

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title                       | Investigating the effect of synthetic nitrogen application rate on the milk production, intake, digestion and nitrogen excretion of dairy cows fed perennial ryegrass or perennial ryegrass-white clover herbage                                                                                                                                                                                                                                                                        |
| Authors                     | Wims, E.;O'Callaghan, Tom F.;McCarthy, B.;Flynn, D.;Serra, E.;Dillon, P.;Ryan, D.;Murphy, J.P.;Dineen, M.                                                                                                                                                                                                                                                                                                                                                                               |
| Publication date            | 2026-06                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Original Citation           | Wims, E, O'Callaghan, T F, McCarthy, B, Flynn, D, Serra, E, Dillon, P, Ryan, D, Murphy, J P & Dineen, M 2026, 'Investigating the effect of synthetic nitrogen application rate on the milk production, intake, digestion and nitrogen excretion of dairy cows fed perennial ryegrass or perennial ryegrass-white clover herbage', Journal of Dairy Science, vol. 109, no. 6, pp. 6156-6169. <a href="https://doi.org/10.3168/jds.2025-27895">https://doi.org/10.3168/jds.2025-27895</a> |
| Type of publication         | Article (peer-reviewed)                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Link to publisher's version | <a href="https://www.scopus.com/pages/publications/105039831332-10.3168/jds.2025-27895">https://www.scopus.com/pages/publications/105039831332 - 10.3168/jds.2025-27895</a>                                                                                                                                                                                                                                                                                                             |
| Rights                      | © 2026, authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®. This is an open access article under the CC BY license ( <a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a> ) - <a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>                                                                                                          |
| Download date               | 2026-10-30 05:56:41                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Item downloaded from        | <a href="https://hdl.handle.net/10468/19475">https://hdl.handle.net/10468/19475</a>                                                                                                                                                                                                                                                                                                                                                                                                     |



# UCC

**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh



## Investigating the effect of synthetic nitrogen application rate on the milk production, intake, digestion, and nitrogen excretion of dairy cows fed perennial ryegrass or perennial ryegrass–white clover herbage

E. Wims,<sup>1,2</sup> T. F. O’Callaghan,<sup>2</sup> B. McCarthy,<sup>1</sup> D. Flynn,<sup>1</sup> E. Serra,<sup>1</sup> P. Dillon,<sup>1</sup> D. Ryan,<sup>1</sup> J. P. Murphy,<sup>1</sup> and M. Dineen<sup>1\*</sup>

<sup>1</sup>Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 P302, Ireland

<sup>2</sup>School of Food and Nutritional Sciences, University College Cork, Co. Cork, T12 K8AF, Ireland

### ABSTRACT

The objective of this experiment was to investigate the effect of synthetic N application rate on milk production, intake, digestion, and N excretion of dairy cows fed perennial ryegrass (PRG) or PRG-white clover (WC) herbage. Twelve ruminally cannulated multiparous Holstein Friesian dairy cows averaging (mean  $\pm$  SD) 66  $\pm$  12 DIM and 492  $\pm$  41 kg BW were initially enrolled during spring in a 14-d acclimation period. Cows were then randomly assigned to 1 of 4 dietary treatments (DT) in an incomplete crossover design with two 22-d experimental periods. This experimental schedule was repeated during autumn when the cows were averaging 216  $\pm$  12 DIM and 512  $\pm$  37 kg of BW. The 4 DT were as follows: (1) PRG receiving 25 kg of synthetic N/ha per cut (G-L); (2) PRG receiving 50 kg of synthetic N/ha per cut (G-H); (3) PRG-WC receiving 0 kg of synthetic N/ha per cut (GC-L); and (4) PRG-WC receiving 25 kg of synthetic N/ha per cut (GC-H). We observed a DT by season interaction effect on milk yield and milk solids yield. In spring, cows fed GC-L had lower milk yield when compared with cows fed G-H (23.4 and 26.8 kg/d, respectively), and lower milk solids yield when compared with cows fed G-L and G-H (1.53, 1.92, and 1.99 kg/d, respectively). In autumn, cows fed GC-H had greater fat yield when compared with cows fed GC-L (0.89 and 0.78 kg/d, respectively) and had greater milk solids yield when compared with cows fed GC-L and G-L, but were similar to cows fed G-H (1.70, 1.53, 1.53, and 1.58 kg/d, respectively). We observed a DT by season interaction effect on DM and OM intake and on OM digestibility (OMD). In spring, DM and OM intake was similar among DT, whereas in autumn cows fed GC-H had greater DM and OM intake when compared with cows fed other DT. Cows fed GC-L

had lower DM digestibility when compared with cows fed other DT in both spring and autumn. In spring, cows fed GC-L had lower OMD when compared with cows fed other DT, whereas in autumn, cows fed G-L had lower OMD when compared with cows fed G-H and GC-H. We observed a DT by season interaction effect on N intake, urinary N concentration, and urinary N output. In spring, cows fed G-H had greater N intake, urinary N concentration, and urinary N output when compared with cows fed other DT. In autumn, cows fed GC-L and GC-H had greater N intake and urinary N output when compared with cows fed G-L and G-H (800, 818, 608, and 626 g/d, respectively, and 447, 439, 265, and 247 g/d, respectively). Urine output did not differ among DT in spring, whereas in autumn, cows fed GC-L and GC-H had greater urine output when compared with cows fed G-L and G-H (66.0, 64.6, 47.3, and 46.8 kg/d, respectively). Overall, reducing synthetic N application rate on PRG-only swards by 25 kg of N/ha per cut generally had no effect on animal performance, intake, or digestibility, and it improved N excretion profiles during the spring. However, these N excretion benefits were not evident during the autumn period. Similarly, cows fed PRG-WC herbage receiving reduced application rates of synthetic N exhibited more favorable N excretion profiles during spring, whereas due to excessively high WC proportions during autumn, cows fed PRG-WC herbage had greater N intake, urinary N output, and urine output when compared with cows fed PRG-only herbage. These results indicate that additional strategies will be required to effectively mitigate urinary N excretion during periods of elevated nitrate-leaching risk.

**Key words:** in vivo digestibility, nitrogen excretion, pasture-based, urinary nitrogen

### INTRODUCTION

Pasture-based systems can require 200 to 300 kg of N input/ha per year to maintain productive growth rates

Received November 5, 2025.

Accepted February 7, 2026.

\*Corresponding author: [michael.dineen@teagasc.ie](mailto:michael.dineen@teagasc.ie)

The list of standard abbreviations for JDS is available at [adsa.org/jds-abbreviations-26](https://adsa.org/jds-abbreviations-26). Nonstandard abbreviations are available in the Notes.

of high-quality swards (Enriquez-Hidalgo et al., 2016; Chapman et al., 2019). Typically, this N is supplied in the form of synthetic N fertilizer to predominantly perennial ryegrass (*Lolium perenne* L.; **PRG**) swards. In Europe, strategic policy initiatives such as the European Green Deal (European Commission, 2019) and Farm to Fork Strategy (European Commission, 2020) have set targets to reduce synthetic N use by 20% by 2030. These reductions aim to mitigate emissions of ammonia, nitrous oxide, and nitrate leaching from agricultural systems, as well as the carbon dioxide emissions associated with the industrial production of synthetic N (Herron et al., 2021). However, the global population is projected to peak at nearly 10.3 billion by 2084 (United Nations, 2024), and when combined with rising standards of living, this is expected to drive a substantial increase in global demand for animal-sourced food products (Alexandratos and Bruinsma, 2012; OECD/FAO, 2025). In the absence of system innovation, reductions in synthetic N use will limit on-farm feed production and subsequent self-sufficiency. This will increase the dependence of farms on imported feeds to maintain human-food production and potentially lead to unintended pollution swapping, undermining the environmental and food security objectives of the European Union policies.

The incorporation of forage legumes such as white clover (*Trifolium repens* L.; **WC**) into PRG swards has been demonstrated to reduce the amount of synthetic N required to maintain productive swards (–100 kg N/ha; Egan et al., 2018; Murray et al., 2024) while simultaneously increasing the milk production efficiency of dairy cows when sward WC proportions are greater than 20% (McCleary et al., 2019). Due to the ability of WC to perform biological N fixation (**BNF**), plant-available N is supplied more gradually and in closer synchrony with plant uptake when compared with synthetic N application (Ledgard et al., 2009). This more balanced N supply often results in a higher proportion of N being assimilated into plant biomass, thereby improving N use efficiency (**NUE**; Ledgard et al., 2009; Lassaletta et al., 2014). Hennessy et al. (2019) suggested that within pasture-based dairy systems, PRG-WC swards increase farm-gate **NUE** when compared with PRG swards, even when estimates of **BNF** are included in such calculations (51% vs. 40%, respectively). Overall, the incorporation of forage legumes into PRG pastures represents a promising innovation strategy to sustain sward productivity, animal performance, and human-food production under increasingly stringent synthetic N application limits.

A key challenge within such systems is that as N supply from **BNF** increases, inputs of synthetic N must be reduced to prevent an overall increase in total N inputs. Ledgard et al. (2009) demonstrated that as total N inputs increase, there is an exponential rise in the nitrate-N that

can be leached, regardless of whether the N input comes from **BNF** or synthetic N. The nutritional physiology of the ruminant does not distinguish among these 2 sources of N, as dietary N in excess of requirements will be excreted, which can lead to concentrated N depositions on the soil in the form of urine patches (200–2,000 kg N/ha; Selbie et al., 2015). This soluble source of N has a high risk of being lost from the production system via nitrate leaching to ground water (Di and Cameron, 2007; Selbie et al., 2015), especially during the late autumn and winter periods that are characterized by high soil moisture status, high rainfall, low pasture growth rates, and low evapotranspiration.

Overall, to reduce nitrate leaching from pasture-based systems while maintaining human-food production, it is important to investigate the effect of reducing synthetic N application rates on swards either containing or not containing WC. Literature comparing the effects of these factors on the N excretion of pasture-fed lactating dairy cows is currently limited, largely due to the logistical challenges associated with conducting total N balance studies. Such experimental techniques also enable precise quantification of nutrient digestibility and can provide mechanistic insight into how the inclusion of WC in the sward typically supports greater **DMI** and milk solids yield (McCleary et al., 2019). Furthermore, it is important to conduct these investigations across the grazing season to capture temporal changes in spring-calving dairy cow requirements, sward nutritive value, and sward WC proportion. Therefore, the objective of this experiment was to investigate the effect of synthetic N application rate, with synthetic N being partially or completely substituted by **BNF** in PRG-WC swards, on the milk production, intake, digestion, and N excretion of dairy cows fed PRG or PRG-WC herbage. We hypothesized that cows fed PRG-WC herbage receiving reduced rates of synthetic N would have lower urinary N excretion when compared with cows fed PRG herbage receiving greater rates of synthetic N. In addition, we hypothesized that the effects would be dependent on season with a more pronounced effect occurring on urinary N excretion during late spring when compared with autumn.

## MATERIALS AND METHODS

This experiment was undertaken at the Teagasc Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland; 52°16'N, 8°25'W; 49 m above sea level) in spring (April–May) and autumn (September–November) of 2022. The soil type was a free draining acid brown earth of sandy loam to loam texture. The experiment was approved by the Health Products Regulatory Authority (Dublin, Ireland) through the ex-

perimental license (AE19132-P150) under the European directive 2010/63/EU and Statutory Instrument no. 543 of 2012 (European Union, 2012). Meteorological data were obtained from a weather station located at Moorepark (Fermoy, Co. Cork, Ireland). The daily air temperature ( $^{\circ}\text{C}$ ), soil temperature ( $^{\circ}\text{C}$  at 100 mm depth), and rainfall (mm) were recorded.

