	Cl150	12.8[b]	14.9[c]	14.2[b]	9.4[c]	0.001	0.001	0.001
(kg of DM/cow)	Cl250	13.6[b]	16.0[b]	13.4[c]	11.5[b]			
	Gr250	16.5[a]	16.9[a]	17.1[a]	15.6[a]			
	SEM	0.16	0.19	0.23	0.19			
Clover DMI	Cl150	3.8	1.8	4.2[b]	5.3[a]	0.23	0.001	0.001
(kg of DM/cow)	Cl250	3.6	1.5	5.0[a]	4.4[b]			
	Gr250	—	—	—	—			
	SEM	0.12	0.17	0.23	0.17			

[a–c]Values in the same column not sharing a common superscript are significantly different.

[1]July only represents samples from 2014.

Herbage DMI. A significant treatment by measurement period interaction ($P < 0.001$) was observed for total DMI (Table 7). In May, the cows on the Cl250 treatment had the greatest DMI. In July, the cows on the Cl250 and Cl150 treatments had similar DMI, which was greater than the cows on treatment Gr250, whereas in September, the cows on treatment Cl150 had the lowest DMI and the cows on treatments Cl250 and Gr250 had similar DMI.

In September, the cows on the Cl150 treatment had a greater clover DMI compared with the cows on the Cl250 treatment. In the Cl250 and Cl150 treatments, clover made up 80 and 110 g/kg of DM, respectively, of the total DMI in May; 270 and 230 g/kg of DM, respectively, in July; and 280 and 360 g/kg of DM, respectively, in September.

DISCUSSION

Sward Productivity

Total annual herbage DM production was not significantly affected by the inclusion of clover into a PRG sward in the current experiment, similar to Enriquez-

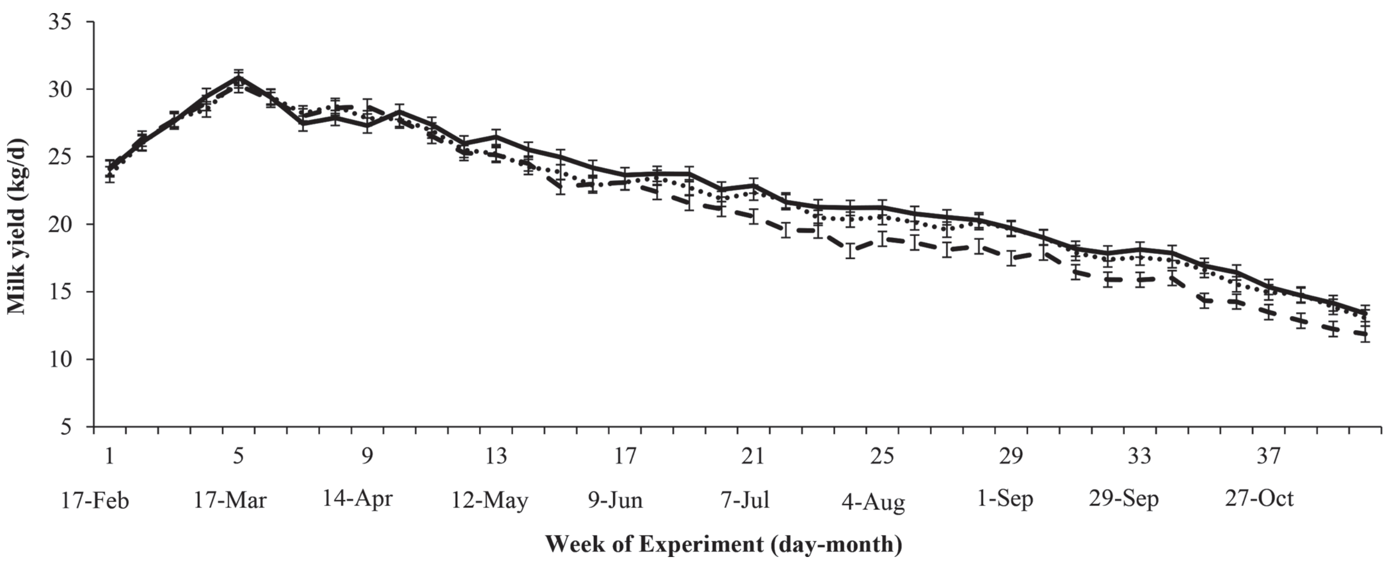

Figure 3. Least squares means of the effect of treatment [*Lolium perenne* L. (PRG) and clover, receiving 150 kg of N/ha per yr, dotted black line; PRG and clover, receiving 250 kg of N/ha per yr, solid black line; and PRG-only sward, receiving 250 kg of N/ha per yr, broken black line] on dairy cow daily milk yield. Data are presented as a mean of 2 yr (February to November 2013 and 2014). Error bars represent standard error of the difference between means.

