The effect of fertilizer nitrogen input to grass-clover swards and calving date on the productivity of pasture-based dairy production

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ABSTRACT

The objective of this systems-scale study was to investigate grazing season timeframes on pasture and milk production and on milk processability of dairy systems with compact spring-calving dairy cows grazing white clover (*Trifolium repens* L.) based grassland. Fifty-four primiparous and multiparous Holstein-Friesian dairy cows were used in a one-factor study with 3 systems ($n = 18$) and repeated over 2 yr (2008/09 and 2009/10). The 3 systems were: early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring (ES100N; 2.1 cows·ha⁻¹; grazing February to November), early spring calving without fertilizer N (ES0N; 1.6 cows·ha⁻¹; grazing February to November) and late spring calving without fertilizer N (LS0N; 1.53 cows·ha⁻¹; grazing April to January). Annual pasture production was affected by an interaction between grazing system and year: Mean annual pasture yields for 2008 and 2009 were ES100N; 10.35 and 9.88, ES0N; 8.88 and 8.63, LS0N; 9.18 and 10.31 t of dry matter (DM)·ha⁻¹ (SEM 0.39). LS0N had higher pasture DM yield in 2009 due to higher clover DM production and biological N fixation compared with the other systems. Clover stolon and root mass in the following February was correlated with stolon and root mass in the previous November with 64% of stolon mass present on LS0N in February ($R^2 = 0.84$). There were no detectable differences in per-lactation milk yield (6,335 kg·cow⁻¹), fat, protein and lactose yields (271, 226, 297 kg·cow⁻¹, respectively), cow liveweight (585 kg) or body condition score (3.02). Although winter grazing favored subsequent clover DM production, biological N fixation and pasture DM production, delaying calving date in spring and extending lactation into the following winter led to inefficient use of this pasture by the grazing herd and lowered the quality of late-lactation milk for processing purposes. Hence, a mean calving

date in mid- to late-February is recommended for zero-fertilizer N input clover-based grassland.

Key words: dairy cow, white clover, nitrogen fertilizer, milk production, milk processability

INTRODUCTION

The environmental effects of mineral nitrogen are of global concern, and there is growing determination to mitigate its use for agricultural purposes (ECE, 2020). Within the European Union, the European Commission (EC) has recently launched the Farm to Fork Strategy as part of the European Green Deal to tackle climate and environmental-related challenges and targets a 20% reduction in fertilizer use by 2030 (EC, 2019). The replacement of synthetic fertilizer N with N fixed by bacteria (biological N fixation; **BNF**) in association with white clover (*Trifolium repens* L.) offers the potential to lower fertilizer N use, nitrous oxide and ammonia emissions and energy use. This in turn helps reduce the carbon footprint and other environmental effects of milk production (Li et al., 2011; Yan et al., 2013) while remaining economically competitive (Humphreys et al., 2017). These benefits are largely dependent on maintaining the clover component of the sward at agronomically desirable levels of approximately 300 g·kg⁻¹ of pasture DM from year to year (Frame and Newbould, 1986).

The optimal temperatures for growth of white clover are higher than perennial ryegrass (*Lolium perenne*) and this makes clover vulnerable to competition by perennial ryegrass particularly during the winter and early spring (Davies, 1992). Previous plot-scale investigations by Laidlaw and Stewart (1987), Laidlaw et al. (1992) and Phelan et al. (2014) showed that carrying high covers of pasture for long periods over the winter and early spring was detrimental to white clover survival and, hence, the subsequent productivity of swards. Furthermore, the application of fertilizer N to grass-clover swards in spring to increase grass production can be detrimental to the white clover component of the sward (Laidlaw et al., 1992; Chapman et al.,

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2017). Conversely, white clover tends to be prominent in the sward from mid-summer onwards (Frame and Newbould, 1986; Laidlaw et al., 1992; Enriquez-Hidalgo et al., 2018), with high levels of BNF, which provides the potential for higher pasture production to extend the grazing season into the late autumn and winter.

Rates of pasture growth are highly seasonal in temperate latitudes (Hurtado-Uria et al., 2013). The period when pasture growth meets the demand by grazing dairy cows is limited. In Western Europe pasture deficits due to low growth rates typically occur in late autumn, winter and early spring. Traditional pasture-based dairy systems are designed to match the herd demand for feed to the production of home-grown feed (grazed and ensiled pasture) with minimal purchased concentrate feed. A long grazing season and a short period of winter housing are key elements of low-cost pasture-based dairy production (Finneran et al., 2012; Hanrahan et al., 2018; Laple et al., 2012). Hence, in Ireland, dairy cows are compactly calved with a mean calving date in February and are typically dried off and housed during the winter.

A key component of seasonal pasture-based milk production is matching calving date to the commencement of pasture growth in spring to produce as much milk as possible off low-cost grazed pasture during the grazing season. Hence, in the absence of applications of fertilizer N in spring, and consequently lower pasture production in spring, a later calving date might better align pasture supply with the requirement for feed by the spring-calving herd. Under such a management strategy producing milk off pasture can be maintained by extending the grazing season into the following winter. In some instances there is a premium price paid for milk produced during the winter months. Nevertheless, the composition of milk changes during lactation (Lucey, 1996) and milk produced by later calving cows with a later turnout date in spring is likely to reflect lactational and nutritional influences in late lactation (O'Brien et al., 1999). This is likely to have consequences for the yield, composition and quality of milk products (Lucey, 1996; O'Brien et al., 2006; Fox et al., 2017).