### **Animals and Experimental Design**

Twelve ruminally cannulated multiparous Holstein Friesian cows averaging (mean  $\pm$  SD)  $66 \pm 12$  DIM and  $492 \pm 41$  kg of BW were initially enrolled during spring in a 14-d acclimation period where all animals grazed together on PRG-only pasture fertilized with 25 kg of synthetic N/ha per regrowth and were supplemented with 0.90 kg DM/d of a standard concentrate. All cows were 4 yr old. Following the acclimatization period, cows were randomly assigned to 1 of 4 dietary treatments (DT) in an incomplete crossover design with two 22-d periods. Cows were first adapted to their DT over a 14-d period before an 8-d measurement period. Cows were then reallocated to another DT for a second 14-d adaptation period, before the final 8-d measurement period. Cows were crossed over where each cow within an initial treatment was randomly crossed over to one of the remaining treatments. During the 14-d dietary adaptation periods, cows were offered their respective DT in an outdoor feed pad facility (mechanical harvesting outlined in the next section, “Dietary Treatments, Herbage Management, and Herbage Measurement”), whereas during the measurement period cows were housed in a metabolism stall facility in individual stalls with free access to water. The above experimental schedule was repeated during autumn when the cows were averaging  $216 \pm 12$  DIM,  $148 \pm 12$  d since conception, and  $512 \pm 37$  kg of BW. During the autumn 14-d acclimation period, all animals grazed together on PRG-only pasture fertilized with 25 kg synthetic N/ha per regrowth and were supplemented with 0.88 kg DM/d of a standard concentrate. The spring experiment was performed from early April to late May and the autumn experiment was performed from late September to early November of 2022. At the beginning of the spring and autumn experiments, the cows were balanced for milk solids yield and BW. The ingredient composition of the standard concentrate on a fresh weight basis was as follows: maize meal, 28.7%; maize distillers, 25.0%; soy hulls, 20.0%; unmolassed beet pulp, 10%; liquid sugar cane molasses, 7%; soybean meal, 3.5%; magnesium oxide, 2.9%; calcium carbonate, 1.2%; sodium chloride, 0.8%; minerals and vitamins, 0.6%; and defluorinated phosphorus, 0.4%. The chemical composition of the standard concentrate was  $89.1\% \pm 1.4\%$  DM,  $16.1\% \pm 0.3\%$  CP (% of DM),  $59.7\% \pm 0.2\%$  soluble N (% of N),

$16.0\% \pm 0.7\%$  NDIN (% of N),  $3.8\% \pm 0.3\%$  ADIN (% of N),  $28.1\% \pm 0.5\%$  amylase- and sodium sulfite-treated NDF corrected for ash residue (**aNDFom**; % DM),  $9.5\% \pm 0.4\%$  water-soluble carbohydrates (**WSC**; % of DM), and  $10.6\% \pm 0.2\%$  ash (% of DM). The 12, 72, and 120 h undigested aNDFom (**uNDFom**) residues (% of aNDFom) were  $60.9\% \pm 4.9\%$ ,  $10.9\% \pm 0.4\%$ , and  $10.9\% \pm 0.4\%$ , respectively.

### **Dietary Treatments, Herbage Management, and Herbage Measurement**

The 4 DT were as follows: (1) PRG receiving 25 kg of synthetic N/ha per cut (**G-L**); (2) PRG receiving 50 kg of synthetic N/ha per cut (**G-H**); (3) PRG-WC receiving 0 kg of synthetic N/ha per cut (**GC-L**); and (4) PRG-WC receiving 25 kg of synthetic N/ha per cut (**GC-H**). The synthetic N application rates were chosen to represent strategies currently used in pasture-based systems. Because the swards were mechanically harvested and delivered to the animals throughout the experiment, the lack of animal manure N being returned to the swards was also accounted for. For the PRG-only swards, the application rates of 25 and 50 kg of synthetic N/ha per cut were selected to represent a moderate rate of synthetic N application (i.e., 125 kg of synthetic N/ha per year) and the maximum allowable rate at the time (i.e., 250 kg synthetic N/ha per year). The same moderate rate of 25 kg of synthetic N/ha per cut was applied to one of the PRG-WC treatments, as these swards can utilize BNF. The final treatment received 0 kg of synthetic N/ha per cut to resemble pasture-based systems that solely rely on BNF rather than synthetic N application. To generate the DT, 2 experimental areas (3 ha each) were established comprising pastures containing PRG only or a mixed PRG and WC sward. The pre-existing swards were established in May of 2020. At establishment, the previous swards were sprayed with glyphosate and plowed, and a fine, firm seedbed was prepared by disking and power harrowing to a depth of 5 cm. Seeds were sown at a 1-cm depth and the ground rolled. The sowing rates for the PRG-only swards were 32 kg/ha PRG (16 kg of AstonEnergy, Goldcrop, Ireland; 16 kg of Abergain, Germinal, Ireland), and, for the PRG-WC swards, 27 kg/ha PRG (13.5 kg of AstonEnergy, 13.5 kg of Abergain) plus 5 kg/ha WC (Coolfin, DLF, Ireland). Before the current experiment, the most recent synthetic N application was 25 kg of synthetic N/ha, applied in September 2021. Each experimental area was subdivided further into 8 equal plots (0.375 ha; 4 plots per DT) to facilitate the application of synthetic N, if applicable. From January to February 2022, even proportions of the experimental area were harvested across all DT to ensure a consistent supply of herbage for the spring experiment. The 4 rep-

licate plots per DT were managed in a staggered rotation so that a consistent supply of herbage of similar regrowth age was available throughout each experiment. After harvesting, either 25 or 50 kg of synthetic N/ha, in the form of protected urea (N-(n-butyl) thiophosphoric triamide; 46% N; Goulding Chemicals Ltd., Marina, Cork, Ireland) was applied to the respective DT areas. The GC-L dietary treatment received no synthetic N. These synthetic N application rates were maintained across the grazing season, after each harvesting event, until mid-September when the closed period for synthetic N application began (DAFM, 2022). During 2022, the G-L, G-H, GC-L, and GC-H plots received 6, 6, 0, and 6 applications of synthetic N, respectively, corresponding to annual rates of 150, 300, 0, and 150 kg of synthetic N/ha. The regrowth interval between synthetic N application and harvesting of each DT was on average 18 d in spring and 34 d in autumn due to seasonal growth patterns. In between the spring and autumn experiments, the same treatment-specific synthetic N application rates were maintained and swards were conserved as silage. In July, the experimental area received 34 kg of K/ha and 13 kg of S/ha in the form of sulfate of potash (42% K and 16% S; Goulding Chemicals Ltd., Marina, Cork, Ireland).

The pastures were harvested twice daily at 0800 and 1530 h using a GrassTech Grazer GT80 (Future Grass Technology, Borris, Co. Carlow, Ireland). Pastures were cut at 4 cm above ground level. During the 14-d adaptation periods, cows were offered herbage twice daily at the time of harvesting. During the 8-d measurement periods, cows were offered herbage 8 times daily at 0630, 0830, 1030, 1230, 1430, 1630, 1830, and 2030 h, with the herbage refrigerated at 4°C between feedings to minimize respiration, as described previously by Dineen et al. (2020). The quantity offered to each animal was recorded, and refusals were collected the following morning at 0730 h and weights were recorded. The feeding rates were adjusted daily to yield refusals of 5% to 10% of intake. After harvesting, herbage offered was sampled for each DT and dried at 90°C for 16 h to determine DM. Refusals were sampled once daily per cow to determine DM. Additional offered and refused samples were frozen at -20°C for later chemical analysis. During both the acclimatization and experimental periods, all cows were also supplemented daily with a standard concentrate (0.90 kg DM/cow during spring and 0.88 kg DM/cow during autumn). Concentrate was fed at milking in 2 equal portions; the quantity offered was recorded, and any refusals were collected and weighed during each 8-d measurement period to calculate concentrate intake. Concentrate samples were collected on alternate days and dried at 60°C for 64 h.

Precutting herbage yield was determined in each plot before harvesting by cutting 2 strips (1.2 m × 10 m) to a

height of 4 cm using an Etesia mower (Etesia UK. Ltd., Warwick, UK). The harvested material was weighed and a 100-g subsample was oven-dried at 90°C for 16 h to determine the DM concentration. Sward density was calculated as described by Dineen et al. (2021); briefly, using a rising plate meter (355 mm diameter and 3.2 kg/m<sup>2</sup> Jenquip, Feilding, New Zealand), 10 compressed herbage height measurements were recorded before and after harvesting for both strips, and sward density was calculated as follows:

$$\begin{aligned} \text{Sward density (kg of DM/cm per ha)} = \\ \frac{\text{Precutting herbage yield (kg of DM/ha)}}{[\text{Precutting compressed height (cm)} \\ - \text{Postcutting compressed height (cm)}]} \end{aligned}$$

Additionally, 30 compressed sward height measurements were taken diagonally across each plot to determine precutting compressed sward height using the previously mentioned rising plate meter. The above measurements were used to calculate precutting herbage yield in accordance with Dineen et al. (2021):

$$\begin{aligned} \text{Precutting herbage yield (kg of DM/ha)} = \\ [\text{Precutting compressed sward height (cm)} \\ - 4 \text{ (cm)}] \times \text{sward density (kg of DM/cm per ha)}. \end{aligned}$$

The postcutting residual compressed sward height was determined by recording 30 measurements across each plot after harvesting using a rising plate meter. The sward WC proportion was determined in each GC-L and GC-H plot before harvesting. Using a Gardena hand shears (Accu 90, Gardena International GmbH, Ulm, Germany), 15 to 20 samples were taken at random locations along a W-shaped transect, cutting to 4 cm. The sample was mixed, and a 70-g subsample was taken and separated into PRG and WC fractions and dried at 90°C for 16 h to determine the proportions on a DM basis.

### Animal Measurements

Cows were milked twice daily at 0730 h and 1530 h. Individual milk yield was recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). During the 8-d measurement periods, milk samples were collected at each milking and subsequently analyzed for fat, CP and lactose concentration using mid-infrared spectroscopy analysis (Milkoscan 7, Foss Electric, Hillerød, Denmark). Milk solids [fat (kg) + protein (kg)], ECM (Tyrell and Reid, 1965), and feed efficiency (ECM/DMI) were calculated. Body weight was measured following

the morning milking on the day before and the day after each measurement period using an electronic portable weighing scale and Winweigh software package (Tru-test Limited, Auckland, New Zealand). Body condition score was recorded concurrently with BW by an experienced observer on a scale of 1 to 5 with 0.25-unit increments (Edmundson et al., 1989).

