The effect of perennial ryegrass ploidy and white clover inclusion on milk production of dairy cows

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Short title: The effect of ploidy and white clover on milk production

Acknowledgements
This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland. The first author was in receipt of a Teagasc Walsh Fellowship. The authors would like to gratefully acknowledge the invaluable assistance of the farm and technical staff based at Teagasc Clonakilty and Teagasc Moorepark.

Summary text
Recent research has reported that perennial ryegrass (PRG) ploidy and white clover inclusion in grazing swards can have a positive effect on milk production. In this study, cows grazing PRG-white clover swards had greater milk yields compared with cows grazing PRG-only swards, while grass ploidy had no effect. This significant increase in milk production from PRG-white clover swards suggests the inclusion of white clover in grazing systems can be effectively used to increase milk production.

Abstract
Grazed grass is considered the cheapest feed available for dairy cows in temperate regions and so to maximize profits, dairy farmers must utilize this high quality feed where possible. Recent research has reported that including white clover (Trifolium repens L.) in grass swards can have a positive effect on milk production. The aim of this study was to quantify the effect of tetraploid and diploid perennial ryegrass (Lolium perenne L.; PRG) swards sown with and without white clover on the milk production of grazing dairy cows. Four grazing treatments were used for this study; tetraploid-only PRG swards, diploid-only PRG swards, tetraploid PRG with white clover swards and diploid PRG with white clover swards. Thirty cows were assigned to each treatment and swards were rotationally grazed at a stocking rate of 2.75 cows/ha and a nitrogen fertilizer application rate of 250 kg/ha annually. There was no significant effect of ploidy on milk production. Over this 4 year study, cows grazing the PRG-white clover treatments had greater milk yields (+ 597 kg/cow.year) and milk solids yield (+ 48 kg/cow.year) compared with cows grazing the PRG-only treatments. This significant
increase in milk production suggests the inclusion of white clover in grazing systems can be effectively used to increase milk production of grazing dairy cows.

**Keywords:** perennial ryegrass ploidy, white clover, milk production

**Introduction**

In suitable temperate regions, grazed grass is the cheapest feed available for dairy cows and within the context of increasing production costs and income volatility, dairy farmers must maximise the use of this high quality feed where possible (Finneran *et al.* 2012). Grazed grass can make up to 80% of dairy cow’s diets, so the production and utilization of grazed grass can significantly increase farm profitability (Macdonald *et al.* 2010). Previous research has shown that perennial ryegrass (*Lolium perenne* L.; PRG) ploidy can affect milk production where cows that grazed tetraploid swards had a higher milk and milk solids yield (kg fat + protein; MS) compared with cows that grazed diploid swards (Wims *et al.* 2013). Tetraploid swards often have a greater nutritional value, in particular, a greater digestibility compared with diploid swards (O'Donovan and Delaby 2005). Recently, there has been renewed interest in the inclusion of white clover (*Trifolium repens* L.) in grazing systems due to the reported benefits of biological nitrogen (N) fixation along with possible milk production benefits (Lüscher *et al.* 2014). Previous studies undertaken have reported a range of biological N fixation from 30 to 170 kg/ha.year by white clover growing in swards with PRG (McNeill and Wood 1990; Ledgard *et al.* 1999). An increase in milk production from cows grazing PRG-white clover swards has also been observed and can be attributed to an overall increase in herbage dry matter (DM) intake (Ribeiro Filho *et al.* 2005) and to the high nutritional value of white clover (Søegaard 1993). The objective of this study was to determine the effect of PRG ploidy and white clover inclusion on milk production of spring calving, pasture-based dairy cows at a farm system scale.