The objective of this study was to examine a system of milk production with later than typical mean calving and turnout to pasture dates (mid-April) with the end of lactation in the following January compared with systems with conventional calving and turnout dates (mid-February) with the end of lactation in mid-November. Aspects examined include the productivity of white clover in grassland, BNF, and pasture and milk production as well as indicators of the processability of late-lactation milk.

MATERIALS AND METHODS

Site Characteristics and Meteorological Data

The study was conducted at Solohead Research Farm in Ireland (52°51'N, 08°21'W; 95 m above sea level) between February 2008 and February 2010. The soils of the farm are 90% poorly drained gleys and 10% grey-brown podzolics overlaying Devonian sandstone at a depth of 5 to 10 m below ground level. The soil has a clay-loam texture, comprising 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13%, and soil pH was 6.6. The land has been under permanent grassland for more than 50 years and was reseeded with perennial ryegrass (*Lolium perenne*) between 1985 and 1995. Between 2001 and 2006, the grassland sward was oversown with white clover as described by Humphreys et al. (2009). The botanical composition of the swards in September 2008 (in g·kg⁻¹ of pasture DM) was predominantly perennial ryegrass (approximately 750 g·kg⁻¹) and white clover (approximately 200 g·kg⁻¹). Unsown species were primarily dandelion (*Taraxacum officinale*), creeping buttercup (*Ranunculus repens*), daisy (*Bellis perennis*) and ribwort plantain (*Plantago lanceolata*), which in total accounted for less than 50 g·kg⁻¹.

Soil temperature (°C at 10 cm depth) and rainfall (mm) were measured every 30 min at an automatic meteorological station on the farm (Campbell Scientific Ltd.).

Experimental Design and Grazing Systems

Fifty-four (12 primiparous and 42 multiparous) Holstein-Friesian spring-calving dairy cows were used in a one-factor study with 3 grazing systems and 18 cows per system per year (from February to February), repeated over 2 years. At the end of lactation, all cows were housed together and fed together as one group. Each spring all cows were divided into 4 main groups on the basis of lactation number (1, 2, 3, and ≥4) and then subdivided into subgroups of 3 on the basis of calving date. From within each subgroup, one cow was randomly assigned to each herd. Herds were then randomly assigned to the 3 following systems:

- **ES100N:** Early spring turnout with an annual N fertilizer input of 100 kg·ha⁻¹. This grazing system was typical of that recommended for conventional dairy production from cows grazing grass-clover swards in Ireland (Humphreys et al., 2009). The mean calving date was February 17 in each year, and lactating cows were primarily at pasture from

February 21, 2008, to November 12, 2008, and from February 20, 2009, to November 18, 2009 (Table 1). Overall annual stocking rate was 2.1 cows·ha⁻¹ (Table 1).

- **ES0N:** Early spring turnout without fertilizer N input. Mean calving date, turnout dates, and housing dates were similar to ES100N (Table 1), but the overall annual stocking rate was 1.6 cows·ha⁻¹ as recommended by Culleton and Fox (2001).
- **LS0N:** Late spring turnout without fertilizer N input. This system had a later mean calving date (April 12) to account for the lower supply of pasture in early spring that occurs in the absence of fertilizer N applications. Cows were primarily at pasture from April 16, 2008, to January 14, 2009, and from April 15, 2009, to January 26, 2010 (Table 1). The overall stocking rate was 1.7 cows·ha⁻¹ between April and September. However, this was reduced to 1.3 cows·ha⁻¹ between September to January by including additional land (3.7 ha) in this system. This simulated the housing of calves and heifers off the main grazing block for the winter.

A total of 34 ha of permanent grassland in both 2008 and 2009 was used for the study. The study area was divided into 6 blocks according to soil type and drainage status in January 2008, with each block containing 3 paddocks. One paddock from each block was randomly assigned to each grazing system, and paddock sizes were adjusted to achieve the land areas that determined the stocking rates (Table 1). Mean paddock sizes were 1.42, 1.88, and 1.76 ha⁻¹ (SD = 0.30) in the ES100N, ES0N, and LS0N, respectively. However, immediate stocking rates within each system changed over time as surplus pasture was removed for silage production (Table 1).

Animal and Grazing Management

Dairy cows were turned out to pasture 3 d after calving in spring and remained outside until drying off at the end of lactation in the following winter. Exceptions were made when ground conditions were too wet (soil moisture content >60%) or when pasture supply was insufficient at pregrazing pasture masses <500 kg of DM·ha⁻¹. Pasture was allocated for a 48-h period to dairy cows under rotational strip-grazing management, and each herd was moved to the next pasture area when