During each 8-d measurement period, cows were fitted with an external harness to which fecal collection aprons were attached. Feces were collected into trays positioned directly behind each animal. Total fecal output from the previous 24 h was weighed and recorded each day. Following weighing, feces was homogenized by hand mixing and 2 subsamples were immediately frozen at  $-20^{\circ}\text{C}$  for later chemical analysis. To facilitate the separation of urine and feces, a latex urine separator was attached to the area around the vulva of each cow. The urine was directed into a 60-L collection drum with 100 mL of 9 M sulfuric acid per 10 L of urine added to prevent volatilization of N. The total urine output from the previous 24 h was weighed and recorded each day. Following weighing, the urine was homogenized and a sample was immediately frozen at  $-20^{\circ}\text{C}$  for later chemical analysis. Data collected during d 15 to 20 of each 22-d experimental period were included in the current analysis.

### Laboratory Analysis and Calculations

All herbage and fecal samples were freeze dried (LS40+chamber, MechaTech Systems Ltd., Bristol, UK) at  $-55^{\circ}\text{C}$  for ~96 h until a constant weight was achieved. Concentrate samples were oven-dried at  $60^{\circ}\text{C}$  for 64 h. Once dried, samples were milled through a 1-mm screen using a Cyclotech 1093 Sample Mill (Foss). Herbage, concentrate, and fecal samples were analyzed for ash (AOAC International, 2000; method 942.05), N using a Leco FP-928 analyzer (Leco Corp., St Joseph, MI), aNDFom (Mertens, 2002), and uNDFom after in vitro incubation with rumen fluid, as described by Dineen et al. (2020). Herbage samples were also analyzed for in vitro OM digestibility (OMD) using the method outlined by Morgan et al. (1989) using the Fibertec System (Foss, Ballymount, Dublin, Ireland); ADF (inclusive of ash residue) using Ankom Technology (Macedon, NY; Lee and Prosky, 1995; method 973.18); WSC according to the procedures of Hall (2014); and soluble N, NDIN, and ADIN according to the procedures of Licitra et al. (1996). Frozen urine samples were thawed and immediately analyzed for N concentration using the Leco FP-928 analyzer.

The total-tract apparent digestibility of DM, OM, aNDFom, and N was determined by first subtracting the fecal output of each nutrient from its respective intake and then dividing by the respective intake. Nitrogen par-

tioning was determined by dividing the amount of N recovered in urine, feces, or milk by the total intake of N. Unaccounted N was calculated as N intake minus the sum of urinary, fecal, and milk N.

### Statistical Analysis

All data were analyzed using a linear mixed effects model within the MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + DT_i + S_j + DTS_{ij} + P_{kj} + DTP_{ikj} + C_{lj} + \varepsilon_{ijkl},$$

where  $Y_{ijkl}$  = dependent variable,  $\mu$  = overall mean,  $DT_i$  = fixed effect of dietary treatment  $i$ ,  $S_j$  = fixed effect of season  $j$ ,  $DTS_{ij}$  = fixed interaction effect of dietary treatment  $i$  and season  $j$ ,  $P_{kj}$  = fixed effect of period  $k$  nested within season  $j$ ,  $DTP_{ikj}$  = fixed interaction effect of dietary treatment  $i$  and period  $k$  nested within season  $j$ ,  $C_{lj}$  = random effect of cow  $l$  nested within season  $j$ , and  $\varepsilon_{ijkl}$  = residual error. The interaction term including DT and P within season was removed from the model when  $P > 0.1$ . Multiple comparisons between treatment means were made using the Tukey–Kramer method. When the fixed interaction effect of DT and season was significant, the SLICE option was used to compare DT within each season. Statistical significance was considered if  $P \leq 0.05$  and a statistical trend if  $0.05 < P \leq 0.10$ .

## RESULTS

Meteorological data for the spring and autumn experimental periods performed during 2022 are presented in Table 1. Mean monthly air and soil temperature during the experimental periods were consistent with the previous 10-yr average. Total rainfall for July and August was below average, whereas September, October, and November exceeded the 10-yr average. Rainfall was 73, 124, and 65 mm greater during September, October, and November, respectively, compared with the previous 10-yr average.

### Dietary Nutritive Value

The precutting yields, WC proportions, and nutritive value of the herbage used during the experiment are presented in Table 2. Herbage precutting yield was greater in spring when compared with autumn (1,684 and 1,322 kg of DM/ha, respectively). Across both seasons, the WC proportion was greater for GC-L swards when compared with GC-H swards. In autumn, WC proportion was greater than in spring, averaging 53% of the sward DM. Herbage DM concentration was greater in spring when

**Table 1.** Mean air temperature, soil temperature and total rainfall during the experimental period compared with the 10-yr average (2012–2021)

| Item                      | Feb. | Mar. | Apr. | May  | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. |
|---------------------------|------|------|------|------|------|------|------|------|------|------|
| Mean air temperature, °C  |      |      |      |      |      |      |      |      |      |      |
| 2022                      | 7.4  | 6.7  | 9.1  | 12.4 | 13.8 | 16.6 | 16.8 | 13.7 | 12.2 | 9.0  |
| 10-yr average             | 5.9  | 6.7  | 8.5  | 11.3 | 14.1 | 16.1 | 15.3 | 13.4 | 10.6 | 7.4  |
| Mean soil temperature, °C |      |      |      |      |      |      |      |      |      |      |
| 2022                      | 7.1  | 7.2  | 10.4 | 14.6 | 16.3 | 18.9 | 19.3 | 15.8 | 12.9 | 9.0  |
| 10-yr average             | 5.8  | 7.2  | 10.0 | 13.6 | 16.9 | 18.8 | 17.4 | 16.4 | 11.5 | 7.7  |
| Total rainfall, mm        |      |      |      |      |      |      |      |      |      |      |
| 2022                      | 96   | 83   | 69   | 44   | 73   | 34   | 28   | 140  | 230  | 165  |
| 10-yr average             | 106  | 74   | 73   | 68   | 77   | 63   | 90   | 67   | 106  | 100  |

compared with autumn (18.1% and 13.4%, respectively). In spring, the herbage CP was greatest for G-H (18.9% DM), intermediate for G-L and GC-H (16.3% and 15.4% DM, respectively), and lowest for GC-L (12.8% DM). In autumn, WC increased herbage CP in the PRG-WC swards, on average, when compared with PRG-only swards (24.8% and 19.2% DM, respectively).

### Animal Performance

We observed a DT by season interaction effect on milk yield ( $P < 0.01$ ; Table 3), ECM yield ( $P < 0.01$ ), fat yield ( $P < 0.01$ ), protein yield ( $P < 0.01$ ), lactose yield ( $P < 0.01$ ), and milk solids yield ( $P < 0.01$ ). In spring, cows fed GC-L had lower yields of ECM, fat, and milk solids when compared with cows fed G-L and G-H, and had lower yields of milk, protein, and lactose when compared with cows fed G-H. In autumn, cows fed GC-H had greater fat yield (+14%) when compared with cows fed GC-L and had greater milk solids yield (+11%) when compared with cows fed GC-L and G-L ( $P < 0.01$ ), but were similar to cows fed G-H ( $P > 0.05$ ). In both spring and autumn, we found no effect of DT or DT by season interaction effect on milk fat, protein, or lactose concentrations, nor on feed efficiency, BW, or BCS ( $P > 0.05$ ; Table 3). In both spring and autumn, we found no effect of DT or DT by season interaction effect on BW or BCS change ( $P > 0.05$ ; data not shown).

### Intake and Apparent Digestibility

The effects of synthetic N application rate and pasture species on intake and apparent total-tract digestibility are presented in Table 4. We observed a DT by season interaction effect on DM ( $P = 0.04$ ), OM ( $P = 0.05$ ), aNDFom ( $P < 0.01$ ), and uNDFom intake ( $P < 0.01$ ). In spring, cows fed GC-L had lower aNDFom intake when compared with cows fed G-L and G-H, but were similar to cows fed GC-H. In spring, cows fed G-L had lower uNDFom intake when compared with cows fed GC-H, but were similar to cows fed G-H and GC-L. In autumn, cows fed GC-H had greater DM and OM intake when

compared with cows fed other DT ( $P < 0.01$ ). In autumn, cows fed GC-L had lower aNDFom intake when compared with cows fed other DT. In autumn, cows fed G-L had greater uNDFom intake when compared with cows fed G-H and GC-L, but were similar to cows fed GC-H.

Cows fed GC-L had lower DM digestibility when compared with cows fed other DT ( $P < 0.01$ ). We found no DT by season interaction effect for DM digestibility ( $P = 0.71$ ), but there was a DT by season interaction effect observed for OM digestibility ( $P = 0.01$ ), aNDFom digestibility ( $P < 0.01$ ) and N digestibility ( $P < 0.01$ ). In spring, cows fed GC-L had lower OM digestibility when compared with cows fed other DT, whereas in autumn, cows fed G-L had lower OM digestibility (−2%) when compared with cows fed G-H and GC-H. In spring, cows fed G-H had greater aNDFom digestibility (800 g/kg) when compared with cows fed GC-L and GC-H (699 and 760 g/kg, respectively) but similar to cows fed G-L (782 g/kg). In autumn, cows fed GC-L had lower aNDFom digestibility when compared with cows fed other DT. In spring, cows fed GC-L had lower N digestibility when compared with cows fed other DT, whereas in autumn, cows fed GC-L had greater N digestibility when compared with cows fed G-L and G-H, but were similar to cows fed GC-H.

### Nitrogen Intake and Whole-Body N Partitioning

The intake, concentration in outputs and partitioning of N are presented in Table 5. There was a DT by season interaction effect observed for N intake ( $P < 0.01$ ), urinary N concentration ( $P < 0.01$ ) and fecal N concentration ( $P = 0.01$ ). In spring, the N intake of cows fed G-H was greatest (533 g/d), cows fed G-L and GC-H were intermediate (436 and 442 g/d, respectively), and cows fed GC-L was lowest (353 g/d). In autumn, cows fed GC-L and GC-H had greater N intake when compared with cows fed G-L and G-H (800, 818, 608, and 626 g/d, respectively). In spring, cows fed G-H had greater urinary N concentration when compared with cows fed other DT (Table 5). In autumn, cows fed GC-L and GC-H had greater urinary N concentration when compared with cows fed G-L, but