**Materials and methods**

The experiment was undertaken at Clonakilty Agricultural College, Clonakilty, Co. Cork, Ireland from February 2014 to November 2017. The experiment was a 2 × 2 factorial design; two PRG ploidies (tetraploid, diploid) and two white clover treatments (PRG-only, PRG-white clover) resulting in 4 grazing treatments (tetraploid-only PRG, diploid-only PRG, tetraploid PRG with white clover and diploid PRG with white clover). Seventy five percent of the area was reseeded (full cultivation (i.e. ploughing and tilling) was used to establish the reseed) in 2012 and 25% in 2013. Twenty blocks, with 4 paddocks in each block, were created and balanced for location, topography and soil type. Paddock size ranged from 0.43 ha to 0.71 ha. Each treatment was randomly assigned a paddock in each block. This created 4 separate farmlets which consisted of 20 paddocks for each grazing treatment. Diploid swards were sown at a rate of 30 kg/ha and tetraploid swards were sown at a rate of 37 kg/ha. In the PRG-white clover paddocks a 50:50 mix of Chieftain and Crusader white clover was sown at a rate of 5 kg/ha. There were 30 cows per treatment and each treatment was stocked at 2.75 cows/ha, with 10 cows of each breed (Holstein-Friesian (HF), Jersey x HF(JEX) and Norwegian Red x JEX) allocated per treatment. Within breed, cows were assigned to treatment based on calving date, parity and economic breeding index. Cows remained on their treatments for the entire grazing season in each year but were re-randomized at the beginning of each year after calving. In the dry period (December and January) cows were housed together and fed similarly. Treatments were rotationally grazed from early-February to mid-November each year and target post-grazing sward height was 4 cm. Nitrogen fertiliser application was 250 kg/ha.year. Each farmlet
was walked weekly to monitor average farm cover (Hanrahan et al. 2017) and when surpluses arose they were removed in the form of baled silage. If a feed deficit occurred across all treatments then all treatments were supplemented with concentrate, on average 343 kg DM of concentrate was fed per cow per year across all treatments. If a feed deficit occurred in an individual treatment, then cows were supplemented with conserved forage produced from within that treatment. Pre-grazing herbage mass (PGHM) in each paddock was determined twice weekly by harvesting 2 strips (10m x 1.5m) using an Etesia mower (Etesia UK Ltd., Warwick, UK) in the area to be grazed next. Herbage samples from all 4 treatments were sampled and analysed at 4 time points (February-March, mid-May to mid-June, mid-June to mid-July, September) across the year for neutral detergent fibre (NDF), and organic matter digestibility (OMD). Pre- and post-grazing heights were measured daily using a rising plate meter (Jenquip, Fielding, New Zealand). Sward white clover content was measured before grazing in each paddock in each rotation by cutting 15 random grab samples to 4 cm with a Gardena hand shears, mixing and weighing 2 70g samples, separating the sample into PRG and white clover fractions and drying at 60°C for 48 hours. Other species were not measured however; their proportion in the sward was low. Milk yield was recorded daily and milk composition weekly by taking milk samples from a consecutive evening and morning milking. Data from 480 cows (120 in 2014, 2015, 2016 and 2017, respectively) was available for analysis. Milk data was analysed using Proc MIXED in SAS (SAS 9.4). Terms included in the model were year, ploidy, white clover inclusion, parity, breed and their interactions.

Results

The effect of PRG ploidy and white clover inclusion on sward characteristics, herbage removal and milk production are presented in Table 1. The white clover content was not statistically different in the diploid-white clover and tetraploid-white clover swards (23.6% and 22.6%, respectively; P = 0.546). There was an effect of ploidy and white clover inclusion on PGHM, with PRG-white clover swards having a lower average PGHM (1,579 kg DM/ha) compared to PRG-only swards (1,678 kg DM/ha) throughout the year (P = 0.001). Diploid swards (1,673 kg DM/ha) had a higher PGHM than tetraploid swards (1,584 kg DM/ha; P = 0.001; Table 1), although there was no significant difference between the tetraploid-only PRG and the diploid PRG with white clover swards (1,631 vs. 1,620 kg DM/ha, respectively). Ploidy did not have a significant effect on pre-grazing sward height, however white clover inclusion in the sward had a significant effect as pre-grazing sward height was 0.24 cm lower for PRG-white clover swards compared with PRG-only swards (8.56 vs. 8.80 cm; P = 0.004). Post-grazing sward height was also significantly affected by ploidy and white clover. Tetraploid-only PRG swards had lower post-grazing sward height compared to diploid only PRG swards (4.10 vs. 4.32 cm; P = <0.001; Table 1). White clover inclusion also affected post-grazing sward height with white clover swards having a significantly lower post-grazing sward height compared to PRG-only swards (3.85 vs. 4.21 cm; P = <0.001). Perennial ryegrass ploidy had a significant effect on OMD and NDF content with tetraploid swards having a higher OMD value and lower NDF value compared to diploid swards. Similarly, white clover inclusion in swards also had a significant positive effect with PRG-white clover swards having a higher OMD and lower NDF content compared to PRG-only swards (OMD: 776 vs. 796 g/kg and NDF: 441 vs. 401 g/kg, respectively; P = <0.001).