Table 1. Details of the 3 clover-based grazing systems¹ in 2008 and 2009

Variable	2008			2009		
	ES100N	ES0N	LS0N	ES100N	ES0N	LS0N
Start of grazing year	Feb. 21	Feb. 19	Apr. 16	Feb. 20	Feb. 22	Apr. 15
End of grazing year	Nov. 12	Nov. 12	Jan. 14	Nov. 18	Nov. 18	Jan. 26
Mean grazing days ² ·cow ⁻¹	220	234	231	218	234	235
Mean grazing days ³ ·ha ⁻¹	462	374	353	458	374	360
Total land area (ha)	8.5	11.3	10.5 (14.2)	8.5	11.3	10.5 (14.2)
Overall stocking rate ⁴ (cows·ha ⁻¹)	2.1	1.6	1.7 (1.3)	2.1	1.6	1.7 (1.3)
Bi-monthly stocking rates when silage areas were accounted for (cows·ha ⁻¹)						
February and March	2.1	1.6		2.1	1.6	
April and May	4.2	2.3	3.4	3.4	2.0	3.2
June and July	2.1	2.8	2.3	2.4	2.4	3.3
August and September	2.1	1.6	1.7	2.1	1.6	1.7
October and November	2.1	1.6	1.3	2.1	1.6	1.3
December and January			1.3			1.3
Mean	2.5	2.0	2.0	2.4	1.8	2.2
Proportion of area harvested for silage						
First cut (April and May)	0.5	0.3	0.5	0.4	0.2	0.5
Second cut (June and July)	0.0	0.4	0.2	0.1	0.3	0.5
N inputs (kg·ha ⁻¹)						
Fertilizer	105	—	—	96	—	—
Slurry	90	65	65	93	69	65
BNF ⁵	113	131	110	90	149	191
Total	308	196	175	279	218	256

¹ES100N = early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

²24-h periods; a value of 0.5 was used when cows were kept indoors at night.

³The number of dairy cow grazing days per ha averaged over the entire area of each system.

⁴From September to January each year, additional land was included in the LS0N system (adjusted land area and stocking rate are in parentheses). During the rest of the year, this area was used for grazing heifers and calves.

⁵BNF = biological nitrogen fixation.

a postgrazing height (**PGH**) of 5 cm was reached. The PGH was measured twice per day with 50 measurements of a Filips rising plate meter (GrassteC Agri Services). Back-fencing was used to stop cows returning to previously grazed areas. Excess pasture production was identified throughout the study and removed as baled silage. These areas were selected when pregrazing masses exceeded 1,600 kg of DM·ha⁻¹ between April and July and >2,000 kg of DM·ha⁻¹ in August (above PGH of 5 cm). Silage areas were closed off between early April and late May (first-cut silage) and between early June and mid-July (second-cut silage; Table 1). The land area available for grazing in each system at various times of the year and associated stocking rates are outlined in Table 1.

The ES100N system received annual mineral N fertilizer input of 100 kg·ha⁻¹. Nitrogen fertilizer was applied as urea between February and April for early grazing and as calcium ammonium nitrate during May of each year. Slurry produced during housing was collected in a common store and reapplied to grassland on a proportional basis to stocking rate on each system (see Table 1) as described by Humphreys et al. (2008).

Concentrates and Silage Fed

Cows received concentrate feed supplementation (26% barley, 26% maize gluten, 35% beet pulp, and 12% soybean meal) at rates of between 3 and 5 kg·cow⁻¹ between February and April and 0 and 4 kg·cow⁻¹ between April to November for cows on ES100N and ES0N. Cows on LS0N received between 4 and 5 kg·cow⁻¹ from September through to drying off in late January. Rates of supplementation were dependent on pasture availability and nutritive value (see results). When housed, cows were fed grass-clover silage ad libitum.

Sward Measurements

Pasture Production and Nutritive Value. Pasture DM production was measured before each grazing event by cutting 4 random strips (each 5 × 0.55 m) using an HRH-536 lawn-mower (Honda) at a cutting height of 5 cm above ground level. Before harvesting grass-clover for silage, an Agria auto-scythe (Etesia UK Ltd.) was used to cut 3 strips (5.0 × 1.1 m). All the harvested pasture samples were bulked and weighed, and a 100-g subsample was dried for 16 h in a forced-draft oven at 95°C to determine DM content. Annual pasture yield (kg of DM·ha⁻¹) was calculated as the sum of pasture removed as pregrazing and presilage cuts. Growth rates for each pregrazing and presilage cut were calculated by dividing the pasture mass by

the regrowth interval. A second 100-g subsample of composite pasture was freeze-dried and milled through a 0.2-mm sieve before chemical analyses for ash content (550°C muffle furnace for 12 h), CP (N content × 6.25; Leco N analyzer FP-528, Leco Corp.), and in vitro organic matter digestibility (**IVOMD**) as described by Morgan et al. (1989). Silage grab samples of approximately 100 g were randomly collected (n = 88) before feeding to housed animals throughout the study. This was analyzed for ash, IVOMD and CP using near infrared spectroscopy (Model 6500, Foss-NIR System). The NIR machine was calibrated on a monthly basis using 12 samples of varying nutritive value determined using laboratory techniques similar to that described above.

White Clover Content in Pasture DM, Biological Nitrogen Fixation, and Stolon and Root DM Mass. The white clover content of the pasture DM (g·kg⁻¹) in each paddock was measured from 30 randomly distributed strips (each 10 × 30 cm) of pasture sampled at a cutting height of 5 cm with electric hand shears (Accu-shears Gardena). This measurement was carried out in April, June, August, and November of each year. All samples were manually separated into clover or other grassland species, and DM was determined as described for pasture production. Annual grass and clover pasture production in each paddock was calculated from the mean annual grass and clover content of each paddock and its respective annual pasture yield.

Clover stolon and root DM mass was measured in February, March, August, and November of each year by cutting 30 random sods (each measuring 10 × 10 cm) to a depth of approximately 8 cm in each paddock. Stolons with attached roots were manually separated from the sods, washed, and analyzed for DM as described above. The annual BNF was estimated from annual clover pasture yield, sward age, and soil type using the model by Høgh-Jensen et al. (2004).