**Table 2.** Precutting yield, height, white clover proportion, and nutritive value of the herbage (mean  $\pm$  SD) used in the experiment<sup>1</sup>

| Item <sup>2</sup>      | Spring          |                 |                 |                 | Autumn          |                 |                 |                 |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                        | G-L             | G-H             | GC-L            | GC-H            | G-L             | G-H             | GC-L            | GC-H            |
| Yield, kg DM/ha        | 1,545 $\pm$ 288 | 1,750 $\pm$ 386 | 1,616 $\pm$ 211 | 1,826 $\pm$ 190 | 1,221 $\pm$ 210 | 1,673 $\pm$ 437 | 1,078 $\pm$ 197 | 1,314 $\pm$ 185 |
| Sward height, cm       | 10.0 $\pm$ 1.4  | 10.5 $\pm$ 0.9  | 9.6 $\pm$ 0.1   | 11.0 $\pm$ 0.5  | 9.4 $\pm$ 1.6   | 10.2 $\pm$ 0.8  | 7.4 $\pm$ 0.1   | 10.0 $\pm$ 1.2  |
| White clover, % DM     | —               | —               | 23.5 $\pm$ 12.7 | 13.3 $\pm$ 5.3  | —               | —               | 64.5 $\pm$ 18.5 | 42.0 $\pm$ 7.6  |
| DM, %                  | 18.0 $\pm$ 2.4  | 16.5 $\pm$ 2.2  | 19.7 $\pm$ 2.7  | 18.1 $\pm$ 2.4  | 14.5 $\pm$ 1.5  | 14.0 $\pm$ 1.4  | 12.4 $\pm$ 1.1  | 12.8 $\pm$ 0.9  |
| CP, % DM               | 16.3 $\pm$ 0.1  | 18.9 $\pm$ 0.3  | 12.8 $\pm$ 1.0  | 15.4 $\pm$ 0.6  | 18.6 $\pm$ 1.1  | 19.7 $\pm$ 0.2  | 25.9 $\pm$ 0.9  | 23.6 $\pm$ 0.2  |
| Soluble N, % N         | 63.1 $\pm$ 4.5  | 67.5 $\pm$ 6.0  | 61.1 $\pm$ 1.8  | 64.1 $\pm$ 4.5  | 60.2 $\pm$ 0.8  | 61.0 $\pm$ 0.1  | 61.6 $\pm$ 2.0  | 61.8 $\pm$ 2.6  |
| NDIN, % N              | 7.0 $\pm$ 1.1   | 6.3 $\pm$ 0.8   | 7.8 $\pm$ 0.7   | 7.2 $\pm$ 1.1   | 6.8 $\pm$ 0.1   | 6.5 $\pm$ 0.1   | 6.0 $\pm$ 0.7   | 6.2 $\pm$ 0.6   |
| ADIN, % N              | 2.2 $\pm$ 0.1   | 1.8 $\pm$ 0.2   | 3.0 $\pm$ 0.4   | 2.7 $\pm$ 0.5   | 2.6 $\pm$ 0.2   | 2.3 $\pm$ 0.4   | 1.9 $\pm$ 0.1   | 2.3 $\pm$ 0.3   |
| aNDFom, % DM           | 32.8 $\pm$ 2.5  | 33.7 $\pm$ 2.9  | 31.7 $\pm$ 2.7  | 32.3 $\pm$ 2.5  | 34.4 $\pm$ 0.8  | 35.4 $\pm$ 0.6  | 24.5 $\pm$ 0.4  | 29.9 $\pm$ 0.2  |
| 12-h uNDFom, % aNDFom  | 47.7 $\pm$ 1.4  | 52.1 $\pm$ 2.7  | 69.9 $\pm$ 7.9  | 54.6 $\pm$ 6.8  | 61.8 $\pm$ 3.2  | 57.6 $\pm$ 2.8  | 59.5 $\pm$ 10.6 | 54.2 $\pm$ 9.2  |
| 30-h uNDFom, % aNDFom  | 25.3 $\pm$ 3.6  | 25.1 $\pm$ 2.7  | 30.6 $\pm$ 3.5  | 29.7 $\pm$ 6.6  | 44.2 $\pm$ 7.8  | 29.2 $\pm$ 4.3  | 33.1 $\pm$ 10.5 | 39.1 $\pm$ 2.5  |
| 120-h uNDFom, % aNDFom | 13.2 $\pm$ 1.1  | 11.6 $\pm$ 0.7  | 14.2 $\pm$ 3.2  | 15.3 $\pm$ 0.5  | 19.3 $\pm$ 0.7  | 16.4 $\pm$ 0.4  | 23.3 $\pm$ 0.7  | 18.6 $\pm$ 0.3  |
| 240-h uNDFom, % aNDFom | 11.0 $\pm$ 1.0  | 11.5 $\pm$ 1.5  | 13.0 $\pm$ 2.7  | 13.0 $\pm$ 1.2  | 17.5 $\pm$ 0.1  | 15.3 $\pm$ 0.1  | 21.8 $\pm$ 1.4  | 17.4 $\pm$ 0.6  |
| ADF, % DM              | 18.8 $\pm$ 1.6  | 19.8 $\pm$ 1.4  | 18.2 $\pm$ 1.1  | 18.3 $\pm$ 1.3  | 23.4 $\pm$ 1.8  | 23.4 $\pm$ 2.2  | 22.7 $\pm$ 3.6  | 22.5 $\pm$ 2.9  |
| WSC, % DM              | 24.9 $\pm$ 1.2  | 21.8 $\pm$ 2.4  | 26.8 $\pm$ 3.5  | 26.6 $\pm$ 2.1  | 16.0 $\pm$ 2.9  | 16.9 $\pm$ 2.4  | 11.7 $\pm$ 0.2  | 14.5 $\pm$ 0.6  |
| Ash, % DM              | 8.0 $\pm$ 0.1   | 8.7 $\pm$ 0.2   | 8.0 $\pm$ 0.1   | 8.1 $\pm$ 0.2   | 10.6 $\pm$ 1.9  | 11.0 $\pm$ 2.4  | 14.4 $\pm$ 3.9  | 12.4 $\pm$ 2.5  |
| In vitro OMD, % OM     | 86.2 $\pm$ 0.9  | 85.9 $\pm$ 0.8  | 85.6 $\pm$ 1.4  | 85.9 $\pm$ 0.8  | 83.0 $\pm$ 0.5  | 83.8 $\pm$ 0.1  | 84.1 $\pm$ 0.8  | 83.7 $\pm$ 0.3  |

<sup>1</sup>G-L = perennial ryegrass receiving 25 kg of synthetic N/ha per cut; G-H = perennial ryegrass receiving 50 kg of synthetic N/ha per cut; GC-L = perennial ryegrass-white clover receiving 0 kg of synthetic N/ha per cut; GC-H = perennial ryegrass-white clover receiving 25 kg of synthetic N/ha per cut.

<sup>2</sup>aNDFom = amylase- and sodium sulfite-treated NDF corrected for ash residue; uNDFom = undigested amylase- and sodium sulfite-treated NDF corrected for ash residue; WSC = water-soluble carbohydrate; OMD = organic matter digestibility.

were similar to cows fed G-H. In spring, cows fed G-H had greater fecal N concentration when compared with cows fed G-L and GC-L, but were similar to cows fed GC-H. We observed a DT by season interaction effect for N output in urine ( $P < 0.01$ ), feces ( $P = 0.02$ ), and milk ( $P < 0.01$ ). In spring, cows fed G-H had greater N output in urine (g/d) when compared with cows fed other DT, whereas in autumn, cows fed GC-L and GC-H had greater N output in urine when compared with cows fed G-L and G-H (447, 439, 265, and 274 g/d, respectively). In autumn, cows fed GC-H had greater N output in feces (g/d) when compared with cows fed G-L and G-H, but were similar to cows fed GC-L (181, 161, 154, and 174 g/d, respectively). In spring, cows fed G-H had greater N output in milk (g/d) when compared with cows fed GC-L and GC-H but similar to cows fed G-L. We observed a DT by season interaction effect for all N outputs when calculated as a proportion of N intake ( $P < 0.01$ ; Table 5). In spring, cows fed G-H partitioned a greater proportion of N intake to urine when compared with cows fed other DT, whereas cows fed GC-L partitioned a greater proportion to feces and milk when compared with cows fed other DT and had no unaccounted N as a proportion of N intake. In autumn, cows fed GC-L and GC-H partitioned a greater proportion of N intake to urine when compared with cows fed G-L and G-H (0.57, 0.54, 0.44, and 0.44 g/g, respectively), whereas cows fed G-L partitioned a greater proportion to feces and milk when compared with cows fed GC-L and GC-H, but were similar to cows fed G-H.

The effect of pasture species and synthetic N application rate on excreta output are presented in Table 6. We observed a DT by season interaction effect for urine output ( $P < 0.01$ ), fecal DM concentration ( $P < 0.01$ ), fecal DM output ( $P = 0.05$ ) and fecal aNDFom output ( $P < 0.01$ ). In spring, urine output did not differ among DT ( $P > 0.05$ ), whereas in autumn cows fed GC-L and GC-H had greater urine output when compared with cows fed G-L and G-H (66.0, 64.6, 47.3, and 46.8 kg/d, respectively). In autumn, cows fed GC-L had greater fecal DM concentration when compared with cows fed other DT, whereas cows fed GC-L and GC-H had greater fecal DM output when compared with cows fed G-H, but were similar to cows fed G-L. In spring, cows fed G-H had lower fecal aNDFom output when compared with cows fed GC-L and GC-H, but were similar to cows fed G-L, whereas in autumn, cows fed GC-L had lower fecal aNDFom output when compared with cows fed G-L, but were similar to cows fed G-H and GC-H.

## DISCUSSION

By identifying the optimal combination of synthetic N application rate and BNF, pasture-based systems can

**Table 3.** Effect of synthetic nitrogen application rate and pasture species on milk production, feed efficiency, BW, and BCS of dairy cows in spring and autumn<sup>1</sup>

| Item                         | Spring             |                   |                   |                    | Autumn             |                    |                   |                   | P-value <sup>2</sup> |       |       |        |
|------------------------------|--------------------|-------------------|-------------------|--------------------|--------------------|--------------------|-------------------|-------------------|----------------------|-------|-------|--------|
|                              | G-L                | G-H               | GC-L              | GC-H               | G-L                | G-H                | GC-L              | GC-H              | SEM                  | DT    | S     | DT × S |
| Milk yield, kg/d             | 25.1 <sup>ab</sup> | 26.8 <sup>a</sup> | 23.4 <sup>b</sup> | 24.2 <sup>ab</sup> | 17.6 <sup>A</sup>  | 17.4 <sup>A</sup>  | 18.4 <sup>A</sup> | 19.5 <sup>A</sup> | 1.08                 | 0.33  | <0.01 | <0.01  |
| ECM yield, <sup>3</sup> kg/d | 27.9 <sup>ab</sup> | 29.2 <sup>a</sup> | 25.3 <sup>c</sup> | 26.0 <sup>bc</sup> | 21.4 <sup>A</sup>  | 22.0 <sup>A</sup>  | 21.5 <sup>A</sup> | 23.7 <sup>A</sup> | 1.15                 | 0.02  | <0.01 | <0.01  |
| Fat, %                       | 4.10               | 3.93              | 3.92              | 3.85               | 4.64               | 4.96               | 4.37              | 4.60              | 0.17                 | 0.14  | <0.01 | 0.19   |
| Protein, %                   | 3.56               | 3.52              | 3.45              | 3.52               | 4.15               | 4.14               | 4.15              | 4.20              | 0.09                 | 0.56  | <0.01 | 0.65   |
| Lactose, %                   | 4.83               | 4.84              | 4.78              | 4.8                | 4.6                | 4.59               | 4.57              | 4.59              | 0.04                 | 0.28  | <0.01 | 0.89   |
| Fat yield, kg/d              | 1.03 <sup>a</sup>  | 1.05 <sup>a</sup> | 0.91 <sup>b</sup> | 0.93 <sup>b</sup>  | 0.80 <sup>AB</sup> | 0.86 <sup>AB</sup> | 0.78 <sup>B</sup> | 0.89 <sup>A</sup> | 0.05                 | <0.01 | 0.02  | <0.01  |
| Protein yield, kg/d          | 0.89 <sup>ab</sup> | 0.95 <sup>a</sup> | 0.81 <sup>b</sup> | 0.85 <sup>b</sup>  | 0.73 <sup>A</sup>  | 0.72 <sup>A</sup>  | 0.75 <sup>A</sup> | 0.80 <sup>A</sup> | 0.04                 | 0.16  | 0.02  | <0.01  |
| Lactose yield, kg/d          | 1.21 <sup>ab</sup> | 1.29 <sup>a</sup> | 1.12 <sup>b</sup> | 1.16 <sup>ab</sup> | 0.81 <sup>A</sup>  | 0.80 <sup>A</sup>  | 0.84 <sup>A</sup> | 0.90 <sup>A</sup> | 0.05                 | 0.25  | <0.01 | <0.01  |
| Milk solids yield, kg/d      | 1.92 <sup>ab</sup> | 1.99 <sup>a</sup> | 1.53 <sup>c</sup> | 1.78 <sup>bc</sup> | 1.53 <sup>B</sup>  | 1.58 <sup>AB</sup> | 1.53 <sup>B</sup> | 1.70 <sup>A</sup> | 0.08                 | <0.01 | 0.02  | <0.01  |
| Feed efficiency <sup>4</sup> | 1.58               | 1.65              | 1.57              | 1.49               | 1.08               | 1.14               | 1.13              | 1.10              | 0.05                 | 0.10  | <0.01 | 0.43   |
| BW, kg                       | 505                | 500               | 508               | 503                | 541                | 545                | 542               | 551               | 13.7                 | 0.68  | 0.04  | 0.20   |
| BCS                          | 2.94               | 2.92              | 2.92              | 2.92               | 3.04               | 2.99               | 3.06              | 3.08              | 0.04                 | 0.59  | 0.02  | 0.57   |