Hidalgo et al. (2014a) and Egan et al. (2017). However, numerically the Cl250 swards produced approximately an extra 1,000 kg of DM/ha compared with the Cl150 and Gr250 swards. Within a farm system similar to the current study, this extra 1,000 kg of DM/ha is a valuable asset and provides additional forage either for grazing or for silage conservation. Ledgard et al. (2001) and Humphreys et al. (2008) reported that a PRG/clover sward with no N fertilizer application produced 80% of the herbage of a PRG-only sward receiving high N fertilizer (>240 kg of N/ha) application. Similar to Enriquez-Hidalgo et al. (2016), in the experiment reported here PRG/clover swards receiving 150 kg of N/ha (Cl150) produced similar total herbage DM yield to the PRG-only swards receiving 250 kg of N/ha (Gr250). This offers a considerable potential saving to the farmer in terms of N fertilizer application in a pasture-based system.

Unlike previous studies which have reported reduced pregrazing HM in PRG/clover swards compared with grass-only sward (Schils et al., 2000; Ribeiro Filho et al., 2005), average pregrazing HM in the current study was similar across treatments. The studies cited above used <70 kg of N/ha compared with 150 or 250 kg of N/ha in the current experiment, which could have accounted for the difference in pregrazing HM. Pregrazing HM in the current study did, however, vary between treatments across the grazing season. In the current study, the Cl250 and Cl150 treatments had 300 kg of DM/ha less pregrazing HM than the Gr250 swards in rotation 1 (February/March), reflecting the lower overwinter clover growth rate (Frame and Newbould, 1986). Perennial ryegrass over winter growth rate has been previously reported to be in the range of 6.9 to 9.4 kg of DM/d (Lawrence, 2015) on simulated grazed swards at Teagasc Moorepark. In the current study, average farm cover (HM present on each sward; kg of DM/ha) at the end of January was 395, 504, and 784 kg of DM/ha for the Cl150, Cl250, and Gr250 treatments, respectively. The difference in farm cover was a result of the difference in over winter growth; the Cl150 and Cl250 swards lost on average 4.4 and 1 kg of DM/ha per d, respectively, in December and January, whereas the Gr250 swards grew on average 6.8 kg of DM/ha per d over the same period. The minimum and optimal temperatures for clover growth are higher than those for PRG (Davies, 1992), which can account for the lower growth in winter and early spring on the PRG/clover swards. The low farm cover on the Cl150 treatment resulted in an extra 80 and 93 kg of DM silage being fed per cow in the rotation 1 compared with the Cl250 and Gr250 treatments, respectively. The difference in HM between treatments had disappeared by the beginning of rotation 2.

Due to lower growth rates on PRG/clover swards in spring compared with PRG swards (Frame and Newbould, 1986), N fertilizer can be applied in that period to counteract the reduction in clover spring growth by boosting PRG growth (Humphreys et al., 2008). Laidlaw (1980), Humphreys et al. (2009), and Enriquez-Hidalgo et al. (2016) reported that the application of <90 kg of N/ha per yr can increase spring herbage production in PRG/clover swards without affecting annual sward clover content in temperate grassland systems in northwest Europe. Later and repeated applications of N fertilizer to PRG/clover swards can be detrimental to sward clover content (Frame and Boyd, 1987a). Average sward clover content was 266 g/kg of DM for the Cl150 treatment, similar to Frame and Boyd (1987a); and 225 g/kg of DM for the Cl250 treatment, which is similar to that reported by Enriquez-Hidalgo et al. (2016) and higher than Frame and Boyd (1987a), Harris et al. (1996), and Ledgard et al. (2001) at that N fertilizer application rate in northwest Europe and New Zealand. The difference in N fertilizer applications between Cl150 and Cl250 resulted in a 41 g/kg of DM reduction in sward clover content. This is much smaller than that reported by Frame and Boyd (1987a) who found a 169 g/kg of DM difference at N fertilizer application levels similar to those in this experiment. The frequent grazing (9 grazing rotations between February and November) implemented in this study combined with relatively low pregrazing HM, especially in rotations 1 to 3, and the consistently low PostGSH (~4 cm) likely minimized the shading of clover by PRG (Nassiri and Elgersma, 1998) and increased sward clover content in subsequent rotations (Hoogendoorn et al., 1992).

