J. Dairy Sci. 104
https://doi.org/10.3168/jds.2020-19753

The effect of *Lolium perenne* L. ploidy and *Trifolium repens* L. inclusion on dry matter intake and production efficiencies of spring-calving grazing dairy cows

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ABSTRACT

The objective of this study was to investigate the effect of perennial ryegrass (*Lolium perenne* L.; PRG) ploidy and white clover (*Trifolium repens* L.) inclusion on milk production, dry matter intake (DMI), and milk production efficiencies. Four separate grazing treatments were evaluated: tetraploid PRG only, diploid PRG only, tetraploid PRG with white clover, and diploid PRG with white clover. Individual DMI was estimated 8 times during the study (3 times in 2015, 2 times in 2016, and 3 times in 2017) using the *n*-alkane technique. Cows were, on average, 64, 110, and 189 d in milk during the DMI measurement period, corresponding to spring, summer, and autumn, respectively. Measures of milk production efficiency were total DMI/100 kg of body weight (BW), milk solids (kg of fat + protein; MSo)/100 kg of BW, solids-corrected milk/100 kg of BW, and MSo/kg of total DMI. Perennial ryegrass ploidy had no effect on DMI; however, a significant increase in DMI (+0.5 kg/cow per day) was observed from cows grazing PRG-white clover swards compared with PRG-only swards. Sward white clover content influenced DMI as there was no increase in DMI in spring (9% sward white cover content), whereas DMI was greater in summer and autumn for cows grazing PRG-white clover swards (+0.8 kg/cow per day) compared with PRG-only swards (14 and 23% sward white clover content, respectively). The greater DMI of cows grazing PRG-white clover swards led to increased milk (+1.3 kg/cow per day) and MSo (+0.10 kg/cow per day) yields. Cows grazing PRG-white clover swards were also more efficient for total DMI/100 kg of BW, solids-corrected milk/100 kg of BW, and MSo/100 kg of BW compared with cows grazing PRG-only swards due to their similar BW but higher milk and MSo yields. The results highlight the potential of PRG-white clover swards to increase DMI at grazing and to improve milk production efficiency in pasture-based systems.
Key words: perennial ryegrass ploidy, white clover, dry matter intake, dairy cow

INTRODUCTION

The conversion of grazed pasture into highly nutritious dairy products is synonymous with grazing regions of the world. This low-cost system has resulted in Ireland, and other countries that rely on pasture-based systems, having a specific advantage due to their high capacity to produce milk from grazed pasture (Dillon et al., 2008) in an environmentally benign manner (Lorenz et al., 2019). Within pasture-based grazing systems, the efficient production of milk is heavily dependent on variable costs, of which feed can make up to 80% (Finneran et al., 2010; Connolly et al., 2011). To maximize profitability in these systems, costs can be reduced by increasing pasture utilization, reducing the proportion of purchased feed, and increasing milk produced from grazed pasture (Ramsbottom et al., 2015; Macdonald et al., 2017; Hanrahan et al., 2018). Therefore, achieving a high DMI per cow from grazed pasture is critical to ensure high animal performance (Dillon et al., 2006).

Low pasture DMI has been identified as a major factor limiting milk production in grazing dairy cows (Bargo et al., 2003). Pasture DMI is influenced by a multitude of environmental, plant, animal, and management factors (Bargo et al., 2003; Dillon, 2006). Some of the main plant and management factors that influence pasture DMI are sward structure (Stakelum and Dillon, 2007), sward nutritive value (Peyraud and Astigarraga, 1998), daily pasture allowance (Pérez-Prieto and Delagarde, 2012), and pregrazing pasture mass (McEvoy et

Received October 8, 2020.
Accepted January 11, 2021.
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al., 2010; Wims et al., 2014). Sward composition can also affect pasture DMI because certain forages (e.g., white clover; *Trifolium repens* L.) are more palatable and digestible than others (perennial ryegrass; *Lolium perenne* L.; **PRG**; Gowen et al., 2003; Dillon, 2006).

Balocchi and López (2010) showed that cows grazing diploid and tetraploid PRG swards preferred to graze tetraploid swards, illustrated by a higher utilization rate and lower postgrazing residual. This may imply a relationship between grazing preference and DMI, as cows have also been shown to have greater pasture DMI when grazing tetraploid swards compared with diploid swards (Lantinga and Groot, 1996; Gowen et al., 2003), although the effect can vary across the season (Gowen et al., 2003). Lantinga and Groot (1996) hypothesized that the increased DMI associated with tetraploid cultivars could be due to their greater palatability or possibly due to a lower resistance to physical breakdown during chewing compared with diploids, although they acknowledged that the mechanisms for increased DMI are not clear. White clover is an important legume in temperate grazing regions for several reasons, including that white clover has been shown to be grazed preferentially over PRG and that it increases DMI in mixed swards (Ribeiro Filho et al., 2003; Rutter et al., 2004; Egan et al., 2018). This preference has been linked to a faster rumen passage rate of white clover compared with PRG due to its lower NDF and ADF content (Minson, 1990; Egan et al., 2018). In contrast, other studies have reported no effect of white clover inclusion in PRG swards on DMI (Ribeiro Filho et al., 2005; Enriquez-Hidalgo et al., 2014; Egan et al., 2017), which can be attributed to the sward white clover content in these studies being insufficient to affect DMI (Enriquez-Hidalgo et al., 2014).