<sup>a-c</sup>Means within row with different superscripts refer to a difference for DT within spring ( $P \leq 0.05$ ).<sup>A,B</sup>Means within row with different superscripts refer to a difference for DT within autumn ( $P \leq 0.05$ ).<sup>1</sup>G-L = perennial ryegrass receiving 25 kg of synthetic N/ha per cut; G-H = perennial ryegrass receiving 50 kg of synthetic N/ha per cut; GC-L = perennial ryegrass-white clover receiving 0 kg of synthetic N/ha per cut; GC-H = perennial ryegrass-white clover receiving 25 kg of synthetic N/ha per cut.<sup>2</sup>DT = dietary treatment; S = season.<sup>3</sup>ECM estimated according to Tyrrell and Reid (1965).<sup>4</sup>Calculated as ECM/DMI.**Table 4.** Effect of synthetic nitrogen application rate and pasture species on the intake and apparent digestibility of DM, OM, aNDFom, and N of dairy cows in spring and autumn<sup>1</sup>

|                     | Spring            |                    |                    |                    | Autumn            |                    |                   |                    | P-value <sup>2</sup> |       |       |        |
|---------------------|-------------------|--------------------|--------------------|--------------------|-------------------|--------------------|-------------------|--------------------|----------------------|-------|-------|--------|
|                     | G-L               | G-H                | GC-L               | GC-H               | G-L               | G-H                | GC-L              | GC-H               | SEM                  | DT    | S     | DT × S |
| Intake, kg/d        |                   |                    |                    |                    |                   |                    |                   |                    |                      |       |       |        |
| DM                  | 17.8 <sup>a</sup> | 17.8 <sup>a</sup>  | 16.4 <sup>a</sup>  | 17.6 <sup>a</sup>  | 19.8 <sup>B</sup> | 19.3 <sup>B</sup>  | 19.4 <sup>B</sup> | 21.6 <sup>A</sup>  | 0.59                 | <0.01 | <0.01 | 0.04   |
| DM, g/kg BW         | 35.4              | 35.6               | 32.5               | 34.9               | 36.7              | 35.5               | 36.1              | 39.4               | 1.26                 | 0.06  | 0.09  | 0.11   |
| OM                  | 16.3 <sup>a</sup> | 16.2 <sup>a</sup>  | 15.0 <sup>a</sup>  | 16.1 <sup>a</sup>  | 17.0 <sup>B</sup> | 17.2 <sup>B</sup>  | 16.6 <sup>B</sup> | 19.0 <sup>A</sup>  | 0.52                 | <0.01 | 0.02  | 0.05   |
| aNDFom              | 5.79 <sup>a</sup> | 5.97 <sup>a</sup>  | 5.18 <sup>b</sup>  | 5.64 <sup>ab</sup> | 6.72 <sup>A</sup> | 6.75 <sup>A</sup>  | 6.50 <sup>A</sup> | 6.50 <sup>A</sup>  | 0.13                 | <0.01 | 0.02  | <0.01  |
| uNDFom              | 0.63 <sup>b</sup> | 0.66 <sup>ab</sup> | 0.68 <sup>ab</sup> | 0.73 <sup>a</sup>  | 1.17 <sup>A</sup> | 1.02 <sup>BC</sup> | 1.02 <sup>C</sup> | 1.11 <sup>AB</sup> | 0.02                 | 0.01  | <0.01 | <0.01  |
| Digestibility, g/kg |                   |                    |                    |                    |                   |                    |                   |                    |                      |       |       |        |
| DM                  | 786 <sup>y</sup>  | 788 <sup>y</sup>   | 766 <sup>z</sup>   | 789 <sup>y</sup>   | 754 <sup>y</sup>  | 767 <sup>y</sup>   | 737 <sup>z</sup>  | 761 <sup>y</sup>   | 5.0                  | <0.01 | <0.01 | 0.71   |
| OM                  | 810 <sup>a</sup>  | 812 <sup>a</sup>   | 794 <sup>b</sup>   | 810 <sup>a</sup>   | 792 <sup>B</sup>  | 808 <sup>A</sup>   | 800 <sup>AB</sup> | 810 <sup>A</sup>   | 3.7                  | <0.01 | 0.22  | 0.01   |
| aNDFom              | 782 <sup>ab</sup> | 800 <sup>a</sup>   | 699 <sup>c</sup>   | 760 <sup>b</sup>   | 805 <sup>A</sup>  | 817 <sup>A</sup>   | 777 <sup>B</sup>  | 812 <sup>A</sup>   | 4.7                  | <0.01 | <0.01 | <0.01  |
| N                   | 709 <sup>ab</sup> | 734 <sup>a</sup>   | 599 <sup>c</sup>   | 688 <sup>b</sup>   | 728 <sup>C</sup>  | 748 <sup>BC</sup>  | 784 <sup>A</sup>  | 755 <sup>AB</sup>  | 8.4                  | <0.01 | <0.01 | <0.01  |

<sup>a-c</sup>Means within row with different superscripts refer to a difference for DT within spring ( $P \leq 0.05$ ).<sup>y,z</sup>Means within row with different superscripts refer to a difference for DT across both seasons ( $P \leq 0.05$ ).<sup>A-C</sup>Means within row with different superscripts refer to a difference for DT within autumn ( $P \leq 0.05$ ).<sup>1</sup>G-L = perennial ryegrass receiving 25 kg of synthetic N/ha per cut; G-H = perennial ryegrass receiving 50 kg of synthetic N/ha per cut; GC-L = perennial ryegrass-white clover receiving 0 kg of synthetic N/ha per cut; GC-H = perennial ryegrass-white clover receiving 25 kg of synthetic N/ha per cut.<sup>2</sup>DT = dietary treatment; S = season.<sup>3</sup>aNDFom = amylase- and sodium sulfite-treated NDF corrected for ash residue; uNDFom = undigested amylase- and sodium sulfite-treated NDF corrected for ash residue.

maintain or improve human-food production in tandem with reducing environmental N emissions. Notably, during the autumn experiment, herbage WC proportions were excessively high for the GC-L and GC-H dietary treatments (64.5% and 42%, respectively). This was likely due to the repeated mechanical harvesting performed on the swards throughout the season, together with the seasonal growth pattern of WC (Walsh et al., 2025). Our goal was to generate swards of 20% to 30% WC; however, this ultimately was outside the control of management.

### Animal Performance

Within the PRG-only diets, moderate reduction in synthetic N application rate (i.e., 25 kg of N/ha per cut) generally had no effect on animal performance, intake, or digestibility. Although cows fed G-L had lower OMD when compared with cows fed G-H during the autumn, there was no effect on milk production or milk composition. In a review by Peyraud and Astigarraga (1998), the authors suggested that reduced application rates of synthetic N on PRG-only swards had almost no effect on OMD and net energy content of swards, whereas in dairy cows fed PRG, the flow of NAN to the duodenum only moderately reduced. Astigarraga et al. (2002) and Mackle et al. (1996) both reported that neither milk yield nor milk composition was affected by lower synthetic N application rates. Murray et al. (2024) also observed no difference in milk production performance between cows fed PRG swards receiving either 250 or 150 kg of synthetic N/ha per year across 3 grazing seasons. In some experiments, reductions in synthetic N application rate were designed to be greater than in the current experiment. For example, the difference between high and low N rates ranged from 20 to 80 kg N/ha per regrowth with the low application rate, at times, receiving 0 kg N/ha (Delagarde et al., 1997; Peyraud et al., 1997). When combined with the local agro-climatic conditions (i.e., soil moisture deficit), pasture CP became excessively low (i.e., ~10% CP), reducing animal performance, likely from a reduction in MP supply (Peyraud and Astigarraga, 1998). Under such conditions, the provision of supplementary protein ingredients, specifically those with greater MP supply, has been demonstrated to improve animal performance (Delaby et al., 1996; Delagarde et al., 1997). Within the current spring and autumn experiments, the PRG-only herbage remained >16% CP, likely resulting in the similar animal performance achieved. Overall, although performance per cow might not be affected by moderate differences in synthetic N application rate, performance per hectare will likely reduce due to reductions in herbage DM production and the optimal stocking rate achievable within these farm systems (Murray et al., 2024).

**Table 5.** Effect of synthetic nitrogen application rate and pasture species on the nitrogen intake and whole-body partitioning of dairy cows in spring and autumn<sup>1</sup>