Sward clover content was 24 to 28 g/kg of DM higher in yr 2 compared with yr 1, regardless of N fertilizer application rate. Similarly, in southern Asia, Yu et al. (2008) found an increase in sward clover content from the first year post sowing to the second year. Nevertheless, as only 2 yr of measurements were undertaken in the current experiment, it is unclear if the clover will continue to increase in subsequent years, or if the clover gradually disappears after the first 2 seasons as previously reported by Wilman and Hollington (1985) and Frame and Boyd (1987b). Williams et al. (2003) observed that sward clover content can be maintained for 8 to 10 yr under moderate levels of N fertilizer in intensively managed swards.

Herbage Chemical Composition

The herbage grown on all treatments in the current study was generally of high quality. Clover inclusion did not increase OMD compared with the Gr250 sward, similar to Humphreys et al. (2009) and Enriquez-Hidalgo et al. (2014a). It has previously been reported by Leach et al. (2000) and Riberio Filho et al. (2003) that herbage digestibility is greater on PRG/clover swards compared with PRG-only swards at low N input levels. Clover has a greater digestibility than PRG (Thomson, 1984), and therefore it would have been expected to enhance the overall herbage digestibility in the current study. The high N fertilizer application (Whitehead, 1995; Salaün et al., 1999) and low pregrazing HM (Beecher et al., 2015) applied in this study may have resulted in the similar OMD.

When grown at lower N fertilizer rates, clover has greater CP concentration than PRG (Rattray and Joyce, 1974; Thomson, 1984; Thomson et al., 1985). In the current study, all treatments had similar CP concentrations, similar to Enriquez-Hidalgo et al. (2014a). Reid (1970) reported that the effect of clover in PRG/clover swards on CP concentration is reduced with increasing levels of N fertilizer application. Increasing N fertilizer application can reduce clover N fixation compared with swards receiving lower levels of N fertilizer. This can result in a reduction of the effect of clover on sward CP concentration (Reid and Castle, 1965).

The NDF concentration of PRG is usually greater than that of legumes (Thomson, 1984; Van Soest, 1994). In the current study, although small, herbage NDF concentration was 20 g/kg lower in the Cl150 and Cl250 treatments compared with the Gr250 treatment, similar to Buxton (1996) and Harris et al. (1997a). Although not measured in the current study, the difference in NDF concentration between PRG and legumes could potentially be due to differences in leaf and stem proportion. Stem has a higher concentration of cell walls than leaf and is therefore less digestible (Buxton, 1996), and clover has a lower proportion of stem than PRG (Ulyatt et al., 1977; Buxton, 1996), which could have accounted for the differenced observed in the current study.

Animal Performance

In the current study, daily milk and milk solids yields were increased as a result of the inclusion of clover in a PRG sward over 2 full lactations. Thomson et al. (1985) and Harris et al. (1998) found similar results; however, this was in short-term component studies. Other studies examining the inclusion of clover in PRG swards over full grazing seasons similar to the current study (Schils et al., 2000; Humphreys et al., 2009; Enriquez-Hidalgo et al., 2014a; Egan et al., 2017) have found varying milk production responses. Schils et al. (2000) reported an increase in milk production per cow on PRG/clover swards compared with PRG only. Humphreys et al. (2009) and Enriquez-Hidalgo et al. (2014a) reported no animal production benefits with PRG/clover swards compared with PRG only. Previous studies (Schils et al., 2000), examining the inclusion of clover in grazing systems, reduced stocking rate as a result of reduced N fertilizer, which reduced milk production per hectare by 25%. Egan et al. (2017) reported a 9% increase in milk production with the inclusion of clover in a PRG sward at a common stocking rate. In the experiment reported here, where the stocking rate was the same across the grass-only and grass-clover treatments, incorporating clover in to the PRG sward increased milk solids yield by 91 (7.1%) and 85 (6.6%) kg/ha from the Cl150 and Cl250 treatments, compared with the Gr250 treatment. This increase in milk solids yield per hectare is financially important to dairy farmers. Greater milk production with the inclusion of clover in the sward is usually due to a combination of higher voluntary DMI and the increased nutritive value of the diet (Clark and Harris, 1996; Harris et al., 1997a, 1998). The largest difference in milk production in the current study occurred in the second half of the grazing season (June onward), similar to Schils et al. (2000) and Woodward et al. (2001). This occurred at the time when the difference in DMI between treatments was greatest. The DMI was 8% greater on the Cl250 and Cl150 treatments compared with the Gr250 treatment in July. Andrews et al. (2007) reported that a sward clover content >200 g/kg of DM is required to observe an animal production benefit from incorporating clover in the sward. In the current study, although Cl150 had a greater sward clover content than Cl250 (290 and 270 g clover/kg of DM, respectively), animals on the Cl250 and Cl150 treatment selected a diet containing 270 and 230 g of clover/kg of DM, respectively, in July. The large content of clover in the swards likely accounted for the increase in DMI (Harris et al., 1997b, 1998).