Animal Production Measurements

Milk Production, Liveweight, and BCS. Cows were milked at 0730 and 1530 h daily throughout lactation in both years of the study. Individual cow milk yield was recorded at each milking. Milk composition from each cow was determined weekly from one successive morning and evening milking sample from each cow using a Milkoscan 203 analyzer (Foss Electric). The liveweight (**LW**) of cows were recorded weekly using weighing scales and the Winweigh software package (Tru-test Limited). Body condition score of each cow was recorded fortnightly using the methodology described by Edmonson et al. (1989).

Days at Pasture and Intake Estimates. Days at pasture were recorded for each cow with a value of 1.0 ascribed to each 24 h period and a value of 0.5 if the animal was at pasture by day only. The amount of concentrate fed per cow was recorded at each milking (Dairymaster) and silage intake was measured as silage fed to cows when housed. Intake of grazed pasture DM by each cow was estimated as the difference between net energy (NE) provided from silage and concentrates and that needed to meet the NE requirements for milk production, maintenance, and pregnancy (Jarrige et al., 1986; Jarrige 1989; O'Mara 1996). Feed costs were based on prices at that time (Teagasc, 2013).

Milk Processability. During the second year of the study, milk samples were collected fortnightly from each system and analyzed for milk processability characteristics. Milk processability was assessed from August 1, 2009 (approximately wk 23 of lactation), until December 2, 2009 (end of lactation), for ES100N and ES0N, and from September 1, 2009 (approximately wk 23 of lactation), until 23 January 2010 (end of lactation), for LS0N. Pooled morning and evening bulk milk samples from each system were collected, stored overnight at 4°C and analyzed in triplicate on the following day. An aliquot of each pooled milk sample was analyzed for fat, protein and lactose concentrations using a Milkoscan 203 analyzer (Foss Electric). Somatic cell count was measured by laser-based flow cytometry (Somacount 300, Bentley Instruments Inc.). Milk samples were also analyzed for casein concentration, NPN, and total protein content. These were measured using the Kjeldahl method [methods 29 (IDF, 1964), 20-4 (ISO/IDF, 2001), and 20-3 (ISO/IDF, 2004), respectively] using a Tecator Digestor Auto and Kjeltac 8400 distiller (Foss Electric). Casein number was expressed as casein divided by total protein multiplied by 100.

Rennet gelation characteristics were determined using low-amplitude strain oscillation (Advanced Rheometer AR550, T. A. Instruments Ltd.). The pH of 100 mL of milk was standardized to 6.55 at room temperature. The temperature of the milk was then brought to 31°C by immersing the milk sample in a water bath, and if required, the pH was readjusted to 6.55. Rennet (Chymax Plus, Pfizer Inc.), diluted to 1:20 with deionized water, was then added to milk at a level 0.18 mL of undiluted rennet/L of milk. The sample was subjected to a low-amplitude shear strain of 0.025 at a frequency of 1 Hz and the elastic shear modulus (G') was measured continuously as a function of time (Guinee et al., 1997). The following variables were calculated from the resultant G'-time profiles: gelation time (GT), defined as the time in seconds for G' to reach a value ≥ 0.2 Pa; maximum curd firming

rate (CFR_{max}), defined as the maximum slope of the G'-time curve; and gel strength defined as the G' value at 50 min from rennet addition.

Statistical Analyses

We conducted an ANOVA with the MIXED procedure in SAS 9.3 (SAS Institute Inc.) using the following model:

$$X_{jkl} = \mu + S_j + Y_k + M_l + SY_{jk} + SM_{jl} + YM_{kl} + STM_{jkl} + e_{jkl},$$

where X_{jkl} is the mean dependent variable, μ is overall mean, S_j is the fixed effect of the j th grazing system, Y_k is the fixed effect of the k th year, M_l is the fixed effect of the l th sampling date and e_{jkl} is residual error term. Year and sampling date were entered as repeat measures using the un@cs covariance structure as recommended by Moser (2004). Paddock was replicate for sward measurements and cow was replicate for animal measurements. Year was used as replicate for sward and animal measurements that were calculated on a herd basis for each system. Grass and clover measurements were analyzed separately using the model. The relationships between November clover stolon and root DM mass and February clover stolon and root DM mass were analyzed for effect of the system with simple linear regression using the GLM procedure in SAS.

Data were analyzed with a repeated measures linear model fitted using the MIXED procedure in SAS 9.3. Responses analyzed were GT, CFR_{max}, gel strength, total protein, casein, NPN, casein number, fat, protein, lactose and SCC. Mean values for main effects or interaction, as appropriate, were compared with a Tukey adjustment for multiplicity effects. Residual checks were made to ensure that the assumptions of the analyses were met.

RESULTS

Meteorological Data

Mean daily soil temperature and monthly rainfall amounts for the study period and the previous 10-yr means are shown in Figure 1. Mean soil temperature was 9.5°C in 2008 and 9.6°C in 2009, whereas the previous 10-yr mean was 10.9°C (range: 10.0–11.8°C; Figure 1a). Total rainfall was 1,228 mm in 2008 and 1,296 mm in 2009, both of which substantially exceeded the previous 10-yr annual average of 1,004 mm (range: 797–1,150 mm; Figure 1b). Monthly rainfall was higher particu-

Figure 1. Mean daily soil temperature (a) and monthly rainfall (b) recorded at the meteorological station at Solohead Research Farm between February 2008 and February 2010. The shaded areas show the recorded values during the study period, and the solid lines show the previous 10-yr mean values (1998 to 2007).

larly in the summer months of both years. Nevertheless, some months, such as February and December in both years had lower rainfall than the previous 10-yr means (Figure 1b).

