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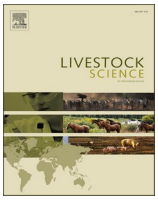

Effects of concentrate supplementation and genotype on milk production and nitrogen utilisation efficiency in late-lactation, spring-calving grazing dairy cows

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HIGHLIGHTS

- HM genotype cows had a superior MR to concentrate compared to LM genotype cows.
- Offering cows concentrate reduced cow level NUE.
- Offering cows concentrate reduced protein % but increased fat + protein kg.

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ABSTRACT

The study objectives were to evaluate the effects of (1) concentrate supplementation (CS), (2) cow genotype, and (3) a potential interaction between CS and cow genotype on milk production, dry matter (DM) intake (DMI) and cow nitrogen (N) utilisation efficiency (NUE) in late lactation (+208 ± 14.1 days in milk), spring-calving grazing dairy cows. The experiment was a complete randomised block design with a 2 × 2 factorial arrangement of treatments and was conducted over a 52-day period. There were two feeding strategies (pasture-only (PO) and pasture + 2.7 kg DM CS) and two genotype groups [lower milk genotype (LM; milk kg PTA = -48 ± 59.9, fat kg PTA = +7 ± 4.7 and protein kg PTA = +3 ± 3.2) and higher milk genotype (HM; milk kg PTA = +190 ± 109.7, fat kg PTA = +12 ± 5.7 and protein kg PTA = +9 ± 3.6)]. Cows in their respective genotype group were randomly assigned to one of two feeding strategies, resulting in four treatment groups ($n = 12$). Cows grazed full time and were allocated 17 kg DM pasture/cow per d. No interactions were observed for any parameters measured. Cows offered CS had increased daily yields of fat + protein (+0.18 kg), lactose (+0.13 kg) and ECM (+2.46 kg) compared to cows offered PO. The HM cows had increased yields of daily fat + protein (+0.13 kg) and lactose (+0.1 kg) compared to the LM cows. Cows offered CS had decreased daily protein (-0.14%) but increased lactose (+0.08%) concentration compared to cows offered PO. The HM cows had decreased daily fat (-0.2%), protein (-0.16%) and casein (-0.07%) concentration compared to the LM cows. Cows offered CS had a reduced daily pasture DMI (-1.41 kg) but an increased daily total DMI (+1.29 kg) and feed N intake (+0.085 kg) compared to cows offered PO. Cows offered CS had decreased NUE (-0.1%) compared to cows offered PO. In conclusion, offering cows 2.7 kg DM CS per day improved milk production in late lactation but resulted in a poorer NUE. The poorer NUE was due to no difference in milk N output and an increase in the partitioning of feed N to urine. The HM cows had an increased milk response to CS with respect to milk fat + protein kg compared to the MR obtained from LM cows.

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1. Introduction

In Ireland, dairy production is mainly aimed at maximising milk production from grazed grassland during a season extending from February to November (Kennedy et al., 2009; O'Brien and Hennessy, 2017). Within these seasonal pasture-based systems, late lactation milk production presents challenges of decreased milk yields (McKay et al., 2019), altered milk composition (O'Brien et al., 1996) and decreased cow level nitrogen (N) utilisation efficiency (NUE). Since European Union milk quota abolition in 2015, Irish milk production has increased by 47% up to 2020. The peak: trough ratio (May milk supply: January milk supply) has increased to 6.5:1 (2020) from 6:1 (2015) (Central Statistics Office, 2015; Central Statistics, 2021), exacerbating seasonality challenges related to milk production cow level NUE.

Challenges associated with late-lactation milk production may be alleviated through adequate nutrition (O'Neill et al., 2012; McKay, 2019). Pasture-based milk production systems utilise concentrate supplement (CS) when there is a reduced availability and quality of grazed pasture (Sairanen et al., 2006). One objective of offering CS to grazing dairy cows is to increase total dry matter (DM) intake (TDMI) and energy intake relative to that achieved with pasture-only (PO) diets (Bargo et al., 2003). Past research has shown that CS has increased milk yield and milk solids yield (Bargo et al., 2003; Kennedy et al., 2003; McKay et al., 2019). Furthermore, Reid et al. (2015) and McKay et al. (2019) found that cow NUE can be increased by offering cows CS in late lactation. Milk yield response (MR) to CS is improved when compared to the MR achieved with grass silage supplementation (Peyraud et al., 2004), with responses ranging from 1.21 kg milk/ kg CS DM (McKay et al., 2019) to 1.36 kg milk/ kg CS DM offered (Bargo et al., 2002) in the autumn. Cow MR to CS is known to be increased in autumn when pasture metabolisable energy content is reduced. However, MR to CS can also be dependent on cow genetic merit (Horan et al., 2005).