Providing forages that promote increased DMI is an opportunity that dairy producers could take to improve the productivity and efficiency of milk production in temperate pasture-based dairy systems. Tetraploid swards and white clover inclusion have been shown to increase DMI by varying degrees over diploid and PRG-only swards (Gowen et al., 2003; Egan et al., 2017, 2018); however, the interaction between PRG ploidy and white clover inclusion on DMI across the grazing season over multiple years has not been well investigated. The hypotheses of this experiment were that (1) tetraploid swards would support higher DMI and milk production than diploid swards, (2) PRG-white clover swards would support higher DMI and milk production than PRG-only swards, and (3) there would be an interaction between PRG ploidy and sward white clover inclusion in terms of DMI. Therefore, the objective of this study was to investigate the effect of PRG ploidy and white clover inclusion on milk production, DMI, and milk production efficiencies over multiple grazing seasons.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was conducted at Teagasc Clonakilty Agricultural College (51°63′N, −08°85′E; 25–70 m above sea level) over 3 yr from 2015 to 2017. A randomized block design was used with a 2 × 2 factorial arrangement of 2 PRG ploidies (tetraploid and diploid) each sown with and without white clover to give 4 grazing treatments as described by McClearn et al. (2019): a tetraploid PRG-only sward (**TGO**), a diploid PRG-only sward (**DGO**), a tetraploid PRG sward with white clover (**TWC**), and a diploid PRG sward with white clover (**DWC**). A dairy grazing platform of 43.6 ha was used, with 75% of the experimental area reseeded in 2012 and 25% reseeded in 2013 by full cultivation (ploughing and tilling). The 4 tetraploid cultivars (Astonenergy, Dunluce, Kintyre, and Twymax, sown at 37.5 kg/ha) and 4 diploid cultivars (Aberchoice, Glenveagh, Tyrella, and Drumbo, sown at 30 kg/ha) were sown as monocultures, with each cultivar sown 10 times across the grazing platform. In the white clover paddocks a 50:50 mix of the medium-leaved white clover cultivars Chieftain and Crusader was sown at 5 kg/ha. This resulted in 4 farmlets being created with 20 paddocks per grazing treatment. Paddocks for each treatment were balanced for location block, soil type, and soil fertility throughout the farm. Each farmlet was 10.9 ha and stocked at 2.75 cows/ha. Thirty spring-calving dairy cows were assigned to each grazing treatment every year based on genotype, parity, calving date, and economic breeding index. Three cow genotypes were used for this experiment: Holstein Friesian, Jersey × Holstein Friesian (JEX), and JEX × Norwegian Red. A total of 208 individual cows were used during the experiment.

Grazing Management

All treatments were grazed in a spring-calving rotational system. Cows were grazed day and night as soon as weather conditions allowed as they calved from February onward. Typically, grazing began in early February and finished in mid November each year. During periods of inclement weather conditions (excessive rainfall), where grazing conditions were poor, on–off grazing was practiced (Kennedy et al., 2009).

Cows were supplemented with 4 kg of concentrate (fresh weight) postcalving; this was gradually reduced and removed from the diet totally when pasture growth on the treatments met demand [47 kg of DM/ha per day; daily pasture allowance/cow (17 kg of DM/cow) × stocking rate (2.75 cows/ha)], usually in mid April. Grazing management was achieved by weekly monitoring of average farm cover for each treatment and using the online application PastureBase to aid in decision making (Hanrahan et al., 2017). Target pregrazing pasture mass (**PrGPM**) was calculated separately for each grazing treatment using the following formula:

$$\text{Target PrGPM} = (\text{stocking rate} \times \text{ideal rotation length} \times \text{daily herbage allowance per cow}) + \text{residual pasture mass},$$

where ideal rotation length during main grazing season = 21 d and daily herbage allowance (>4.0 cm) = 17 to 18 kg of DM/cow per day (O'Donovan and McEvoy, 2016).

If a pasture deficit occurred across all treatments, then concentrate supplementation was increased for all groups. However, if a pasture deficit occurred in less than all 4 treatments, then silage produced from each treatment farmlet was used to supplement the deficit to each individual treatment group.

Residency time within paddocks (between 1 and 2 d) was determined by targeting a postgrazing sward height (**PoGSH**) of 3.5 to 4.0 cm for the first and final grazing rotation and a target of 4.0 to 4.5 cm throughout the main grazing season. If residency time was predicted to be greater than 36 h, then the paddock was subdivided. Cows within treatments were moved to their following paddocks when the target PoGSH was reached. No mechanical correction, by mowing of paddocks postgrazing, took place over the 4 yr, and all excess forage was removed and conserved as silage. Inorganic nitrogen was applied equally across all 4 treatments in the form of urea or calcium ammonium nitrate at a rate of 250 kg of nitrogen/ha per year. Inorganic phosphorus and potassium were applied at similar rates across all 4 treatments based on yearly soil test results.

Herbage Measurements

Grazing data were collected from all paddocks grazed during the DMI measurement periods across the 3 yr. Pregrazing pasture mass was determined before grazing by harvesting 2 strips (approximately 10 m × 1.2 m) to a height of 4.0 cm using an Etesia mower (Etesia UK Ltd.). The harvested forage was weighed and a 100-g subsample was dried at 90°C for 15 h to determine DM. Ten sward heights were taken before and after each strip of forage was harvested using a Jenquip rising platemeter and used to calculate sward density as follows:

$$\text{Sward density (kg of DM/cm)} = \text{pasture yield (kg of DM/ha)}/[\text{precutting height (cm)} - \text{postcutting height (cm)}].$$

Pregrazing sward height (**PrGSH**) and PoGSH were also measured across whole paddocks before and after grazing using a platemeter, taking compressed sward heights at 30 locations prerazing and 50 compressed sward heights following grazing.

Pregrazing pasture mass above 4.0 cm was calculated using sward density according to the following equation (Delaby et al., 1998):

$$\text{Pregrazing pasture mass (kg of DM/ha)} = [\text{pregrazing sward height (cm)} - 4.0 \text{ cm}] \times \text{sward density (kg of DM/cm per ha)}.$$

Chemical Analysis of Pasture

Selected pasture representative of that grazed by the cows was manually collected using Gardena hand shears (Accu 60; Gardena International GmbH) before grazing in each paddock during the DMI measurement periods. This was done following close observation of the grazing animals' defoliation height from previous days. Samples were stored at −20°C, freeze-dried, and milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss) before conducting chemical analysis. The pasture samples were analyzed for DM content, ash content, ADF, NDF (Van Soest, 1963), CP (AOAC, 1990), and in vitro OM digestibility (Morgan et al., 1989).