Pasture Production and Nutritive Value

Averaged over both years annual pasture DM production on ES100N was similar to LS0N and was higher ($P < 0.05$) on both of these systems than ES0N (Table 2). Grass DM production was higher ($P < 0.001$) on ES100N than on the other systems in both years. Clover DM production was affected by an interaction between grazing system and year. There was no difference in clover DM production between systems in 2008.

Clover DM production was higher in 2009 than 2008 on the ES0N and LS0N and significantly so ($P < 0.05$) on LS0N. In 2009 LS0N had higher ($P < 0.05$) clover DM production than ES100N and was not different from ES0N. Mean postgrazing sward height was not affected by grazing system (mean = 4.97 cm, SEM = 0.042, $P > 0.05$).

There were no ($P > 0.05$) differences between systems in mean IVOMD (Figure 2a), CP (Figure 2b) and ash content, which averaged $102 \text{ g} \cdot \text{kg}^{-1}$ (SEM ± 8.23) for each system averaged over both years. There were no significant differences in composition of silage fed to cows during this study. The mean composition ($\pm$ SD) of silage DM was $78 \text{ g} \cdot \text{kg}^{-1}$ ash (± 13.3), $784 \text{ g} \cdot \text{kg}^{-1}$ OMD (± 6.3), and $122 \text{ g} \cdot \text{kg}^{-1}$ CP (± 18.9).

Clover Content of Pasture DM, Biological Nitrogen Fixation, Stolon and Root DM Mass

Clover content of pasture DM was affected by an interaction between grazing system, month, and year ($P < 0.001$; Table 3). Systems ES0N and LS0N had similar clover contents throughout 2008, whereas LS0N had higher clover content in August 2009 ($P < 0.01$; Table 3). In 2008, ES100N had lower clover content than the other 2 systems in June, whereas in 2009, ES100N had lower clover contents in both June and August ($P < 0.01$; Table 3). Biological N fixation was affected by an interaction between grazing system and year ($P < 0.01$; Table 2). The mean BNF was significantly lower in ES100N than the other systems in 2009 ($P < 0.05$; Table 2).

Clover stolon and root DM mass were affected by an interaction between grazing system and year, being similar across systems in 2008 but significantly lower in ES100N in 2009 ($P < 0.01$; Table 3). There were positive correlations between stolon and root DM mass in November and stolon and root DM mass in

the following February in both years (Figure 3). Stolon and root DM mass in February was 64% of that in the previous November on LS0N ($R^2 = 0.84$, $P < 0.01$) and approximately 50% on ES0N ($R^2 = 0.34$, $P < 0.05$) and ES100N ($R^2 = 0.59$, $P < 0.01$).

Days at Pasture, Feed Intake per Cow, and Silage Surpluses and Deficits

The number of days at pasture per cow for ES100N was ($P < 0.001$) less than ES0N and LS0N (219 vs. 234 and 233 d, respectively; Tables 1). On the other hand, the number of grazing days per hectare was higher with higher stocking rate (Table 1). Cows on LS0N system consumed a mean additional 136 kg·cow⁻¹ of concentrates compared with ES100N and ES0N ($P < 0.001$). Intake of grazed pasture per cow was higher on ES0N and silage DM intake was correspondingly lower (Table 4). Combined over 2 yr, ES100N had a silage DM deficit of 0.44 kg·cow⁻¹, whereas ES0N and LS0N had silage surpluses of 0.09 and 1.03 kg of DM·cow⁻¹, respectively.

Table 2. The effect of grazing system¹ on pasture DM production and annual estimated biological nitrogen fixation (BNF, kg·ha⁻¹)

Variable	Grazing system			SEM
	ES100N	ES0N	LS0N	
Pasture DM production (t·ha ⁻¹)				
2008				
Grass	8.22	6.41	7.11	
Clover	2.13	2.47	2.07	
Total	10.35	8.88	9.18	
2009				
Grass	8.18	5.83	6.73	
Clover	1.69	2.80	3.58	
Total	9.88	8.63	10.31	
BNF (kg·ha ⁻¹)				
2008	113	131	110	
2009	90	149	191	
System				
Grass				0.293***
Clover				0.262*
Total pasture				0.389*
BNF				16.6*
Year				
Grass				0.239
Clover				0.137
Total pasture				0.319
BNF				10.8*
System × Year				
Grass				0.414
Clover				0.373*
Total pasture				0.553
BNF				18.8**

¹ES100N = early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Milk Production, Liveweight, BCS, and Milk Processability

There was no ($P > 0.05$) difference in per-lactation milk yield ($6,335 \pm 119.8 \text{ kg}\cdot\text{cow}^{-1}$), fat ($271 \pm 6.0 \text{ kg}\cdot\text{cow}^{-1}$), protein ($226 \pm 4.0 \text{ kg}\cdot\text{cow}^{-1}$), and lactose ($297 \pm 5.9 \text{ kg}\cdot\text{cow}^{-1}$) yields per cow between the 3 grazing systems. There was no ($P > 0.05$) difference in dairy

cow LW during ($585 \pm 6.7 \text{ kg}$) or at the end of lactation ($585 \pm 6.7 \text{ kg}$); likewise for BCS during (3.02 ± 0.029) or at the end of lactation (2.98 ± 0.044). Somatic cell counts in late lactation were not influenced by the grazing system ($P > 0.05$; Table 5). The LS0N system had lower ($P < 0.001$) concentrations of fat, protein, casein and total protein between wk 23 and the end of lactation compared with ES100N and ES0N (Table 5). There

Figure 2. Effect of pasture nutritive value of grass-clover in vitro organic matter digestibility (IVOMD) (a) and crude protein (b) for each system, ES100N (□), ES0N (×) and LS0N (●). Error bars show the SEM for the interaction between grazing rotation and system, $P > 0.05$. ES100N = early spring calving with annual fertilizer N input of $100 \text{ kg}\cdot\text{ha}^{-1}$ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

were no significant differences in lactose, casein number and NPN of late-lactation milk between the systems. Rheological measurements indicated that CFR_{max} , and GT were ($P < 0.001$) shorter for milk produced on LS0N compared with ES100N and ES0N (Table 5). Gel strength at 50 min was ($P < 0.001$) greater for ES100N and ES0N than LS0N (Table 5).