Perennial ryegrass ploidy had no effect on cumulative milk yield, but there was a tendency for cows grazing tetraploid swards to have higher cumulative MS yield than cows grazing diploid swards (464
Cumulative milk yield and MS per cow were significantly affected by white clover inclusion with cows grazing white clover swards producing, on average, an extra 597 kg milk/cow.year and 48 kg MS/cow.year compared to PRG-only swards ($P = <0.001$). There was no interaction effect observed between ploidy and white clover inclusion. Average sward white clover content of the PRG-white clover swards and daily milk yield per cow for PRG-only and PRG-white clover swards is presented in Figure 1. As sward white clover content increases through the year, milk yield differences begin to occur when white clover content of the swards reaches approximately 15%) and this difference is maintained throughout the rest of lactation.

**Discussion**

Pre-grazing herbage mass was influenced by ploidy, with diploid-only PRG swards tending to have a higher PGHM compared to tetraploid-only PRG swards. This is similar to previous studies with diploid cultivars having significantly higher PGHM (Gowen et al. 2003; Wims et al. 2013). Perennial ryegrass-white clover swards had significantly lower PGHM compared to PRG-only swards which is similar to previous studies (Schils et al. 2000b; Ribeiro Filho et al. 2005). In contrast Egan et al. (2018) reported similar PGHM for PRG-white clover and PRG-only swards. This study found lower PGHM in rotation 1 (data not presented) in spring due to lower winter growth rates from PRG-white clover swards (Guy et al. 2018), but this wasn’t reflected throughout the remainder of the year. Although there were differences in PGHM, large variances in PGHM are required to have an impact on milk yield and previous studies have recorded no significant effect on milk yield when cows graze varying PGHM (Tuñon et al. 2011; Wims et al. 2014).

Post-grazing sward height was affected by ploidy and white clover inclusion. Tetraploid swards had lower post-grazing sward heights compared to diploid swards; this was similar to Wims et al. (2012). The lower post-grazing sward height observed with tetraploid swards are associated with their high leaf:stem ratio along with a higher energy content which can increase grazing efficiency (Balocchi and López 2010). Previous research has shown that cows have a preference for grazing tetraploid over diploid cultivars; associated with a longer grazing time, lower post-grazing height and higher digestibility of the tetraploid cultivars (Smith et al. 2001; Stilmant et al. 2005). White clover swards also had significantly lower post-grazing heights compared with PRG-only swards. This was observed across the year and was not a reflection of lower PGHM as Guy et al. (2018) reported lower post-grazing heights in PRG-white clover swards throughout summer when PRG-white clover swards had similar PGHM compared to PRG-only swards. Previous studies have also shown this with cows actively selecting white clover over perennial ryegrass in swards (Rutter et al. 2004; Cosgrove et al. 2006). Cow preference for white clover swards can also result in lower post-grazing sward heights (Phillips and James 1998; Ribeiro Filho et al. 2005).