Clover Contribution

White clover content was estimated in each paddock before grazing TWC and DWC paddocks during the DMI measurement periods. Gardena hand shears were used to take 15 random pasture samples cut to 4.0 cm throughout the paddock. This was mixed, and two 70-g cut samples were weighed and separated by hand into PRG, white clover, and other plant fractions and dried at 60°C for 48 h to give proportions on a DM basis.

Animal Measurements

Cows were milked twice daily at approximately 0700 and 1530 h. Weekly milk production was derived from individual milk yields (kg) recorded at each milking (Dairymaster). Milk fat, protein, and lactose contents were determined once weekly from a consecutive p.m. and a.m. milk sample for each cow and tested using infrared spectrophotometry (Milkoscan 203, Foss Electric). Milk solids (kg of fat + protein; **MSo**) yield per cow and solids-corrected milk (**SCM**; Tyrrell and Reid, 1965) were also calculated. For the purpose of this experiment, only milk production data relating to the weeks of the DMI measurement periods are presented. Full-lactation milk production results can be found in McClearn et al. (2019). Cows were weighed in the week before, during the week of, and in the week after the DMI measurement period upon exit from the milking parlor using an electronic scale (Tru-Test Ltd.). Body condition score was assessed, in a similar manner, by the same individual throughout the study on a scale of 1 to 5 in increments of 0.25 (where 1 = emaciated and 5 = extremely fat) as outlined by Edmonson et al. (1989).

DMI and Production Efficiencies

Individual DMI was estimated 8 times during the study (3 times in 2015, 2 times in 2016, and 3 times in 2017) using the *n*-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989). During each DMI measurement period cows were, on average, 64, 110, and 189 DIM, corresponding to spring, summer, and autumn, respectively, each year. All cows were dosed twice daily after milking for 11 consecutive days with a paper bullet containing 760 mg of C32-alkane (*n*-dotriacontane). From d 7 to 11 of dosing, fecal samples were collected from each cow twice daily before a.m. and p.m. milking, either in the paddock the hour before milking by observing the cows and collecting the fecal sample when cows 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 then thawed, bulked together, dried at 60°C for 48 h, milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss), and analyzed for alkane concentration per the methodology of Mayes et al. (1986). Briefly, after solvent extraction and purification, the alkanes were analyzed using gas chromatography (Dove and Mayes, 2006). For each treatment approximately 15 individual pasture samples were manually collected using Gardena hand shears, mimicking the grazing defoliation pattern observed on previously grazed swards, before each grazing event on d 7 to 11. The pasture samples were stored at −18°C. Frozen pasture samples were bowl-chopped, freeze-dried at −50°C to a constant weight, milled through a 1-mm screen using a Cyclotech 1093 Sample Mill, and analyzed for alkane concentration similarly to the feces samples. Cows were fed 0.18 kg of DM concentrate/cow during the DMI measurement periods with the exception of 2 DMI periods: spring 2015, when they received 0.88 kg of DM/cow, and spring 2016, when they received 3.55 kg of DM/cow. Pasture composed the remainder of the diet for all DMI measurement periods (no silage was fed during the DMI measurement periods). The alkane content of the concentrate fed was analyzed as described previously. In the TWC and DWC paddocks, pasture samples were taken and manually separated into PRG and white clover fractions, frozen, and milled as described previously. These fractions were then analyzed to provide individual alkane concentrations for PRG and white clover. The ratio of dosed C32-alkane to pasture C33 (tritriacontane) was used to estimate DMI using the equation described by Mayes et al. (1986) as follows:

$$\text{Daily pasture intake}\ (\text{kg of DM/cow}) = \frac{F_i/F_j \times (D_j + I_s \times S_j) - I_s \times S_i}{P_i - (F_i/F_j \times P_j)},$$

where F_i, S_i, and P_i are the concentrations (mg/kg of DM) of the natural odd-chain *n*-alkanes in feces, supplement, and pasture, respectively; F_j, S_j, and P_j are the concentrations (mg/kg of DM) of the even-chain *n*-alkane in feces, supplement, and pasture, respectively; D_j is the dose rate (mg/d) of the even-chain *n*-alkane, and I_s is the daily supplement intake (kg of DM/d). When calculating pasture DMI for the TWC and DWC treatments, a weighted average pasture C33 concentration, based on the proportion of PRG and white clover in the sward during the DMI measurement period, was used to account for the different C33 concentrations of PRG and white clover (Dove and Mayes, 1991).

Measures of milk production efficiency were calculated based on the net energy system (Faverdin et al., 2011), where 1 Unité Fourragère Lait (**UFL**) of energy is defined as the net energy content of 1 kg of standard barley for milk production, equivalent to 1,700 kcal. The measures of milk production efficiency were energy (UFL) intake, total DMI (**TDMI**)/100 kg of BW, SCM/100 kg of BW, MSo/100 kg of BW, and Mso/kg of TDMI.

Statistical Analysis

Grazing and nutritive value characteristics were analyzed using PROC MIXED of SAS (SAS Institute Inc., 2010), with the effects of year, DMI measurement period, PRG ploidy, and white clover treatment included as fixed effects, and the associated interactions, such as PRG ploidy × white clover treatment, DMI measurement period × white clover treatment, DMI measurement period × PRG ploidy, and the triple interaction DMI measurement period × PRG ploidy × white clover treatment, were included in the model. Tukey's test was used to determine differences between treatment means. Statistical significance was considered at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

Daily milk yield, pasture DMI (**PDMI**), TDMI, energy intake, energy balance, TDMI/100 kg of BW, SCM/100 kg of BW, MSo/100 kg of BW, and MSo/kg of TDMI were analyzed using PROC MIXED of SAS (SAS Institute Inc., 2010), taking into account the effects of year, DMI measurement period, PRG ploidy, white clover treatment, genotype, and parity as fixed effects, and the associated interactions PRG ploidy × white clover treatment, DMI measurement period × white clover treatment, DMI measurement period × PRG ploidy, DMI measurement period × PRG ploidy × white clover treatment, year × PRG ploidy, year × white clover treatment, and genotype × parity, were included in the model. Individual cow was the experimental unit, considered as a random effect in the model, and DMI measurement period (spring, summer, or autumn) was the repeated measure. Tukey's test was used to determine differences between treatment means. Statistical significance was considered at $P \leq 0.05$, and trends were considered at $0.05 < P \leq 0.10$.