DISCUSSION

Pasture and Milk Production

In previous studies at this site, the average number of grazing days per year was 255 per cow (Humphreys et al., 2008, 2009). Likewise, with similar input of fertilizer

Table 3. The effect of grazing system¹ on clover content of pasture DM production and mass of clover stolon and root

Variable	Grazing system			SEM
	ES100N	ES0N	LS0N	
Clover content of pasture DM ($g \cdot kg^{-1}$)				
2008				
April	109	233	161	
June	285	390	393	
August	301	326	258	
November	115	152	117	
Mean	203	275	232	
2009				
April	58	62	119	
June	165	322	294	
August	321	421	557	
November	112	161	156	
Mean	164	242	282	
Clover stolon and root DM ($kg \cdot ha^{-1}$)				
2008				
February	769	904	705	
March	737	969	888	
August	599	785	778	
November	632	934	694	
Mean	684	898	766	
2009				
February	339	527	608	
March	344	710	797	
August	581	807	1,066	
November	610	885	1,052	
Mean	469	732	881	
2010				
February	400	572	709	
System				
Clover content				25.8*
Stolon and root mass				43.3***
Year				
Clover content				16.8
Stolon and root mass				34.8
Month				
Clover content				19.9***
Stolon and root mass				50.6
System $\times$ Year				
Clover content				29.1*
Stolon and root mass				60.3**
System $\times$ Month				
Clover content				34.5
Stolon and root mass				86.7
Year $\times$ Month				
Clover content				23.4***
Stolon and root mass				69.6**
System $\times$ Year $\times$ Month				
Clover content				40.6***
Stolon and root mass				120.5

¹ES100N = early spring calving with annual fertilizer N input of $100 \text{ kg} \cdot \text{ha}^{-1}$ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Effect of grazing system¹ on silage DM ensiled, annual budgets of feed intake per cow with the end-of-year surpluses or deficits (negative values) per cow, purchases (negative values) and sales (positive values) of concentrate DM and silage DM per cow and per hectare over 2 yr

Item	2008			2009		
	ES100N	ES0N	LS0N	ES100N	ES0N	LS0N
Silage DM ensiled ²	0.83	0.96	1.41	0.71	1.15	2.12
Intake per cow ³ (t·cow ⁻¹)						
Grazed pasture DMI	3.23	3.41	3.12	3.13	3.33	3.15
Concentrate DMI	0.50	0.50	0.72	0.65	0.58	0.62
Silage DMI	1.46	1.32	1.35	1.47	1.31	1.30
Silage DM surplus or deficit ⁴	-0.36	-0.04	0.54	-0.53	0.23	1.52
Purchases/sales of feed per cow (€·cow ⁻¹)						
Concentrate DM	-165	-165	-237	-213	-190	-206
Silage DM	-46	-5	70	-69	29	198
Purchases/sales of feed (€·ha ⁻¹)						
Concentrate DM	-347	-264	-363	-448	-304	-315
Silage DM	-97	-8	107	-144	47	302

¹ES100N = early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

²Proportional loss during ensilage assumed to be 0.25 (McGeachan, 1989, 1990).

³Daily herbage intake at pasture, estimated from energy balances.

⁴Silage DM ensiled minus silage intake.

N to grass-clover swards and using the same methods of measuring pasture DM production, Humphreys et al. (2009) recorded a mean annual pasture DM production of 11.5 t·ha⁻¹ averaged over 4 yr between 2003 and 2006. Hence the lengths of the grazing season and annual yields of pasture on ES100N (10.35 and 9.88

t·ha⁻¹) in the present study were lower than in earlier similar studies at this site. Lower pasture production can be attributed to annual average soil temperatures in both years that were >1.0°C below average and annual volumes of rainfall that were >20% above average on this site with impeded drainage that is prone to

Figure 3. Relationship between clover stolon and root DM mass in November and the following February for 3 grazing systems, ES100N (□, long-dash fitted line: $y = 0.538x$, $R^2 = 0.59$, $P = 0.002$), ES0N (x, dotted fitted line: $y = 0.553x$, $R^2 = 0.34$, $P = 0.03$) and LS0N (●, solid fitted line: $y = 0.641x$, $R^2 = 0.84$, $P = 0.004$). Data are from both years of the study (2008–2009 and 2009–2010). ES100N = early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

Table 5. Milk composition and processing characteristics of milk from spring-calved cows in late lactation 2009–2010 (wk 23 to end of lactation)¹