Predicted transmitting ability (PTA) values indicate the additive genetic component of a trait that an animal is expected to transmit to its offspring relative to the base population (Wattiaux, 2011). Past research completed on effects of milk production PTA (Kennedy et al., 2003) suggested that higher milk production PTA cows that were supplemented had an increased MR to CS (Kennedy et al., 2003) and DMI (Horan et al., 2006). Furthermore, higher milk production PTA cows are more capable of partitioning nutrients ingested from CS towards milk production and less towards N excretion and body tissue gain (Ferris et al., 1999; McCarthy et al., 2007; Cheng et al., 2014). There is limited research, however, investigating the MR to concentrates in higher milk genotype cows during late lactation.

Dairy cow NUE is an important topic because of the polluting effect that N has on air (NH_3) and waterways (NO_3) (Owens et al., 1994; Environmental Protection Agency, 2017). The NUE of dairy cows is poor (Ipharraguerre and Clark, 2005), and can be compounded in grazing systems as high quality pasture is naturally high in crude protein (CP; Van Vuuren et al., 1990). Dairy cow NUE also reduces throughout lactation (Castillo, 2001). This reduction has been due to a naturally declining milk yield along with an increase in pasture N levels in autumn (Mulligan et al., 2004). The studies of Reid et al. (2015) and McKay et al. (2019) show that cow NUE can be improved by offering CS. To our knowledge, no studies have investigated NUE of cows divergent in milk, fat, and protein kg PTA within the Economic Breeding Index (EBI) or the potential interaction between genotype and CS. This research would be important for farmers who seek to improve milk production, while simultaneously reducing the environmental impact of dairy production through on farm management strategies.

The study objectives were to evaluate the effects of (1) offering CS, (2) cow genotype, and (3) a potential interaction between CS and cow genotype on milk production and NUE in late-lactation, spring-calving grazing dairy cows.

2. Materials and methods

2.1. Animal ethics

All procedures described in this experiment were approved by the Animal Research Ethics Committee at University College Dublin (UCD) and conducted under experimental licence from the Health Products Regulatory Authority under the European Directive 2010/63/EU and S. I. No. 543 of 2012. Each person who carried out procedures on experimental cows during this experiment, were authorised to do so by the Health Products Regulatory Authority. Procedures conducted on the experimental cows were deemed "mild" in severity banding. Hence, no pain, suffering, or distress was observed in experimental cows, and no humane endpoints were required. This experiment was conducted at UCD Lyons Farm, Celbridge, Naas, Co. Kildare, Ireland, W23 ENY2 (53^0 17' 56" N, 6^0 32' 18" W).

2.2. Cows, treatments and experimental design

Thirty-six multiparous and 12 primiparous Holstein Friesian dairy cows were selected from the spring-calving herd at UCD Lyons Farm. A complete randomised block design experiment (2 × 2 factorial arrangement of the treatments), with two feeding strategies (PO and pasture + 2.7 kg DM CS) and two cow genotypes (Table 1; lower milk genotype (LM; milk kg PTA = -48.1 ± 59.88, fat kg PTA = +6.6 ± 4.67 and protein kg PTA = +3.2 ± 3.15) and higher milk genotype (HM; milk kg PTA = +190.3 ± 109.75, fat kg PTA = +11.5 ± 5.73 and protein kg PTA = +8.9 ± 3.57)], was conducted over a 52-day (d) period from 10th September to 31st October 2018. All cows were offered 100% of their energy requirements through PO, given their expected milk production at the start of the study. Additionally, half of the cows (n = 24) were offered 2.7 kg DM CS/cow per d; a level of CS that is similar to the industry standard where cows are pasture based and are in late lactation. Sample sizes were determined by means of a power test using the CV of ruminal ammonia concentration (Whelan et al., 2013). Cows were blocked on parity and balanced on d in milk (DIM; 208 ± 14.1), BCS and overall EBI (within genotype groups), which is the Irish dairy total merit index (www.icbf.com). Cows within genotype groups were randomly assigned to one of two feeding strategies, resulting in four treatment groups (n = 12): (1) LM cows offered PO (LM-); (2) LM cows offered pasture + CS (LM+); (3) HM cows offered PO (HM-); and (4) HM cows offered pasture + CS (HM+). Cows grazed full time and were allocated 17 kg DM pasture/cow per d. Supplementary concentrates were manufactured by Gain Feeds, where all ingredients were ground to form a pellet (Table 2). Concentrate supplementation was dispensed in the milking parlour using the Feedrite automatic system linked to cow electronic identification (Dairymaster). A supplementary magnesium bolus (Opti Mag 3, Norbrook) was administered to cows offered PO on d 22 of the study due to a high risk of hypomagnesaemia. Each bolus had a release rate of 3 g magnesium per d.