RESULTS

Sward Measurements

Pregrazing pasture mass differed significantly ($P = 0.027$; Table 1) between grazing treatments during the DMI measurement periods as diploid swards had an average PrGPM of 1,761 kg of DM/ha (>4.0 cm) compared with 1,597 kg of DM/ha for tetraploid swards. White clover inclusion had no effect on PrGPM, and there was no interaction between PRG ploidy and white clover inclusion. Pregrazing sward height was not significantly different between PRG ploidy and swards with or without white clover. Postgrazing sward height differed between PRG ploidy and when white clover was included. Tetraploid swards were grazed lower than diploid swards (0.41 cm; $P < 0.001$), and PRG-white clover swards (TWC and DWC) were grazed lower than PRG-only swards (TGO and DGO; 0.27 cm; $P = 0.011$). Pasture allowance (>4.0 cm) was not affected by PRG ploidy or white clover inclusion ($P < 0.05$ for PRG ploidy and white clover inclusion, respectively). White clover content was not affected by PRG ploidy but differed significantly between seasons ($P < 0.001$), being lowest in spring (8.9%), intermediate in summer (14.2%), and highest in autumn (23.7%). Season also had an effect on PrGSH, PoGSH, and pasture allowance ($P < 0.01$). Pregrazing sward height was lower in spring than in autumn ($P < 0.001$), but PrGSH in summer was not significantly different from that in spring or autumn. Postgrazing sward height was lowest in spring ($P < 0.05$), whereas no difference was observed in PoGSH between summer and autumn ($P = 0.699$). Pasture allowance was similar in spring and summer but greater in autumn ($P = 0.003$).

Sward nutritive values are shown in Table 2. Neither PRG ploidy nor white clover inclusion affected OM digestibility content; however, OM digestibility declined from spring to summer and subsequently from summer to autumn regardless of sward type. Crude protein content did not differ between PRG ploidy but was significantly higher in white clover swards ($P < 0.001$). There was a trend for seasonal variation in CP content, with the highest values observed in spring, intermediate in summer, and lowest in autumn ($P = 0.058$). Neutral detergent fiber content was similar between tetraploid and diploid swards, but there was a trend for NDF to be lower ($P = 0.062$) when white clover was present in the sward. Differences were also observed throughout the year, with NDF content being highest in summer for all swards types. Acid detergent fiber content did not differ with PRG ploidy or white clover inclusion and was relatively similar between seasons ($P > 0.05$). Ash content did not differ between PRG ploidy but was significantly higher in white clover swards ($P < 0.001$). Unité Fourragère Lait was not different between tetraploid and diploid or between PRG-only and PRG-white clover swards ($P > 0.05$).

Milk Production

Cows grazing PRG-white clover swards had higher daily milk (23.1 vs. 21.7 kg/cow) and MSo (1.85 vs. 1.75 kg/cow) yields compared with those grazing PRG-only swards during the DMI measurement periods, with this effect evident in each season ($P < 0.001$; Table 3). Tetraploid swards tended ($P = 0.059$) to have higher daily milk yield than diploid swards (22.6 vs. 22.2 kg/cow, respectively), with the greatest effect being observed in autumn from cows grazing tetraploid swards. Daily SCM was greater for tetraploid swards compared with diploid swards (23.1 vs. 22.6 kg/cow per day, respectively; $P < 0.001$) and PRG-white clover swards compared with PRG-only swards (23.5 vs. 22.2 kg/cow per day, respectively; $P < 0.001$). Daily MSo yield was significantly affected by PRG ploidy ($P = 0.016$), with higher MSo yields observed from cows grazing tetraploid swards compared with diploid swards (1.82 vs.

Table 1. Effect of perennial ryegrass ploidy and white clover inclusion on average and within-season grazing sward characteristics

	Treatment[2]					*P*-value[3]			
Item[1]	TGO	DGO	TWC	DWC	SEM	P	WC	S	P × WC
Pregrazing pasture mass (kg of DM/ha)	1,641	1,794	1,553	1,728	72.6	0.027	0.297	0.698	0.879
Spring	1,626	1,654	1,512	1,751	121.3				
Summer	1,733	2,016	1,464	1,637	145.0				
Autumn	1,563	1,712	1,683	1,797	115.8				
Pregrazing sward height (cm)	8.75	9.20	8.70	9.01	0.238	0.116	0.615	<0.001	0.767
Spring	7.91	8.35	7.97	8.62	0.397				
Summer	9.13	9.93	8.16	8.47	0.474				
Autumn	9.21	9.32	9.97	9.93	0.379				
Postgrazing sward height (cm)	3.88	4.38	3.69	4.03	0.106	<0.001	0.011	<0.001	0.436
Spring	3.63	3.93	3.38	3.95	0.173				
Summer	3.94	4.65	3.66	4.06	0.214				
Autumn	4.08	4.56	4.04	4.07	0.166				
Pasture allowance (kg of DM/cow)	15.9	16.0	15.1	16.0	0.59	0.419	0.494	0.003	0.449
Spring	15.9	15.5	14.2	15.0	0.97				
Summer	14.9	15.2	14.5	15.3	1.16				
Autumn	17.0	17.2	16.6	17.7	0.93				
White clover content (%)	—	—	17.2	14.0	1.80	0.203	—	<0.001	—
Spring	—	—	9.4	8.5	2.93				
Summer	—	—	14.7	13.7	3.61				
Autumn	—	—	27.6	19.8	2.68				

[1]Spring, summer, and autumn correspond to 64, 110, and 189 DIM, respectively.

[2]TGO = tetraploid perennial ryegrass only; DGO = diploid perennial ryegrass only; TWC = tetraploid + white clover; DWC = diploid + white clover.

[3]P = ploidy; WC = white clover; S = season.