Item ²	System			Pooled SEM	P-value
	ES100N	ES0N	LS0N		System
Fat (g·kg ⁻¹)	49.3	47.8	46.2	0.23	***
Protein (g·kg ⁻¹)	38.2	38.6	36.7	0.31	***
Lactose (g·kg ⁻¹)	45.1	45.0	44.7	0.12	NS
Casein (g·kg ⁻¹)	29.1	29.4	28.6	0.09	***
Total protein (g·kg ⁻¹)	37.8	38.2	36.8	1.20	***
Casein number ³ (g·kg ⁻¹)	77.9	77.2	76.7	0.66	NS
NPN (g·kg ⁻¹)	0.26	0.27	0.28	0.30	NS
SCC	203	195	198	7.49	NS
GT (s)	1,243	1,141	1,106	3.91	***
CFR _{max} (Pa·min ⁻¹)	3.41	3.50	3.22	0.005	***
GS (Pa)	79.5	78.6	71.0	0.18	***

¹ES100N = early spring calving with annual fertilizer N input of 100 kg·ha⁻¹ applied in spring; ES0N = early spring calving without fertilizer N; LS0N = late spring calving without fertilizer N.

²SCC = somatic cell count values reported are cells/mL ÷ 1,000; GT = gelation time; CFR_{max} = maximum curd firming rate; GS = gel strength.

³Casein number = (casein ÷ total protein) × 100.

****P* < 0.001.

waterlogging; excess rainfall lowered pasture production and caused inferior grazing conditions compared with the earlier studies. Furthermore, the stocking rate imposed on ES100N was based on previous research at this site (Humphreys et al., 2009) and during the present study required the purchase of an additional 0.45 t silage DM per cow (Table 4). Nevertheless, taking into account the adverse weather conditions during the present study, the stocking rate and feed demand on ES100N was reasonably well aligned with the pasture production potential at this site.

The 2 zero-fertilizer N input systems had lower milk output per hectare than ES100N. However, milk output per hectare was constrained by the stocking rates of dairy cows imposed on systems in this study. In contrast to ES100N there was a surplus of silage generated on both zero-fertilizer N systems averaged over both years, which indicates that stocking rates on both of these systems were suboptimal to fully utilize available pasture, particularly LS0N. Averaged over both years annual pasture DM production on ES100N was similar to LS0N and certainly in 2009 LS0N produced sufficient pasture to carry a similar stocking rate of dairy cows to ES100N.

Milk Production per Cow

There were no significant differences in annual milk production and milk composition per cow between the 3 grazing systems in the current study. Similarly, Glassey et al. (2013) reported no difference in annual milk production per cow by cows grazing grass-clover swards receiving and not receiving input of fertilizer

N. The IVOMD and CP of grazed swards did not differ between the systems, which partly accounts for the absence of a difference in dairy cow performance in the present study. A recent meta-analysis (Dineen et al., 2018) found that a mean sward white clover content of approximately 320 g·kg⁻¹ is required to increase in daily milk and milk solid yields per cow. The annual white clover content (range: 164–282 g·kg⁻¹; Table 3) across all systems in the present study was below the level required for higher milk production per cow.

Clover Production and Biological Nitrogen Fixation

In the former study (Humphreys et al., 2009) clover content of pasture DM averaged 219 g·kg⁻¹ (range: 203–239 g·kg⁻¹ DM) and annual clover DM production averaged 2.52 t·ha⁻¹ (range: 2.10–2.85 t·ha⁻¹) averaged over the 4 years in a system with similar stocking rates and fertilizer N input to ES100N. The annual clover contents of pasture DM on ES100N in the present study (164 and 203 g·kg⁻¹; Table 3) were lower than the former study. Likewise, the annual yields of clover pasture DM in the present study (range: 1.69 to 2.13 t·ha⁻¹; Table 2) were lower than the previous study, partly due to lower clover content of pasture DM and partly due to lower annual pasture DM production. It seems that the adverse weather conditions experienced during the present study lowered the clover DM production proportionally more than overall pasture DM production on ES100N.

The absence of fertilizer N input increased clover DM production on ES0N from 2.47 t·ha⁻¹ in 2008 to 2.80 t·ha⁻¹ in 2009 (Table 2). Likewise, clover DM produc-

tion on LSON increased from $2.07 \text{ t} \cdot \text{ha}^{-1}$ in 2008 to $3.58 \text{ t} \cdot \text{ha}^{-1}$ in 2009. Early spring has previously been identified as the optimal time to apply fertilizer N to grass-clover swards (Laidlaw, 1980; Harris and Clark, 1996; Humphreys et al., 2008). Little or no BNF takes place and clover growth rates are low at this time of year (Davies, 2001). Fertilizer N applied to ES100N and higher recycling of N in excreta increased spring and annual pasture production compared with ES0N. Higher grass production following the application of fertilizer N was associated with lower clover DM production on ES100N in 2009, which can be attributed to greater competition from the grass component of the sward. It is well established that there is a negative relationship between N fertilization and the clover content of swards and hence, BNF (Laidlaw et al., 1992; Harris and Clark, 1996; Burchill et al., 2014). On the other hand, grazing during the first half of the winter (December 2008 and January 2009) combined with no fertilizer N input substantially increased clover DM production on LSON in 2009 compared with ES0N. The higher pasture production on LSON in 2009 can be attributed to higher annual clover DM production along with higher BNF.

It is apparent that the higher clover DM production and BNF on LSON in 2009 was influenced by a carry-over effect from the previous year. The mass of stolon surviving over the winter is an important indicator of clover persistence (Frame and Newbould, 1986; Collins et al., 1991; Wachendorf et al., 2001). The LSON system had higher stolon masses than the other systems in February 2009 and February 2010. There was a net loss of stolon mass of 22% between November 2008 and February 2009 on LSON compared with 55% on the 2 early spring grazing systems, which had an earlier closing date (Table 1). As a consequence of this, LSON had higher stolon mass throughout 2009. The net loss of stolon over the winter 2009-10 was similar in the 3 systems (approximately 34%). Nevertheless, the LSON had higher stolon mass than the other systems at the end of the study in February 2010 as a consequence of having higher stolon mass during the 2009 growing season.