| Item                          | Spring             |                   |                    |                    |                   | Autumn             |                   |                    |       |       | P-value <sup>2</sup> |        |
|-------------------------------|--------------------|-------------------|--------------------|--------------------|-------------------|--------------------|-------------------|--------------------|-------|-------|----------------------|--------|
|                               | G-L                | G-H               | GC-L               | GC-H               | G-L               | G-H                | GC-L              | GC-H               | SEM   | DT    | S                    | DT × S |
| N intake, g/d                 | 436 <sup>b</sup>   | 533 <sup>a</sup>  | 353 <sup>c</sup>   | 442 <sup>b</sup>   | 608 <sup>b</sup>  | 626 <sup>b</sup>   | 800 <sup>a</sup>  | 818 <sup>a</sup>   | 19.0  | <0.01 | <0.01                | <0.01  |
| N in urine, g/100 g DM        | 0.42 <sup>b</sup>  | 0.58 <sup>a</sup> | 0.32 <sup>b</sup>  | 0.42 <sup>b</sup>  | 0.57 <sup>b</sup> | 0.60 <sup>AB</sup> | 0.70 <sup>A</sup> | 0.70 <sup>A</sup>  | 0.03  | 0.01  | <0.01                | <0.01  |
| N in feces, g/100 g DM        | 3.45 <sup>c</sup>  | 3.78 <sup>a</sup> | 3.52 <sup>bc</sup> | 3.69 <sup>ab</sup> | 3.43 <sup>A</sup> | 3.46 <sup>A</sup>  | 3.45 <sup>A</sup> | 3.59 <sup>A</sup>  | 0.05  | <0.01 | <0.01                | 0.01   |
| N in milk, g/100 g            | 0.56               | 0.55              | 0.54               | 0.55               | 0.65              | 0.65               | 0.65              | 0.66               | 0.01  | 0.60  | <0.01                | 0.64   |
| N output, g/d                 |                    |                   |                    |                    |                   |                    |                   |                    |       |       |                      |        |
| N to urine                    | 111 <sup>b</sup>   | 188 <sup>a</sup>  | 78 <sup>b</sup>    | 115 <sup>b</sup>   | 265 <sup>B</sup>  | 274 <sup>B</sup>   | 447 <sup>A</sup>  | 439 <sup>A</sup>   | 14.0  | <0.01 | <0.01                | <0.01  |
| N to feces                    | 130 <sup>a</sup>   | 137 <sup>a</sup>  | 130 <sup>a</sup>   | 136 <sup>a</sup>   | 161 <sup>BC</sup> | 154 <sup>C</sup>   | 174 <sup>AB</sup> | 181 <sup>A</sup>   | 6.0   | 0.03  | <0.01                | 0.02   |
| N to milk                     | 140 <sup>ab</sup>  | 148 <sup>a</sup>  | 126 <sup>b</sup>   | 133 <sup>b</sup>   | 115 <sup>A</sup>  | 112 <sup>A</sup>   | 118 <sup>A</sup>  | 126 <sup>A</sup>   | 6.0   | 0.16  | 0.01                 | <0.01  |
| N unaccounted                 | 70 <sup>y</sup>    | 62 <sup>y</sup>   | 5 <sup>z</sup>     | 56 <sup>y</sup>    | 66 <sup>y</sup>   | 79 <sup>y</sup>    | 61 <sup>z</sup>   | 74 <sup>y</sup>    | 10.6  | <0.01 | 0.02                 | 0.06   |
| N output, proportion N intake |                    |                   |                    |                    |                   |                    |                   |                    |       |       |                      |        |
| N to urine                    | 0.25 <sup>b</sup>  | 0.35 <sup>a</sup> | 0.24 <sup>b</sup>  | 0.26 <sup>b</sup>  | 0.44 <sup>B</sup> | 0.44 <sup>B</sup>  | 0.57 <sup>A</sup> | 0.54 <sup>A</sup>  | 0.014 | <0.01 | <0.01                | <0.01  |
| N to feces                    | 0.29 <sup>bc</sup> | 0.27 <sup>c</sup> | 0.40 <sup>a</sup>  | 0.31 <sup>b</sup>  | 0.27 <sup>A</sup> | 0.25 <sup>AB</sup> | 0.22 <sup>C</sup> | 0.22 <sup>BC</sup> | 0.008 | <0.01 | <0.01                | <0.01  |
| N to milk                     | 0.32 <sup>b</sup>  | 0.29 <sup>c</sup> | 0.37 <sup>a</sup>  | 0.31 <sup>bc</sup> | 0.19 <sup>A</sup> | 0.18 <sup>AB</sup> | 0.15 <sup>C</sup> | 0.16 <sup>BC</sup> | 0.010 | <0.01 | <0.01                | <0.01  |
| N unaccounted                 | 0.15 <sup>a</sup>  | 0.10 <sup>a</sup> | 0.00 <sup>b</sup>  | 0.12 <sup>a</sup>  | 0.10 <sup>A</sup> | 0.12 <sup>A</sup>  | 0.07 <sup>A</sup> | 0.08 <sup>A</sup>  | 0.020 | <0.01 | 0.99                 | <0.01  |

<sup>a-c</sup>Means within row with different superscripts refer to a difference for DT within spring ( $P \leq 0.05$ ).

<sup>y-z</sup>Means within row with different superscripts refer to a difference for DT across both seasons ( $P \leq 0.05$ ).

<sup>A-C</sup>Means within row with different superscripts refer to a difference for DT within autumn ( $P \leq 0.05$ ).

<sup>1</sup>G-L = perennial ryegrass receiving 25 kg of synthetic N/ha per cut; G-H = perennial ryegrass receiving 50 kg of synthetic N/ha per cut; GC-L = perennial ryegrass-white clover receiving 0 kg of synthetic N/ha per cut; GC-H = perennial ryegrass-white clover receiving 25 kg of synthetic N/ha per cut.

<sup>2</sup>DT = dietary treatment; S = season.

Under scenarios where synthetic N application rates must be reduced, it is evident that legumes will be required to maintain herbage production and animal performance (Murray et al., 2024). In a meta-analysis, Dineen et al. (2018) reported that daily milk yield and milk solids yield were increased by 1.4 and 0.12 kg, respectively, in cows grazing PRG-WC swards compared with cows grazing PRG-only swards, when the mean sward WC proportion was 31.6% of DM. These findings are also consistent with more recent experiments (McCleary et al., 2019; McAuliffe et al., 2022; Murray et al., 2024), likely reflecting improvements in herbage nutritive value. During the current experiment, in spring, cows fed GC-L had lower milk solids yield when compared with cows fed PRG-only herbage. The CP of the GC-L herbage was 12.8%, which likely resulted in the reduced DM, OM and aNDFom digestibility and numerically lowest DMI. Although WC has been shown to enhance the performance of spring-calving dairy cows, this effect is most evident in mid- to late-lactation, when WC makes a greater contribution to the sward than in spring (McCleary et al., 2019). Andrews et al. (2007) reported that a minimum WC inclusion rate of 20% of DM is required to observe a milk production benefit, whereas Rochon et al. (2004) suggested that a WC proportion of 30% of DM is optimal for maximizing animal performance. The lower animal performance when cows were fed GC-L during spring (i.e., 0 kg of N/ha) highlights the importance of optimizing the combination of synthetic N application rate and BNF rather than total elimination of synthetic N. However, it is important to note that within a normal grazing system, some organic N would be recycled to the pasture in the form of urinary and fecal N, whereas under the circumstances of the current experiment, herbage was mechanically harvested and excreta were not returned to the swards.

During the autumn experiment, cows fed GC-H had greater milk solids yield when compared with cows fed G-L and GC-L. The greater milk solids yield was likely achieved through greater DMI rather than increased digestibility. Both McCarthy et al. (2023) and Lee et al. (2004) reported greater DMI for cows fed PRG-WC when compared with cows fed PRG. Lee et al. (2004) demonstrated a linear increase in DMI with increasing WC proportions. However, the increase in DMI when cows are fed WC is not always consistent and might be related to measurement technique (i.e., marker versus directly measured), sward WC proportion, or time of year (Enriquez-Hidalgo et al., 2014; Egan et al., 2017; Hurley et al., 2021). It is not obvious why the cows fed GC-L did not also increase DMI and milk yield when compared with the PRG-only DT. As mentioned previously, the PRG-WC swards had excessive proportions of WC during the autumn. This led to differences in

**Table 6.** Effect of synthetic nitrogen application rate and pasture species on the excreta output of dairy cows in spring and autumn<sup>1</sup>

| Item <sup>3</sup>  | Spring             |                   |                   |                   | Autumn            |                    |                   |                    | P-value <sup>2</sup> |       |       |        |
|--------------------|--------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|--------------------|----------------------|-------|-------|--------|
|                    | G-L                | G-H               | GC-L              | GC-H              | G-L               | G-H                | GC-L              | GC-H               | SEM                  | DT    | S     | DT × S |
| Urine output, kg/d | 26.8 <sup>a</sup>  | 32.1 <sup>a</sup> | 24.4 <sup>a</sup> | 27.0 <sup>a</sup> | 47.3 <sup>b</sup> | 46.8 <sup>b</sup>  | 66.0 <sup>a</sup> | 64.6 <sup>a</sup>  | 2.09                 | <0.01 | <0.01 | <0.01  |
| Fecal FW, kg/d     | 40.3               | 37.4              | 39.4              | 38.1              | 41.7              | 41.0               | 38.8              | 45.6               | 2.33                 | 0.25  | 0.29  | 0.10   |
| Fecal DM, %        | 9.5 <sup>a</sup>   | 9.9 <sup>a</sup>  | 9.7 <sup>a</sup>  | 9.8 <sup>a</sup>  | 11.8 <sup>b</sup> | 11.1 <sup>b</sup>  | 13.5 <sup>a</sup> | 11.2 <sup>b</sup>  | 0.42                 | 0.02  | <0.01 | <0.01  |
| Fecal DM, kg/d     | 3.8 <sup>a</sup>   | 3.6 <sup>a</sup>  | 3.7 <sup>a</sup>  | 3.7 <sup>a</sup>  | 4.8 <sup>ab</sup> | 4.4 <sup>b</sup>   | 5.2 <sup>a</sup>  | 5.1 <sup>a</sup>   | 0.17                 | 0.04  | <0.01 | 0.05   |
| Fecal OM, kg/d     | 3.1                | 2.9               | 3.0               | 3.0               | 3.5               | 3.2                | 3.3               | 3.6                | 0.12                 | 0.06  | <0.01 | 0.68   |
| Fecal aNDFom, kg/d | 1.29 <sup>ab</sup> | 1.15 <sup>b</sup> | 1.42 <sup>a</sup> | 1.34 <sup>a</sup> | 1.30 <sup>a</sup> | 1.20 <sup>ab</sup> | 1.08 <sup>b</sup> | 1.21 <sup>ab</sup> | 0.04                 | 0.07  | 0.10  | <0.01  |

<sup>a,b</sup>Means within row with different superscripts refer to a difference for DT within spring ( $P \leq 0.05$ ).

<sup>AB</sup>Means within row with different superscripts refer to a difference for DT within autumn ( $P \leq 0.05$ ).

<sup>1</sup>G-L = perennial ryegrass receiving 25 kg of synthetic N/ha per cut; G-H = perennial ryegrass receiving 50 kg of synthetic N/ha per cut; GC-L = perennial ryegrass-white clover receiving 0 kg of synthetic N/ha per cut; GC-H = perennial ryegrass-white clover receiving 25 kg of synthetic N/ha per cut.

<sup>2</sup>DT = dietary treatment; S = season.

<sup>3</sup>FW = fresh weight; aNDFom = amylase- and sodium sulfite-treated NDF corrected for ash residue.

herbage aNDFom concentration, with the GC-H herbage being nutritionally optimal (30% of DM), which might have allowed increased DMI to be achieved, whereas the aNDFom concentration of the GC-L herbage might have been suboptimal (25% of DM; Stockdale et al., 1987), with a lower aNDFom digestibility being observed. The reduced *in vivo* aNDFom digestibility was consistent with the *in vitro* results, which showed greater aNDFom residues at 120 and 240 h for GC-L herbage. Therefore, this excessive proportion of WC and low aNDFom concentration in the GC-L herbage might have limited animal performance. Interestingly, for all DT, cows exhibited greater DMI during autumn when compared with spring. A comparable seasonal increase was observed by Dwan et al. (2025) for cows grazing PRG and PRG-WC swards, consistent with evidence from a large-scale study by Berry et al. (2007), indicating that DMI can tend to increase across the grazing season.