In this 4 year study, PRG ploidy had no effect on daily or total milk yield although there was a tendency for cows grazing tetraploid swards to have greater daily and total MS yield. There are contrasting results from the literature with regard to the effect of ploidy on milk production. Wims et al. (2013) found that cows grazing tetraploid cultivars had higher milk and MS yield compared with cows grazing diploid cultivars during both the vegetative and reproductive growth stages. While Gowan et al. (2003) reported no significant effect of ploidy on milk production, however in their study one tetraploid cultivar outperformed the other three cultivars (1 tetraploid, 2 diploid) in terms of milk production. This may indicate the variations between individual cultivars may be higher than...
between ploidy. Short-term studies have shown increased milk yield for cows grazing tetraploid swards compared to diploid swards (Castle and Watson 1971; Lantinga and Groot 1996). However, in longer term, full lactation studies (Gowen et al. 2003; O'Donovan and Delaby 2005) no effect of ploidy was observed. This may indicate the beneficial effects of tetraploid cultivars may be diluted across the year and there are only seasonal advantages from tetraploid cultivars. Cumulative milk yield and MS yield was significantly increased due to the inclusion of white clover in the grazing sward. Numerous studies have found similar results (Schils et al. 2000a; Humphreys et al. 2009; Egan et al. 2018) these were all long-term studies and observed varying increases in milk production from white clover swards with a range of artificial N application rates (90-250 kg N/ha). The difference in milk production from the PRG-white clover swards was observed from May onwards in each year. This is consistent with an increasing white clover content in the sward and is similar to other studies (Schils et al. 2000b; Woodward et al. 2001). The increase in milk production is typically based on two factors; an increase in DM intake from PRG-white clover swards compared to PRG-only swards and an increase in herbage nutritive value in PRG-white clover swards compared to PRG-only swards. Increased DM intake from white clover swards has been reported in previous studies, but again sward white clover content needs to be greater than 20% to see an effect (Harris and Thomas, 1973; Schils et al. 2000). The higher nutritive value of PRG-white clover swards compared to PRG-only swards reported in this study (Table 1) may have contributed to the increase in MS production through increased DM intake. It is well reported that sward nutritive value is typically higher in white clover swards compared to PRG-only swards, with higher crude protein levels and lower NDF content (Søegaard 1993; Enriquez-Hidalgo et al. 2018). This higher quality grazing material from PRG-white clover swards may have been instrumental in the increase in MS production. However, the tetraploid PRG-only swards had a higher nutritive value compared to diploid PRG-only swards and did not impact MS production.

Conclusions

The results from this 4 year full-lactation study suggest the inclusion of white clover in grazing swards can increase milk and MS yield. This study highlighted the well-known benefits of including white clover in PRG swards and it showed no significant interaction effect between ploidy and white clover inclusion in terms of white clover establishment, nutritive value or milk production.

Declaration of Conflict of Interest

The authors declare no conflicts of interest.
References


Table 1. Sward characteristics and milk production of four different sward types including a tetraploid-only PRG (T), diploid-only PRG (D), tetraploid PRG with white clover (TC) and diploid PRG with white clover (DC) swards including effects of ploidy (P), white clover (WC) and interactive effects.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T</th>
<th>D</th>
<th>TC</th>
<th>DC</th>
<th>S.E.M.</th>
<th>P</th>
<th>WC</th>
<th>P * WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-grazing herbage mass (kg DM/ha)</td>
<td>1,631</td>
<td>1,725</td>
<td>1,537</td>
<td>1,620</td>
<td>30.2</td>
<td>0.003</td>
<td>0.001</td>
<td>0.849</td>
</tr>
<tr>
<td>Pre-grazing herbage height (cm)</td>
<td>8.71</td>
<td>8.88</td>
<td>8.54</td>
<td>8.57</td>
<td>0.085</td>
<td>0.199</td>
<td>0.004</td>
<td>0.398</td>
</tr>
<tr>
<td>Post-grazing herbage height (cm)</td>
<td>4.10</td>
<td>4.32</td>
<td>3.78</td>
<td>3.92</td>
<td>0.039</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.271</td>
</tr>
<tr>
<td>White clover content (%)</td>
<td>-</td>
<td>-</td>
<td>22.6</td>
<td>23.6</td>
<td>1.17</td>
<td>0.546</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OMD (g/kg DM)</td>
<td>779.5</td>
<td>771.7</td>
<td>803.5</td>
<td>788.8</td>
<td>3.12</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.265</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>430.5</td>
<td>451.4</td>
<td>389.9</td>
<td>411.3</td>
<td>3.55</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.958</td>
</tr>
<tr>
<td>Milk yield (kg/cow.year)</td>
<td>5,235</td>
<td>5,198</td>
<td>5,866</td>
<td>5,784</td>
<td>47.9</td>
<td>0.205</td>
<td>&lt;0.001</td>
<td>0.628</td>
</tr>
<tr>
<td>Milk solids yields (kg/cow.year)</td>
<td>440</td>
<td>434</td>
<td>488</td>
<td>481</td>
<td>3.6</td>
<td>0.077</td>
<td>&lt;0.001</td>
<td>0.982</td>
</tr>
</tbody>
</table>

Figure 1. Daily milk yield for grass-only (tetraploid-only PRG and diploid-only PRG) and grass-clover, (tetraploid PRG with white clover and diploid PRG with white clover) and average sward white clover content by week of year.