Table 2. Effect of perennial ryegrass ploidy and white clover inclusion on average and within-season grazing sward nutritive value

	Treatment[2]					*P*-value[3]			
Item[1]	TGO	DGO	TWC	DWC	SEM	P	WC	S	P × WC
OM digestibility (g/kg of DM)	786	784	786	784	1.1	0.162	0.979	<0.001	0.814
Spring	789	790	789	788	1.7				
Summer	787	786	789	786	2.1				
Autumn	781	770	780	779	1.7				
CP (g/kg of DM)	185	183	212	214	5.2	0.979	<0.001	0.058	0.630
Spring	198	196	219	214	8.2				
Summer	182	177	209	218	10.3				
Autumn	176	175	206	215	8.2				
NDF (g/kg of DM)	391	406	362	372	11.4	0.753	0.062	0.008	0.639
Spring	358	361	342	364	18.1				
Summer	414	415	399	394	22.6				
Autumn	400	423	380	358	18.1				
ADF (g/kg of DM)	216	221	220	218	4.4	0.691	0.864	<0.001	0.488
Spring	194	195	201	198	7.0				
Summer	223	232	229	231	8.8				
Autumn	231	235	229	226	7.0				
Ash (g/kg of DM)	78	77	82	83	1.6	0.934	0.009	<0.001	0.439
Spring	73	71	75	74	2.6				
Summer	80	80	83	89	3.2				
Autumn	82	80	87	86	2.6				
UFL[4]	1.05	1.05	1.05	1.04	0.005	0.432	0.366	<0.001	0.588
Spring	1.07	1.08	1.07	1.07	0.007				
Summer	1.05	1.05	1.05	1.04	0.009				
Autumn	1.03	1.02	1.02	1.02	0.007				

[1]Spring, summer, and autumn correspond to 64, 110, and 189 DIM, respectively.

[2]TGO = tetraploid perennial ryegrass only; DGO = diploid perennial ryegrass only; TWC = tetraploid + white clover; DWC = diploid + white clover.

[3]P = ploidy; WC = white clover; S = season.

[4]UFL = Unité Fourragère Lait.

Table 3. Effect of perennial ryegrass ploidy and white clover inclusion on average and within-season milk, solids-corrected milk (SCM) and milk solids yield, and milk composition

Item[1]	Treatment[2]				SEM	P-value[3]			
	TGO	DGO	TWC	DWC		P	WC	S	P × WC
Milk yield (kg/d)	21.8	21.6	23.4	22.7	0.23	0.059	<0.001	<0.001	0.237
Spring	25.0	24.8	26.3	25.6	0.30				
Summer	22.8	23.6	24.5	24.0	0.35				
Autumn	17.8	17.1	19.3	18.7	0.29				
SCM yield (kg/d)	22.4	22.1	23.9	23.1	0.24	0.023	<0.001	<0.001	0.243
Spring	24.8	24.7	25.9	25.6	0.32				
Summer	23.2	23.4	25.3	24.1	0.39				
Autumn	19.0	18.0	20.4	19.6	0.32				
Fat content (%)	4.39	4.37	4.37	4.32	0.051	0.558	0.358	<0.001	0.635
Spring	4.23	4.31	4.20	4.26	0.067				
Summer	4.28	4.31	4.37	4.18	0.079				
Autumn	4.68	4.53	4.54	4.52	0.066				
Protein content (%)	3.75	3.74	3.71	3.70	0.022	0.419	0.047	<0.001	0.892
Spring	3.53	3.53	3.48	3.48	0.028				
Summer	3.72	3.72	3.75	3.71	0.033				
Autumn	4.01	3.96	3.91	3.90	0.028				
Milk solids[4] (kg/d)	1.76	1.74	1.88	1.81	0.019	0.015	<0.001	<0.001	0.157
Spring	1.93	1.93	2.01	1.96	0.026				
Summer	1.82	1.84	2.00	1.89	0.031				
Autumn	1.53	1.45	1.63	1.57	0.025				

[1]Spring, summer, and autumn correspond to 64, 110, and 189 DIM, respectively.

[2]TGO = tetraploid perennial ryegrass only; DGO = diploid perennial ryegrass only; TWC = tetraploid + white clover; DWC = diploid + white clover.

[3]P = ploidy; WC = white clover; S = season.

[4]Milk solids = kg of fat + protein.

1.78 kg/cow, respectively). Milk fat content was not affected by PRG ploidy or white clover inclusion. Milk protein content was lower for cows grazing PRG-white clover swards ($P = 0.047$) but did not differ between PRG ploidy (Table 3).

DMI and Production Efficiencies

Perennial ryegrass ploidy did not affect TDMI (17.0 vs. 16.9 kg of DM/cow for tetraploid and diploid, respectively) or PDMI (16.3 vs. 16.1 kg of DM/cow for tetraploid and diploid, respectively; Table 4). However, cows grazing PRG-white clover swards had significantly higher PDMI ($P = 0.003$) and TDMI ($P = 0.002$) compared with those grazing PRG-only swards, with an average increase in both PDMI and TDMI of +0.5 kg (16.5 vs. 16.0 kg of DM/cow for PDMI and 17.2 vs. 16.7 kg of DM/cow for TDMI for PRG-white clover and PRG-only cows, respectively; Table 4). The effect of white clover varied over the season as there was no difference in TDMI between PRG-only and PRG-white clover swards in spring (17.0 vs. 16.8 kg of DM/cow, respectively), whereas PRG-white clover swards had a greater TDMI in summer and autumn (17.4 kg of DM/cow) compared with PRG-only swards (16.6 kg of DM/cow). Cows grazing tetraploid swards tended to have a higher daily energy intake compared with diploids (17.5 vs. 17.2 UFL/d, $P = 0.089$), and cows had a higher daily energy intake when grazing PRG-white clover swards compared with PRG-only swards (17.6 vs. 17.2 UFL/d, $P = 0.008$). However, daily energy balance was not affected by PRG ploidy or white clover inclusion ($P > 0.05$), and daily energy balance increased throughout the year ($P < 0.001$).