Numerous authors suggest that the inclusion of WC increases the digestibility of the swards (Egan et al., 2018; Murray et al., 2024); however, there is a lack of data in the literature measuring *in vivo* digestibility of lactating dairy cows fed WC. Niderkorn et al. (2017) reported no effect of varying WC proportions on DM or OM digestibility in stall-fed sheep whereas Hurley et al. (2021) observed greater DM and OM digestibility for PRG-WC than PRG swards in stall-fed sheep. In the current experiment, there was a lack of evidence to support that WC inclusion increased the DM, OM, or aNDFom digestibility of the diet when compared with lactating dairy cows fed PRG-only. Further investigation should examine the effect of WC on rumen digestion kinetics and fermentation characteristics in lactating dairy cows to elucidate the mechanisms underlying the increases in DMI and animal performance typically observed. Altogether, these findings suggest that in legume-containing swards, synthetic N application rates can be reduced without compromising animal performance but should not be eliminated.

### Nitrogen Excretion

In spring, within the PRG-only diets, reduction in synthetic N application rate reduced N intake, urinary N concentration and urinary N output; however, there was no effect on these variables during autumn. This is likely due to the greater increase in grass CP concentrations during spring (+2.6% CP) when compared with autumn (+1.1% CP) as a result of increased synthetic N application rate (Peyraud and Astigarraga, 1998). As reported by O'Connor et al. (2019), increased synthetic N application rate also had a greater effect on grass WSC concentration during spring (−3.1% WSC) when compared with autumn (−0.9% WSC). During late-autumn, pasture growth rates decline and soil N mineralization increases

when compared with spring and summer (Johnston and Jenkinson, 1989), resulting in a lower responsiveness of grass CP concentration to increasing synthetic N application rate. This highlights the limitation of relying on reduced synthetic N application rate on PRG-only swards to reduce urinary N excretion from lactating dairy cows during autumn, which is the high-risk period for urinary N leaching to occur (Selbie et al., 2015). Furthermore, pursuing this strategy will also reduce animal performance per hectare through reduced herbage production (Murray et al., 2024).

We hypothesized that cows fed PRG-WC herbage receiving reduced application rates of synthetic N would have lower urinary N excretion when compared with cows fed PRG-only herbage receiving greater rates of synthetic N. In addition, we hypothesized that the effects would be dependent on season with a more pronounced effect occurring on urinary N excretion during late spring when compared with autumn. In agreement with our hypothesis, cows fed PRG-WC herbage receiving reduced application rates of synthetic N had lower N intake, urinary N concentration, and urinary N output when compared with PRG-only herbage receiving greater rates of synthetic N (i.e., G-H) during spring. This response again was likely mediated through lower PRG-WC herbage CP concentrations. During spring, the low WC proportions and low BNF likely resulted in a lower sward N supply. This was not the case during autumn, as WC proportions were excessively high, with high rates of BNF likely supplying ample N to the sward. This was reflected in the high PRG-WC herbage CP concentrations (>23%) during autumn, leading to greater N intake, urinary N output, and proportion of N intake partitioned toward urine in cows fed PRG-WC when compared with cows fed PRG-only. The high N intake and urinary N excretion of cows fed PRG-WC during autumn would increase N loading onto pasture through urine patches and elevate the risk of N leaching from pasture-based systems (Selbie et al., 2015). In addition, reducing synthetic N application on PRG-WC swards increased WC proportions and herbage CP concentration in the current experiment, which could further elevate the risk of N leaching. However, it is unlikely that swards rotationally grazed throughout the year would achieve similar WC proportions to what was observed in the current experiment, where repeated mechanical harvesting likely increased WC proportions. Further investigation is therefore needed to determine if an optimal combination of synthetic N application rate and BNF, through a more moderate proportion of WC in the sward, can reduce N excretion during the autumn.

Interestingly, during autumn, PRG-WC herbage had a lower DM concentration when compared with PRG-only herbage. When cows were fed these PRG-WC herbages, they increased their urine output by 39%, on average,

when compared with cows fed PRG-only. This diuretic response has recently been reported in cows fed forage plantain and chicory (Mangwe et al., 2019; Minnée et al., 2020). Minnée et al. (2020) indicated that the increased daily urine output is primarily driven by increased water consumption due to lower herbage DM, with no additional diuretic mechanisms confirmed. Critically, when this diuretic effect occurs in cows fed plantain, a large reduction in urinary N concentration typically occurs. In the current experiment, however, cows fed PRG-WC had greater urinary N concentrations than those fed PRG-only, indicating that the dilution effect of increased urine output was insufficient to offset the greater N intake of cows fed PRG-WC herbage. Therefore, strategies to replicate the dilution effect of plantain by offering lower-DM feeds, as proposed by Eady et al. (2025), are likely to reduce urinary N concentration only when the inclusion of lower-DM feeds does not increase N intake. Ultimately, other intervention strategies, such as the inclusion of alternative forage species and more moderate WC proportions, might be needed to overcome autumn urinary N loading in pasture-based systems.

## CONCLUSIONS

In this experiment, moderate reductions in synthetic N application rate on PRG-only swards generally had no effect on animal performance, intake, or digestibility across both spring and autumn. During spring, cows fed PRG-only herbage receiving reduced synthetic N exhibited more favorable N excretion profiles; however, these benefits were not evident during the autumn period. In swards containing WC, synthetic N application rates can be reduced without compromising animal performance but cannot be eliminated. Similarly, cows fed PRG-WC herbage receiving reduced synthetic N exhibited more favorable N excretion profiles during spring; however, due to excessively high WC proportions during autumn, cows fed PRG-WC herbage had greater N intake, urinary N output, proportion of N intake partitioned toward urine, and urine output when compared with cows fed PRG only. Overall, these findings indicate that additional strategies, including alternative pasture species or management practices such as duration-controlled grazing, will be required to effectively mitigate urinary N excretion during periods of elevated nitrate-leaching risk.

## NOTES

This work was funded by the Department of Agriculture, Food and the Marine's Competitive Research Funding Programme (2021R482 - PASTURE-NUE). The experiment was approved by the Health Products Regulatory Authority (Dublin, Ireland) through the

experimental license (AE19132-P150) under the European directive 2010/63/EU and Statutory Instrument no. 543 of 2012 (European Union, 2012). The technical assistance of Michelle Liddane, Pat O'Connor, Andy McGrath, Christina Fleming, Jim Flynn, Matthieu Fort, Robert Serem, Akash Tomy, Chris Heffernan, Neil Maher, Mathilde Chaize, Charlie Dwan, Elia Tavernier, Ben Lahart, Michael Kennedy, Kevin Dolan, and all the farm staff at the Teagasc Moorepark farm (Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland) is greatly appreciated. The experiment was approved by the Health Products Regulatory Authority (Dublin, Ireland) through the experimental license (AE19132-P150) under the European directive 2010/63/EU and Statutory Instrument no. 543 of 2012 (European Union, 2012). The authors have not stated any conflicts of interest.

**Nonstandard abbreviations used:** aNDFom = amylase- and sodium sulfite-treated NDF corrected for ash residue; BNF = biological N fixation; DT = dietary treatment; FW = fresh weight; GC-H = PRG-WC receiving 25 kg of synthetic N/ha per cut; GC-L = PRG-WC receiving 0 kg of synthetic N/ha per cut; G-H = PRG receiving 50 kg of synthetic N/ha per cut; G-L = PRG receiving 25 kg of synthetic N/ha per cut; NUE = N use efficiency; OMD = organic matter digestibility; PRG = perennial ryegrass; uNDFom = undigested amylase- and sodium sulfite treated NDF corrected for ash residue; WC = white clover; WSC = water-soluble carbohydrates.

## REFERENCES

- Alexandratos, N., and J. Bruinsma. 2012. World Agriculture Towards 2030/2050: The 2012 Revision. ESA Working Paper No. 12-03. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Andrews, M., D. Scholefield, M. T. Abberton, B. A. McKenzie, S. Hodge, and J. A. Raven. 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: Productivity, environmental impact and economic considerations. *Ann. Appl. Biol.* 151:11–23. <https://doi.org/10.1111/j.1744-7348.2007.00137.x>.
- AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International.
- Astigarraga, L., J.-L. Peyraud, and L. Delaby. 2002. Effect of nitrogen fertiliser rate and protein supplementation on the herbage intake and the nitrogen balance of grazing dairy cows. *Anim. Res.* 51:279–293. <https://doi.org/10.1051/animres:2002022>.
- Berry, D. P., B. Horan, M. O'Donovan, F. Buckley, E. Kennedy, M. McEvoy, and P. Dillon. 2007. Genetics of grass dry matter intake, energy balance, and digestibility in grazing Irish dairy cows. *J. Dairy Sci.* 90:4835–4845. <https://doi.org/10.3168/jds.2007-0116>.
- Chapman, D., I. Pinxterhuis, S. Ledgard, and T. Parsons. 2019. White clover or nitrogen fertiliser for dairying under nitrate leaching limits? *Anim. Prod. Sci.* 60:78–83. <https://doi.org/10.1071/AN18577>.
- DAFM (Department of Agriculture, Food and the Marine). 2022. European Union (Good Agricultural Practice for Protection of Waters) Regulations 2022 (S.I. No. 113 of 2022).
- Delaby, L., J.-L. Peyraud, R. Vérité, and B. Marquis. 1996. Effect of protein content in the concentrate and level of nitrogen fertiliza-