Table 1
Genotype profile of cows in the experiment [1].

	Lower milk genotype	National percentile	Higher milk genotype	National percentile
Genetic parameter				
Milk kg	-48.1	Bottom 20%	190.3	Top 10%
Fat kg	6.6	Top 30%	11.5	Top 1%
Protein kg	3.2	Bottom 50%	8.9	Top 1%
Fat %	0.14	Top 5%	0.06	Top 30%
Protein %	0.09	Top 5%	0.04	Top 30%

[1] National percentiles apply to the year 2018.

Table 2
Chemical composition of concentrate supplement (CS) and pasture, and ingredient inclusion level of CS offered [1].

	CS	Pasture
Chemical composition, g/kg DM unless stated		
Dry matter, g/kg	870.0	155.8
Ash	68.0	88.5
Crude protein	190.9	260.5
Neutral detergent fibre	223.2	413.3
Acid detergent fibre	107.4	188.7
Water soluble carbohydrates	-	63.5
Ether extract	23.3	30.0
Starch	235.5	-
Gross energy, MJ/kg DM	17.7	17.3
Ingredient inclusion level of concentrates, g/kg DM [2]		
Barley	180.0	
Maize	180.0	
Maize distiller grain with solubles	150.0	
Sugar beet pulp pellets 8mm	95.0	
Soyabean meal 47%	200.0	
Soya hulls	95.0	
Soyabean oil	5.0	
Palm oil blend	10.0	
Monocalcium diphosphate	5.6	
Sugarcane molasses	45.0	
Calcium carbonate	8.0	
Sodium chloride	8.8	
Magnesium oxide	7.5	
Gain cattle premix [3]	10.0	

[1] Chemical composition analysis results are the average of 8 weekly results.
[2] All grains were ground.
[3] Gain cattle premix consisted of the following: 10.2 g/kg calcium; 5.4 g/kg phosphorus; 3.6 g/kg sodium; 11.1 g/kg potassium; 6.9 g/kg chlorine; 5.8 g/kg magnesium; 0.1 g/kg copper; 0.3 g/kg zinc; 16,000 IU/kg vitamin A; 4,000 IU/kg vitamin D; and 20 IU/kg vitamin E.

2.3. Grassland management

Cows grazed (to 4 cm) as a single group and were fed fresh allocations of pasture twice daily (8.5 kg DM per cow) post am and pm milking (17 kg DM per d, total). Pre-grazing herbage mass was determined daily before cows entered a new allocation using the "quadrant and shears" method as described by Whelan et al. (2012a). The average pre-grazing herbage mass (> 4 cm) was 1,376 ± 204 kg DM per hectare. Post-grazing herbage mass was also measured daily, a total of 50 measurements were taken across each grazing area using a rising plate meter (diameter 355 mm and 3.2 kg/m^2; Jenquip) by walking in a W-shape across the field. Post-grazing herbage mass (> 4 cm) was 382 ± 204 kg DM per hectare.

2.4. Data and sample collection

2.4.1. Pasture and concentrate

On a daily basis, pasture samples were collected using the "quadrant and shears" method (Whelan et al., 2012a). Then, on a weekly basis, daily pasture samples were pooled for chemical analyses (DM, gross energy, ether extract, ash and CP), neutral detergent fibre, acid detergent fibre and water-soluble carbohydrates. Weekly changes in pasture quality over the experiment are shown in Fig. 1. Cows had ad libitum access to fresh water. Concentrate supplement samples were collected weekly for DM and then ground for analyses.