None of the production efficiencies calculated were affected by PRG ploidy ($P > 0.05$); however, when white clover was present in the swards, there were significant increases in TDMI/100 kg of BW, SCM/100 kg of BW, MSo/100 kg of BW, and MSo/TDMI ($P < 0.05$; Table 5). Total DMI/100 kg of BW was significantly greater for cows grazing PRG-white clover swards compared with cows grazing PRG-only swards (3.53 vs. 3.44 kg, respectively; $P = 0.013$). Similarly, SCM/100 kg of BW and MSo/100 kg of BW were greater for cows grazing PRG-white clover swards compared with those grazing PRG-only swards (4.83 vs. 4.58 kg, respectively, $P < 0.001$; and 0.38 vs. 0.36 kg, respectively, $P < 0.001$; Table 5).

DISCUSSION

One of the major factors affecting milk production from grazed pasture is dairy cow total DM and energy intake (Bargo et al., 2003; Dillon, 2006). Increasing the

Table 4. Effect of perennial ryegrass ploidy and white clover inclusion on average and within-season total and pasture DMI, energy intake, and energy balance

Item[1]	Treatment[2]				SEM	P-value[3]			
	TGO	DGO	TWC	DWC		P	WC	S	P × WC
Pasture DMI (kg)	16.1	15.8	16.5	16.4	0.15	0.251	0.003	<0.001	0.538
Spring	15.9	14.9	15.4	15.1	0.22				
Summer	16.3	16.0	17.0	16.7	0.27				
Autumn	16.1	16.6	17.1	17.3	0.22				
Total DMI (kg)	16.8	16.6	17.2	17.1	0.15	0.257	0.002	0.300	0.549
Spring	17.4	16.5	16.9	16.7	0.22				
Summer	16.9	16.6	17.6	17.3	0.26				
Autumn	16.1	16.7	17.2	17.4	0.21				
Energy intake (UFL[4]/d)	17.3	17.0	17.7	17.5	0.15	0.089	0.008	0.002	0.701
Spring	18.2	17.2	17.6	17.3	0.22				
Summer	17.3	16.9	18.1	17.5	0.27				
Autumn	16.5	16.8	17.4	17.6	0.21				
Energy balance (UFL/d)	1.7	1.5	1.3	1.4	0.16	0.746	0.107	<0.001	0.477
Spring	1.5	0.6	0.4	0.2	0.24				
Summer	1.4	1.0	1.2	1.1	0.30				
Autumn	2.1	3.0	2.4	2.9	0.24				

[1]Spring, summer, and autumn correspond to 64, 110, and 189 DIM, respectively.

[2]TGO = tetraploid perennial ryegrass only; DGO = diploid perennial ryegrass only; TWC = tetraploid + white clover; DWC = diploid + white clover.

[3]P = ploidy; WC = white clover; S = season.

[4]UFL = Unité Fourragère Lait.

conversion efficiency of grazed pasture into product, through increased DMI by manipulating pasture sward composition, is one option that could be used to achieve this. An overview of the overall lactation milk production, reproductive performance, annual pasture production, and economic performance from this study has been reported previously (McClearn et al., 2019, 2020). Briefly, McClearn et al. (2019, 2020a,b) reported no difference in milk production or profitability from cows grazing either tetraploid or diploid swards but did report large increases in milk and MSo production (+596 and +48 kg, respectively) and profitability (+€305/ha) for cows grazing PRG-white clover swards compared with PRG-only swards. This paper investigated the mechanisms underlying the increase in milk production from PRG-white clover swards, namely increased DMI.

Effect of Sward Type on Milk Production

Although McClearn et al. (2019) reported no significant difference in daily or cumulative milk and MSo yields between tetraploid and diploid swards over the full lactation of this study, there was a tendency for increased milk yield and a significant increase in MSo yield when cows grazed tetraploid swards compared with diploid swards during the DMI measurement periods. There is conflicting evidence as to the effect of PRG ploidy on milk production per cow, as some stud-

Table 5. Effect of perennial ryegrass ploidy and white clover inclusion on BW and milk production efficiencies

Item[1]	Treatment[2]				SEM	P-value[3]		
	TGO	DGO	TWC	DWC		P	WC	P × WC
BW (kg)	495	487	488	489	4.5	0.220	0.426	0.097
TDMI (kg/100 kg of BW)	3.43	3.45	3.54	3.51	0.034	0.969	0.013	0.357
SCM (kg/100 kg of BW)	4.57	4.59	4.90	4.75	0.055	0.219	<0.001	0.055
Milk solids[4] (kg/100 kg of BW)	0.36	0.36	0.39	0.37	0.004	0.165	<0.001	0.029
Milk solids/TDMI (kg)	0.105	0.106	0.110	0.108	0.0010	0.564	0.006	0.236

[1]TDMI = total DMI; SCM = solids-corrected milk.

[2]TGO = tetraploid perennial ryegrass only; DGO = diploid perennial ryegrass only; TWC = tetraploid + white clover; DWC = diploid + white clover.

[3]P = ploidy; WC = white clover; S = season.

[4]Milk solids = kg of fat + protein.

ies have shown increased milk yield for cows grazing tetraploid compared with diploid swards (Castle and Watson, 1971; Lantinga and Groot, 1996; Wims et al., 2013), whereas other studies have shown no difference in milk yield of cows grazing tetraploid or diploid swards (Gowen et al., 2003; O'Donovan and Delaby, 2005). Similar to Gowen et al. (2003), in the current study, milk yield was similar in the first 2 DMI measurement periods (1.8% increase in spring and −0.6% decrease in summer for tetraploid compared with diploid swards), whereas there was a greater difference in milk yield in autumn (3.6% increase from tetraploid compared with diploid swards). The build-up of dead material in the sward from the consistently higher PoGSH associated with diploid swards during the grazing season (Guy et al., 2018a,b; McClearn et al., 2019) can affect sward nutritive value, particularly in autumn, and may be one of the reasons for the lower milk and MSo yields of cows grazing diploid swards during autumn, although there was no difference in nutritive value between PRG ploidy in this study. Another reason for the inconsistent milk response to ploidy between studies may be the different cultivars used in studies. Gowen et al. (2003) stated that the cultivars used in a particular study may not represent all of the beneficial chemical or morphological traits of a particular PRG ploidy and therefore cannot be taken to be representative of all cultivars within a particular PRG ploidy category. Also, the single weekly time point taken during the DMI measurement period may not fully represent the variation in pasture nutritive value that may occur within a season (Guy et al., 2018b).