- tion on the performance of dairy cows in pasture. *Ann. Zootech.* 45:327–341. <https://doi.org/10.1051/animres:19960405>.
- Delagarde, R., J. L. Peyraud, and L. Delaby. 1997. The effect of nitrogen fertilization level and protein supplementation on herbage intake, feeding behaviour and digestion in grazing dairy cows. *Anim. Feed Sci. Technol.* 66:165–180. [https://doi.org/10.1016/S0377-8401\(96\)01098-X](https://doi.org/10.1016/S0377-8401(96)01098-X).
- Di, H. J., and K. C. Cameron. 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor—A lysimeter study. *Nutr. Cycl. Agroecosyst.* 79:281–290. <https://doi.org/10.1007/s10705-007-9115-5>.
- Dineen, M., L. Delaby, T. Gilliland, and B. McCarthy. 2018. Meta-analysis of the effect of white clover inclusion in perennial ryegrass swards on milk production. *J. Dairy Sci.* 101:1804–1816. <https://doi.org/10.3168/jds.2017-12586>.
- Dineen, M., B. McCarthy, P. Dillon, F. Coughlan, N. Galvin, and M. E. Van Amburgh. 2021. The effect of concentrate supplement type on milk production, nutrient intake, and total-tract nutrient digestion in mid-lactation, spring-calving dairy cows grazing perennial ryegrass (*Lolium perenne* L.) pasture. *J. Dairy Sci.* 104:11593–11608. <https://doi.org/10.3168/jds.2021-20148>.
- Dineen, M., B. McCarthy, P. Dillon, P. A. LaPierre, S. Fessenden, C. Matthews, N. Galvin, and M. E. Van Amburgh. 2020. Rumen metabolism, omasal flow of nutrients, and microbial dynamics in lactating dairy cows fed fresh perennial ryegrass (*Lolium perenne* L.) not supplemented or supplemented with rolled barley grain. *J. Dairy Sci.* 103:11332–11348. <https://doi.org/10.3168/jds.2020-18437>.
- Dwan, C., D. Hennessy, H. Irish, L. Shalloo, F. Buckley, L. Delaby, N. Galvin, and B. Lahart. 2025. Evaluating the effect of white clover inclusion and dairy cow genetic merit on enteric methane emissions within rotational grazing systems. *J. Dairy Sci.* 108:9844–9860. <https://doi.org/10.3168/jds.2024-26106>.
- Eady, C., A. J. Conner, J. S. Rowarth, G. D. Coles, M. H. Deighton, and D. J. Moot. 2025. An examination of the ability of plantain (*Plantago lanceolata* L.) to mitigate nitrogen leaching from pasture systems. *N. Z. J. Agric. Res.* 68:130–157. <https://doi.org/10.1080/00288233.2024.2373220>.
- Edmundson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72:68–78. [https://doi.org/10.3168/jds.S0022-0302\(89\)79081-0](https://doi.org/10.3168/jds.S0022-0302(89)79081-0).
- Egan, M., N. Galvin, and D. Hennessy. 2018. Incorporating white clover (*Trifolium repens* L.) into perennial ryegrass (*Lolium perenne* L.) swards receiving varying levels of nitrogen fertilizer: Effects on milk and herbage production. *J. Dairy Sci.* 101:3412–3427. <https://doi.org/10.3168/jds.2017-13233>.
- Egan, M., M. B. Lynch, and D. Hennessy. 2017. Including white clover in nitrogen fertilized perennial ryegrass swards: Effects on dry matter intake and milk production of spring calving dairy cows. *J. Agric. Sci.* 155:657–668. <https://doi.org/10.1017/S0021859616000952>.
- Enriquez-Hidalgo, D., T. Gilliland, M. H. Deighton, M. O'Donovan, and D. Hennessy. 2014. Milk production and enteric methane emissions by dairy cows grazing fertilized perennial ryegrass pasture with or without inclusion of white clover. *J. Dairy Sci.* 97:1400–1412. <https://doi.org/10.3168/jds.2013-7034>.
- Enriquez-Hidalgo, D., T. J. Gilliland, and D. Hennessy. 2016. Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. *Grass Forage Sci.* 71:559–574. <https://doi.org/10.1111/gfs.12201>.
- European Commission. 2019. The European Green Deal. European Commission, Brussels, Belgium.
- European Commission. 2020. A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System. European Commission, Brussels, Belgium.
- European Union. 2012. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off. J. Eur. Union L* 276:33.
- Hall, M. B. 2014. Selection of an empirical detection method for determination of water-soluble carbohydrates in feedstuffs for application in ruminant nutrition. *Anim. Feed Sci. Technol.* 198:28–37. <https://doi.org/10.1016/j.anifeeds.2014.08.009>.
- Hennessy, D., S. McAuliffe, M. Egan, and E. Ruelle. 2019. What is the effect of incorporating white clover (*Trifolium repens* L.) into grazed grassland on the farm-gate nitrogen balance? *Grassland Sci. Eur.* 24:143–145.
- Herron, J., D. Hennessy, T. P. Curran, A. Moloney, and D. O'Brien. 2021. The simulated environmental impact of incorporating white clover into pasture-based dairy production systems. *J. Dairy Sci.* 104:7902–7918. <https://doi.org/10.3168/jds.2020-19077>.
- Hurley, M., E. Lewis, M. Beecher, B. Garry, C. Fleming, T. Boland, and D. Hennessy. 2021. Dry matter intake and in vivo digestibility of grass-only and grass-white clover in individually housed sheep in spring, summer and autumn. *Animals (Basel)* 11:306. <https://doi.org/10.3390/ani11020306>.
- Johnston, A. E., and D. S. Jenkinson. 1989. The nitrogen cycle in UK arable agriculture. *Fertiliser Society Proceedings No. 286*. International Fertiliser Society.
- Lassaletta, L., G. Billen, B. Grizzetti, A. Anglade, and J. Garnier. 2014. 50 year trends in nitrogen use efficiency of world cropping systems: The relationship between yield and nitrogen input to cropland. *Environ. Res. Lett.* 9:105011. <https://doi.org/10.1088/1748-9326/9/10/105011>.
- Ledgard, S., R. Schils, J. Eriksen, and J. Luo. 2009. Environmental impacts of grazed clover/grass pastures. *Ir. J. Agric. Food Res.* 48:209–226.
- Lee, J., S. Woodward, G. Waghorn, and D. Clark. 2004. Methane emissions by dairy cows fed increasing proportions of white clover (*Trifolium repens*) in pasture. *Proc. N. Z. Grassl. Assoc.* 66:151–155. <https://doi.org/10.33584/jnzs.2004.66.2552>.
- Lee, S. C., and L. Prosky. 1995. International survey on dietary fiber: Definition, analysis, and reference materials. *J. AOAC Int.* 78:22–36. <https://doi.org/10.1093/jaoac/78.1.22>.
- Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57:347–358. [https://doi.org/10.1016/0377-8401\(95\)00837-3](https://doi.org/10.1016/0377-8401(95)00837-3).
- Mackie, T. R., C. R. Parr, and A. M. Bryant. 1996. Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. *N. Z. J. Agric. Res.* 39:341–356. <https://doi.org/10.1080/00288233.1996.9513194>.
- Mangwe, M. C., R. H. Bryant, M. R. Beck, N. Beale, C. Bunt, and P. Gregorini. 2019. Forage herbs as an alternative to ryegrass-white clover to alter urination patterns in grazing dairy systems. *Anim. Feed Sci. Technol.* 252:11–22. <https://doi.org/10.1016/j.anifeeds.2019.04.001>.
- McAuliffe, S., J. F. Mee, E. Lewis, N. Galvin, and D. Hennessy. 2022. Feeding system effects on dairy cow rumen function and milk production. *Animals (Basel)* 12:523. <https://doi.org/10.3390/ani12040523>.
- McCarthy, K. M., N. Walsh, C. van Wylick, M. McDonald, A. G. Fahy, M. B. Lynch, K. M. Pierce, T. M. Boland, H. Sheridan, M. Markiewicz-Keszycska, and F. J. Mulligan. 2023. The effect of a zero-grazed perennial ryegrass, perennial ryegrass and white clover, or multispecies forage on the dry matter intake, milk production and nitrogen utilization of dairy cows in mid-late lactation. *Livest. Sci.* 272:105234. <https://doi.org/10.1016/j.livsci.2023.105234>.
- McClean, B., T. J. Gilliland, L. Delaby, C. Guy, M. Dineen, F. Coughlan, and B. McCarthy. 2019. Milk production per cow and per hectare of spring-calving dairy cows grazing swards differing in *Lolium perenne* L. ploidy and *Trifolium repens* L. composition. *J. Dairy Sci.* 102:8571–8585. <https://doi.org/10.3168/jds.2018-16184>.
- Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* 85:1217–1240. <https://doi.org/10.1093/jaoac/85.6.1217>.
- Minnée, E. M. K., C. M. T. Leach, and D. E. Dalley. 2020. Substituting a pasture-based diet with plantain (*Plantago lanceolata*) reduces nitrogen excreted in urine from dairy cows in late lactation. *Livest. Sci.* 239:104093. <https://doi.org/10.1016/j.livsci.2020.104093>.

- Morgan, D., G. Stakelum, and J. Dwyer. 1989. Modified neutral detergent cellulase digestibility procedure for use with the “Fibertec” system. *Isr. J. Agric. Res.* 28:91–92.
- Murray, Á., L. Delaby, T. J. Gilliland, and B. McCarthy. 2024. Nitrogen offset potential in a multiyear farmlet-scale study: Milk and herbage production from grazed perennial ryegrass–white clover swards. *J. Dairy Sci.* 107:2129–2142. <https://doi.org/10.3168/jds.2023-23822>.
- Niderkorn, V., C. Martin, A. Le Morvan, Y. Rochette, M. Awad, and R. Baumont. 2017. Associative effects between fresh perennial ryegrass and white clover on dynamics of intake and digestion in sheep. *Grass Forage Sci.* 72:691–699. <https://doi.org/10.1111/gfs.12270>.
- O’Connor, A., A. P. Moloney, P. O’Kiely, T. M. Boland, and M. McGee. 2019. Effects of fertiliser nitrogen rate to spring grass on apparent digestibility, nitrogen balance, ruminal fermentation and microbial nitrogen production in beef cattle and in vitro rumen fermentation and methane output. *Anim. Feed Sci. Technol.* 254:114198. <https://doi.org/10.1016/j.anifeedsci.2019.06.002>.
- OECD/FAO (Organisation for Economic Co-operation and Development/Food and Agriculture Organization of the United Nations). 2025. OECD-FAO Agricultural Outlook 2025–2034. OECD/FAO. <https://doi.org/10.1787/601276cd-en>.
- Peyraud, J. L., and L. Astigarraga. 1998. Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: Consequences on animal nutrition and N balance. *Anim. Feed Sci. Technol.* 72:235–259. [https://doi.org/10.1016/S0377-8401\(97\)00191-0](https://doi.org/10.1016/S0377-8401(97)00191-0).
- Peyraud, J. L., L. Astigarraga, and P. Faverdin. 1997. Digestion of fresh perennial ryegrass fertilized at two levels of nitrogen by lactating dairy cows. *Anim. Feed Sci. Technol.* 64:155–171. [https://doi.org/10.1016/S0377-8401\(96\)01056-5](https://doi.org/10.1016/S0377-8401(96)01056-5).
- Rochon, J. J., C. J. Doyle, J. M. Greef, A. Hopkins, G. M. Molle, M. Sitzia, D. Scholefield, and C. J. Smith. 2004. Grazing legumes in Europe: A review of their status, management, benefits, research needs and future prospects. *Grass Forage Sci.* 59:197–214. <https://doi.org/10.1111/j.1365-2494.2004.00423.x>.
- Selbie, D. R., L. E. Buckthought, and M. A. Shepherd. 2015. The challenge of the urine patch for managing nitrogen in grazed pasture systems. *Adv. Agron.* 129:229–292. <https://doi.org/10.1016/bs.agron.2014.09.004>.
- Stockdale, C. R., A. Callaghan, and T. E. Trigg. 1987. Feeding high energy supplements to pasture-fed dairy cows. Effects of stage of lactation and level of supplement. *Aust. J. Agric. Res.* 38:927–940. <https://doi.org/10.1071/AR9870927>.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow’s milk. *J. Dairy Sci.* 48:1215–1223. [https://doi.org/10.3168/jds.S0022-0302\(65\)88430-2](https://doi.org/10.3168/jds.S0022-0302(65)88430-2).
- United Nations. 2024. World Population Prospects 2024: Summary of Results. UN DESA/POP/2024/TR/No. 9. United Nations, New York, NY.
- Walsh, H., D. Patton, J. J. Collins, L. Delaby, K. Pierce, and B. Horan. 2025. Transitioning to low chemical nitrogen perennial ryegrass–white clover pastures on wetland grazing dairy systems. *Anim. Prod. Sci.* 65:AN24284. <https://doi.org/10.1071/AN24284>.

## ORCIDS

- E. Wims, <https://orcid.org/0000-0001-8072-4575>  
 T. F. O’Callaghan, <https://orcid.org/0000-0003-2684-7253>  
 B. McCarthy, <https://orcid.org/0000-0001-8072-9325>  
 E. Serra, <https://orcid.org/0000-0003-2006-3757>  
 P. Dillon, <https://orcid.org/0000-0002-2511-8625>  
 D. Ryan, <https://orcid.org/0009-0009-4956-8063>  
 J. P. Murphy, <https://orcid.org/0000-0002-8291-2710>  
 M. Dineen <https://orcid.org/0000-0003-4585-2680>