It seems that grazing during the winter by the cows on LSON favored clover stolon survival, perhaps by allowing greater light penetration to the base of the sward during the winter (Phelan et al., 2014). A similar result was found in Northern Ireland by Laidlaw and Stewart (1987) and Laidlaw et al. (1992), who found that grazing by sheep over the winter improved clover content of pasture DM in the following spring relative to no grazing between November and March. The beneficial effect on clover was attributed to lower competition from the

accompanying grass. On the other hand, in Switzerland, Lüscher et al. (2001) found that frequent defoliation (7 times between November and March) caused a net loss in clover stolon DM mass by the spring. The differences in responses in the latter study and those conducted on the island of Ireland could be attributed to climatic differences between Ireland (relatively mild weather during the winter) and Switzerland and perhaps to the much greater severity of the defoliation treatments in the latter study.

Milk Constituents and Processing Characteristics

The measurements of the processability of late-lactation milk indicated that there were no detectable differences in lactose, casein number, or NPN between the 3 systems. Somatic cell count was not influenced by grazing systems; mean SCC values were within the range of normality ($<200 \times 10^3 \text{ cells} \cdot \text{mL}^{-1}$) previously reported by O'Brien et al. (2006) and Guinee and O'Brien (2010). The decrease in milk volume with the later stages of lactation coincided with increases in total protein and fat levels, and a reduction in that of lactose similar to that reported by Guinee et al. (2007). However, the late-lactation milk of the LSON system had lower concentrations of fat, protein, casein, and total protein relative to ES100N and ES0N. While LSON had lowest mean concentrations of milk composition and nitrogen fractions, these results were still within the range of values reported previously for spring-calving herds (Mehra et al., 1999; O'Brien et al., 1999).

The current study found that LSON had shorter coagulation times and weaker gel structures. The low firmness of LSON gels made from late-lactation milk may be due to proteolysis of casein by plasmin (Lucey, 1996). The higher casein content of milk is expected to enhance coagulation properties with higher cheese yields (Guinee et al., 2006). In contrast to previous studies, the current study found that GT was longer for ES100N and ES0N milk (Guinee et al., 1997; Auldist et al., 2010). However, the higher protein content in the late-lactation milk from the latter systems formed a firmer gel than LSON, which was influenced by higher concentrations of protein in the milk (Guinee et al., 1997). Guinee et al. (2006) found that during cheese-making where curds are cut based on time, an increase in milk protein content is likely to coincide with an increase in the moisture content of the curd, because the stiffer gel at cutting has a lower ability to rearrange and lower rate of syneresis. Consequently, both ES100N and ES0N potentially would have higher cheese yield. Cheddar cheese yield increases by approximately 0.25 to 0.30 kg/100 kg of milk for every 0.1 g/100 g in-

crease in milk protein in the range 3.0 to 4.5 g/100 g, while retaining the protein-to-fat ratio constant at 0.96 (Guinee et al., 2006).

Calving Date and Grazing Season Timeframe

A key component of seasonal pasture-based milk production is achieving good synchronicity between calving date and the demand of the lactating herd for pasture and the commencement of pasture growth in spring. Whereas grazing during the winter in LSON promoted BNF and pasture production in the following year in the present study, the later calving strategy resulted in less pasture consumed per cow and higher supplementation with concentrates compared with ESON (Table 4). These concentrates were mostly fed during the winter. Furthermore, on LSON there was an excessive supply of pasture available when lactating cows were turned out to pasture in mid-April, making it necessary to close some of the area available for grazing for silage in April (Table 1). Hence, there was poor synchronicity between the herd demand for pasture and pasture availability, resulting in a less efficient and higher cost system. Furthermore, extending lactation into the early winter lowered the quality of late-lactation milk for processing purposes.

In terms of producing low-cost milk for processing into long shelf-life products, the ESON was more efficient than LSON. Under such circumstances it is advisable that calving date is not changed from mid-February, even under zero-fertilizer N input. It's possible that the beneficial effects of winter grazing on subsequent clover productivity could be achieved on an ESON-type system by grazing later and to a lower PGH before housing for the winter, rather than delaying calving date. Such an approach warrants further investigation. The economic feasibility of the LSON approach depends on the purpose for which the milk is used, such as liquid milk and short shelf-life products, and any additional price paid to promote the production of this milk during the winter months.

CONCLUSIONS

The zero-fertilizer N input systems had lower milk production per ha than ES100N because milk output was constrained by stocking rate. Pasture production on LSON in 2009 had the potential to carry a similar stocking rate of dairy cows to that of ES100N. Delaying calving date until mid-April on LSON resulted in inefficient use of pasture for milk production. Extending lactation into early winter lowered the quality of late-lactation milk for processing purposes. It is rec-

ommended that a mean calving date in mid-February under zero-fertilizer N input is a better option than a later calving date. It is possible that the beneficial effects of winter grazing on clover productivity could be achieved by grazing later and to a lower post-grazing height before housing for the winter. Such an approach could carry a higher stocking rate of dairy cows under zero-fertilizer N input; perhaps in the region of 2.0 to 2.5 cows ha⁻¹.

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