White clover inclusion in the sward increased milk and MSo yields per cow during the DMI measurement periods, which agrees with multiple studies that have shown increased milk yield per cow when white clover is included in PRG swards (Harris et al., 1997; Dineen et al., 2018; Egan et al., 2018). Some studies have reported no effect of white clover inclusion on milk yield (Ribeiro-Filho et al., 2005; Enriquez-Hidalgo et al., 2014) as the white clover content of the sward was insufficient [sward white clover content was 27 and 20% in Ribeiro-Filho et al. (2005) and Enriquez-Hidalgo et al. (2014), respectively] to achieve an increase in milk yield (Enriquez-Hidalgo et al., 2014). In Ribeiro-Filho et al. (2005), the PRG-white clover swards had a lower PrGPM, and this is the likely reason for the lack of an effect of white clover on milk yield. The greatest differences in milk and MSo yields occurred in summer and autumn corresponding to higher sward white clover contents. This agrees with Egan et al. (2018), who achieved high annual sward white clover contents (24.6%) and demonstrated the seasonal effect of white clover inclusion on milk production, as milk and MSo yields increased from June onward. McClearn et al. (2019) divided the lactation into 3 distinct periods (period 1 = wk 1–14 of lactation, period 2 = wk 15–28 of lactation, and period 3 = wk 29–42 of lactation) and showed that milk and MSo yields were greater for PRG-white clover swards than for PRG-only swards in periods 2 and 3 of lactation, whereas there was no difference in milk or MSo yields in period 1.

Effect of Sward Type on DMI

Sward type and structure have been shown to have a large influence on pasture DMI, with differences in DMI observed due to PRG cultivar, ploidy, heading date, and different levels of PrGPM (Stakelum and Dillon, 2007; Pérez-Prieto and Delagarde, 2012; Wims et al., 2014). The current study observed an increase in DMI due to white clover inclusion but not from differences in PRG ploidy. Gowen et al. (2003) measured PDMI of 4 PRG cultivars at 3 time points in the grazing season and observed higher DMI from tetraploid compared with diploid cultivars at 2 time points (corresponding to grazing rotations 5 and 7) and in the absence of nutritive value differences. Lantinga and Groot (1996) also observed similar increases in DMI and hypothesized that there is a positive relationship between high leaf content and DMI, which can be amplified in tetraploid cultivars. Furthermore, previously observed PRG ploidy differences in DMI have been accounted for by a higher palatability and possibly a lower resistance to chewing for tetraploids due to their higher leaf/stem ratio (Lantinga and Groot et al., 1996; Gowen at al., 2003). This has more recently been confirmed by Guy et al. (2018a), who, from a subset of paddocks used in the current experiment, reported greater leaf proportion for tetraploid swards. Even though cows grazing tetraploid swards grazed lower into the sward (−0.42 cm) during the DMI measurement periods, indicating greater palatability, pasture allowance was similar between PRG ploidy due to the higher PrGPM for diploids. Higher PrGPM has been shown to reduce DMI compared with lower PrGPM (Roca-Fernández et al., 2011; Wims et al., 2014). This is probably because cows offered lower PrGPM have been shown to graze lower into the sward (Curran et al., 2010), possibly due to higher nutritive value in the base of the sward and a lower level of dead material compared with swards with higher PrGPM. Although the diploid swards in the present study had higher PrGPM compared with tetraploids, the differences were not as extreme as in Roca-Fernández et al. (2011), and this is reflected in the similar nutritive values recorded between PRG ploidy during the DMI measurement periods. Also, cows grazing diploid swards did not achieve the same PoGSH, which showed that they were not forced to graze the lower nutritive value material in the base of the sward and may explain the lack of a PRG ploidy effect on DMI in this study. Additionally, it has been hypothesized that there is a relationship between water-soluble carbohydrate (**WSC**) content and DMI. Tas et al. (2005) found no difference in DMI between 8 diploid PRG cultivars despite differences in WSC content. Water-soluble carbohydrate content is typically higher in tetraploid compared with diploid cultivars (Byrne et al., 2018); however, Lantinga and Groot (1996) reported similar WSC levels in tetraploid and diploid cultivars but still observed higher DMI from tetraploids. Water-soluble carbohydrate content was not measured in this experiment, and newer cultivars used in Byrne et al. (2018) are likely to have increased WSC levels compared with the cultivars used by Lantinga and Groot (1996); this could explain the inconsistent response in WSC content and DMI between ploidy.

The inclusion of white clover in grazing swards typically increases nutrient value and voluntary DMI (Harris et al., 1997; Ribeiro Filho et al., 2003; Egan et al., 2018). Harris et al. (1997) showed that in white clover swards, cows had a significant increase in DMI when white clover content of the diet was 50% compared with 20%, with no difference in DMI when white clover content of the diet was 50% compared with 80%. Ribeiro Filho et al. (2003) found that cows grazing swards with 40% white clover had a DMI increase of 1.5 kg of DM/cow per day compared with cows grazing PRG-only swards. Therefore, the clear increase in PDMI and TDMI from white clover swards in the present study (+0.5 kg of DM/cow for both PDMI and TDMI) corroborates these previous findings. This also explains why there was no difference in DMI between PRG-only and PRG-white clover swards in spring, when sward white clover content was low, and the higher DMI response when white clover content increased in summer and autumn (+0.7 and +0.9 kg of DM/cow, respectively). Although passage rate was not directly measured, Moseley and Jones (1984) reported that the higher DMI associated with PRG-white clover swards was linked to a more rapid loss of particulate matter from the rumen (57% vs. 49% for white clover and PRG, respectively), particularly in the first 3 h postfeeding, due to a faster reduction in particle size and passage rate of white clover from the rumen. Recently, Niderkorn et al. (2017) reported that sheep had an increased eating rate just after feeding with a mixture of PRG and white clover compared with either PRG or white clover on their own. Niderkorn et al. (2017) also suggested that white clover incorporation in the diet may result in a faster digestion of the soluble fraction of the forage and a shorter retention time in the rumen. Beever et al. (1986) reported that the increase in DMI associated with white clover is likely due to an increased rate of digestion (rates of OM disappearance were 0.14 and 0.07%/h for white clover and PRG, respectively, using the in sacco technique), associated with a greater ease with which indigestible particles arising from white clover may leave the rumen compared with PRG.