Voluntary DMI of forages can vary depending on several factors, including the forage's resistance to breakdown (Minson, 1990). Particles must be reduced to a size <1 mm before they can readily flow from the rumen (Moseley and Jones, 1984); chewing during eating and ruminating are the most important way in which forage particles are reduced in size (Minson, 1990; Wilson and Kennedy, 1996). Wilson and Kennedy (1996) reported that a shorter period of ruminating is required for clover swards to reach a particle size small enough to pass through the rumen. When available, clover is eaten in greater quantities then grasses because it has a lower resistance to breakdown during eating and ruminating (Minson, 1990). The lower resistance to breakdown of clover over PRG (Minson, 1990) can be attributed to a lower NDF concentration (Dewhurst et al., 2003; Enriquez-Hidalgo et al., 2014b; Egan et al., 2017), which was observed in the current study. Van

Soest et al. (1991) and Van Soest (1994) reported that NDF is more closely related to the daily ruminating time and DMI than any other chemical fraction.

The inclusion of clover in PRG swards can reduce milk fat concentration (Thomson et al., 1985; Harris et al., 1997b) due to a faster rate of passage of feed through the rumen (Ulyatt, 1981; Thomson et al., 1985) and lower diet structural carbohydrate content (Davies, 1992). In the study reported here, milk fat concentration was not different on Cl250 and Gr250, similar to Riberio Filho et al. (2003), Ribeiro Filho et al. (2005), and Enriquez-Hidalgo et al. (2014a). There was no difference in milk protein concentration between treatments in the current study, similar to Phillips (1998), Leach et al. (2000), Enriquez-Hidalgo et al. (2014a), and Egan et al. (2017). This is not uncommon in a PRG/clover sward. The CP concentration of all swards was similar (232 g/kg of DM), and additionally, the herbage CP concentration in all treatments exceeded the CP requirements of dairy cows (150 to 180 g/kg of DM) for milk production (NRC, 2001). The inclusion of clover in PRG swards can result in increased levels of N concentration of the total herbage, because of the higher digestible protein in clover compared with PRG (Thomson, 1984). In the current study, however, no difference was observed in herbage CP content. In general, the diet of grazing ruminants contains an excess of CP due to an inefficient utilization of N present in the diet (Macduff et al., 1990; Ledgard et al., 2009), which when excreted can result in N loss to the environment. Contrasting findings have been made in terms of N losses from mixed PRG/clover swards compared with PRG N fertilizer swards from previous research, mainly due to a reduction in N fertilizer application on the mixed PRG/clover swards (Macduff et al., 1990; Ledgard et al., 2009). Where direct comparisons have been made, mixed clover swards and PRG swards with similar N inputs leached similar quantities of NO_3^- (Cuttle et al., 1992). The experiment presented here found similar herbage production on all 3 treatments indicating potential to reduce N fertilizer application to 150 kg of N/ha on grass-clover swards, even at a stocking rate of 2.74 cows/ha.

CONCLUSIONS

The frequent and tight grazing management used in the current study maintained a sward clover content of >225 g/kg of DM. Applying an extra 100 kg of N fertilizer/ha reduced sward clover content by 40 g/kg. Including clover in PRG swards receiving 250 kg of N/ha per year increased herbage production by 1,000 kg of DM/ha. The PRG/clover treatment receiving 150 kg of N/ha had similar herbage production to the PRG-only treatment receiving 250 kg of N/ha. Herbage production over the winter on the grass-clover treatments was lower than that of the Gr250 treatment. The lower winter growth rates on the Cl150 treatment resulted in an extra 80 to 93 kg of silage/cow been fed in the spring period on the Cl150 treatment. Milk production was greater for the cows grazing the Cl150 and Cl250 treatments in mid to late lactation when sward clover content was greatest. The increase in milk production was a result of greater DMI in July, and also reduced NDF concentration and high sward clover content. The results from the current 2-yr full-lactation study suggest that the inclusion of clover in an intensive grass-based milk production system can increase milk and milk solids yield. The results of this study also indicate the potential for intensive dairy farmers to reduce N fertilizer inputs when clover is included into a PRG sward.

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