Rumen physical fill has been linked to reduced DMI (Dado and Allen, 1995; Allen, 1996; Niderkorn et al., 2017) and is mainly determined by cell wall components (i.e., NDF). Mertens (1987) suggested that DMI could be predicted using dietary NDF, although there is evidence that using NDF alone is inadequate due to the inherent variability of NDF in different forages (Allen, 1996; Roche et al., 2008; Beecher et al., 2018). Beecher et al. (2018) reported no relationship between NDF and DMI but did report a relationship (R^2 = 0.50) between in vivo NDF digestibility and DMI. Therefore, DMI could be higher in swards with a lower NDF content or possibly greater digestibility of NDF, such as PRG-white clover. In the current experiment there was a trend for the NDF content of PRG-white clover swards to be lower (−22 g/kg), and this may have been one of the reasons for the increased DMI on PRG-white clover swards. Nonetheless, some previous studies have reported no increase in DMI from PRG-white clover swards (Thomson et al., 1985; Egan et al., 2017) but did still find an increase in milk production. They attributed this to the increase in nutritive value from PRG-white clover swards compared with PRG-only swards, and in particular to the lower NDF content. The focus in recent years has moved to undigested amylase- and sodium sulfite-treated NDF corrected for ash (**uNDFom**), as it has been demonstrated to be a useful predictor of in vivo digestibility (Dineen et al., 2021). Dineen et al. (2020) reported that cows consuming PRG-only swards, which had an amylase- and sodium sulfite-treated NDF corrected for ash (**aNDFom**) content of 36% of DM and a uNDFom content of 10% of aNDFom, had a rumen uNDFom pool size of 4.8 kg but that the physical fill capacity of the rumen did not restrict DMI, as when cows were fed a rolled barley supplement, uNDFom rumen pool size increased (5.5 kg). Dineen et al. (2021) analyzed 46 samples of PRG and reported a uNDFom content of 13.2% of aNDFom. However, very limited data are available on the uNDFom characteristics of white clover. Further work on the NDF digestion characteristics of PRG-white clover swards is required to elucidate the mechanisms involved in the increased DMI associated with these swards. McClearn et al. (2019) calculated total feed intake per cow per lactation and showed that cows consumed 320 kg more DM from PRG-white clover swards than from PRG-only swards and attributed 68% of the increase in MSo produced (+32 kg) to increased DMI, with the remaining 32% likely due to the increase in sward nutritive value and associated benefits (Table 2). The current experiment corroborates previous studies and substantiates a causal factor for the increase in milk production for cows grazing PRG-white clover swards—namely, additional DMI and energy intake—although the mechanism for this increase in DMI remains unclear.

Effect of Sward Type on Milk Production Efficiencies

The overall levels of TDMI/100 kg of BW, SCM/100 kg of BW, MSo/100 kg of BW, and MSo/kg of TDMI achieved within the study (3.48, 4.70, 0.370, and 0.107 kg, respectively) are similar to results of previous studies investigating various pasture-based production systems (Mackle et al., 1996; McCarthy et al., 2014; Coffey et al., 2017). The results of this study show that milk production efficiency measures improved when white clover was included in PRG swards. Total DMI/100 kg of BW, SCM/100 kg of BW, MSo/100 kg of BW, and MSo/kg of TDMI increased by 2.4, 5.1, 5.5, and 3.2%, respectively, when cows grazed PRG-white clover swards compared with PRG-only swards. The increased milk production efficiency of cows grazing PRG-white clover swards was due to a combination of increased TDMI (illustrated by the 2.4% increase in TDMI/100 kg of BW) and increased sward nutritive value and production (illustrated by the 3.2% increase in MSo/kg of TDMI) compared with cows grazing PRG-only swards. The cumulative effect of these factors is evidenced in the 5.1% to 5.5% increase in SCM/100 kg of BW and MSo/100 kg of BW, respectively, as cows across all grazing treatments were of similar BW (Table 5).

Several studies have highlighted the importance of achieving large intakes of high nutritive value pasture per unit of BW and efficiently converting this feed into MSo (Buckley et al., 2005; Coffey et al., 2017). The results from the current study highlight the potential of white clover to increase DMI of grazing dairy cows and thus improve the efficiency of milk production in pasture-based systems.

CONCLUSIONS

The results of this study show that PRG ploidy did not significantly affect DMI or milk yield but did have an effect on MSo yield during the DMI measurement period. The results also show that significant increases in DMI and sward nutritive value can be gained from using PRG-white clover swards rather than PRG-only swards, which can ultimately promote increased milk and MSo yields if sward white clover content is at sufficient levels. The driving factors for these increases are greater efficiency in TDMI/100 kg of BW and MSo/100 kg of BW that result from white clover being included in the grazing swards. The results from this study should give pasture-based dairy farmers confidence that including white clover in PRG swards at sufficient levels can increase DMI at grazing and, subsequently, milk production and milk production efficiency.

ACKNOWLEDGMENTS

This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland (Dublin, Ireland). The first author received a Teagasc Walsh Scholarship (Teagasc, Carlow, Ireland). The authors gratefully acknowledge the invaluable assistance of the work placement students and the farm and technical staff based at Teagasc Clonakilty Agricultural College and Teagasc Moorepark. The authors declare no conflicts of interest.

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