2.4.2. Milk

Cows were milked twice daily at 0700 hours (h) and 1500 h. Milk output was recorded and milk sampling was facilitated using the Weighall milk metering and sampling system (Dairymaster). Milk samples for each individual cow were collected and analysed once per week (wk) on the same occasion for milk composition parameters; thereby controlling any time-related confounding effects. Test day milk fat + protein kg was then determined.

2.4.3. Body weight and body condition score

Individual cow body weights were measured twice daily using electronic scales as the cows exited the milking parlour through the automatic cow-drafting unit (Dairymaster), and then, a weekly average was calculated. Body condition score was assessed by two fully trained operators following morning milking once weekly using a scale of 1 to 5 with 0.25 increments according to Edmonson et al. (1989).

2.4.4. Ruminal fluid

A sample of ruminal fluid was collected using the Flora Rumen Scoop Oral Oesophageal Sampler (Prof-Products) once per wk at 1600 h as cows left the milking parlour post evening milking. To avoid saliva contamination during ruminal fluid sampling, the rumen scoop sampling chamber was only opened after the scoop entered the rumen. Before removing the sampling chamber from the rumen, the sampling chamber was closed. The pH of the ruminal fluid was measured immediately (Phoenix Instrument EC-25 pH/ Conductivity Portable Meter). Once collected, samples were strained through four layers of cheesecloth, a 4 mL aliquot was collected using an automatic pipette, mixed with 1 mL of 500 g/L trichloroacetic acid, and cooled on ice. These were stored (-20 °C) pending analysis for ruminal volatile fatty acid (VFA) and NH_3-N concentrations.

2.4.5. Nitrogen partitioning study

A N partitioning study was conducted during wk 3 of the experiment (221 ± 14.1 DIM). Pasture DMI and N excretion were determined using the *n*-alkane technique of Dove and Mayes (2006) over a period of 6 d. Cows were dosed with a paper bolus impregnated with 500 mg of the *n*-alkane *n*-dotriacontane, for a period of 12 d following am and pm milking. From d 7 to 12 (6 d), samples of the CS, pasture, milk, and faeces were collected. Pasture samples were collected during the morning and evening using a quadrant and handheld shears (Gardena Accu 90, Gardena GmbH). These samples were immediately dried at 55 °C for 48 h. Faecal samples were collected whenever possible, where cows naturally defecated, and, if not, samples were collected per rectum and placed in a forced-air oven at 55 °C for 72 h. Samples of milk were collected during am and pm milking each of the 6 d for each cow. Then, am and pm samples were pooled in proportion to am and pm milk yield so that a single cow's milk sample was composed of am and pm milk sample; daily milk N output/cow was then determined.

2.5. Sample analyses

2.5.1. Feed and faecal sample analysis

Pasture, CS and faecal samples were dried in a forced air oven at 55 °C and were ground in a hammer mill fitted with a 1 mm screen (Lab Mill, Christy Turner, Ltd.). The ash content was determined following combustion in a muffle furnace (Nabertherm GmbH) at 550 °C for 5.5 h (AOAC, 2005b). The N content of samples was determined by combustion on LECO and CP content calculated (N × 6.25; FP 528 Analyzer, Leco Corp.) (AOAC, 2005c). The DM content of samples was determined after drying overnight at 105 °C (16 h; AOAC, 2005a). Gross energy of feed was determined by bomb calorimetry (Parr 1281 Bomb Calorimeter, Parr Instrument Company). The ether extract of feed samples was determined using Soxtex instruments and light petroleum ether. Neutral and acid detergent fibre were determined using the sodium sulphite method of Van Soest et al. (1991) adopted for use in the Ankom 220 Fiber Analyzer (Ankom Technology). This method included a thermostable α-amylase and 20 g of Na_2S but residual ash was not determined. Starch content of feed samples was analysed using the Megazyme Total Starch Assay Procedure (product no: K-TSTA; Megazyme International Ireland Ltd.). The concentration of water-soluble carbohydrates was determined as described by Dubois et al. (1956).

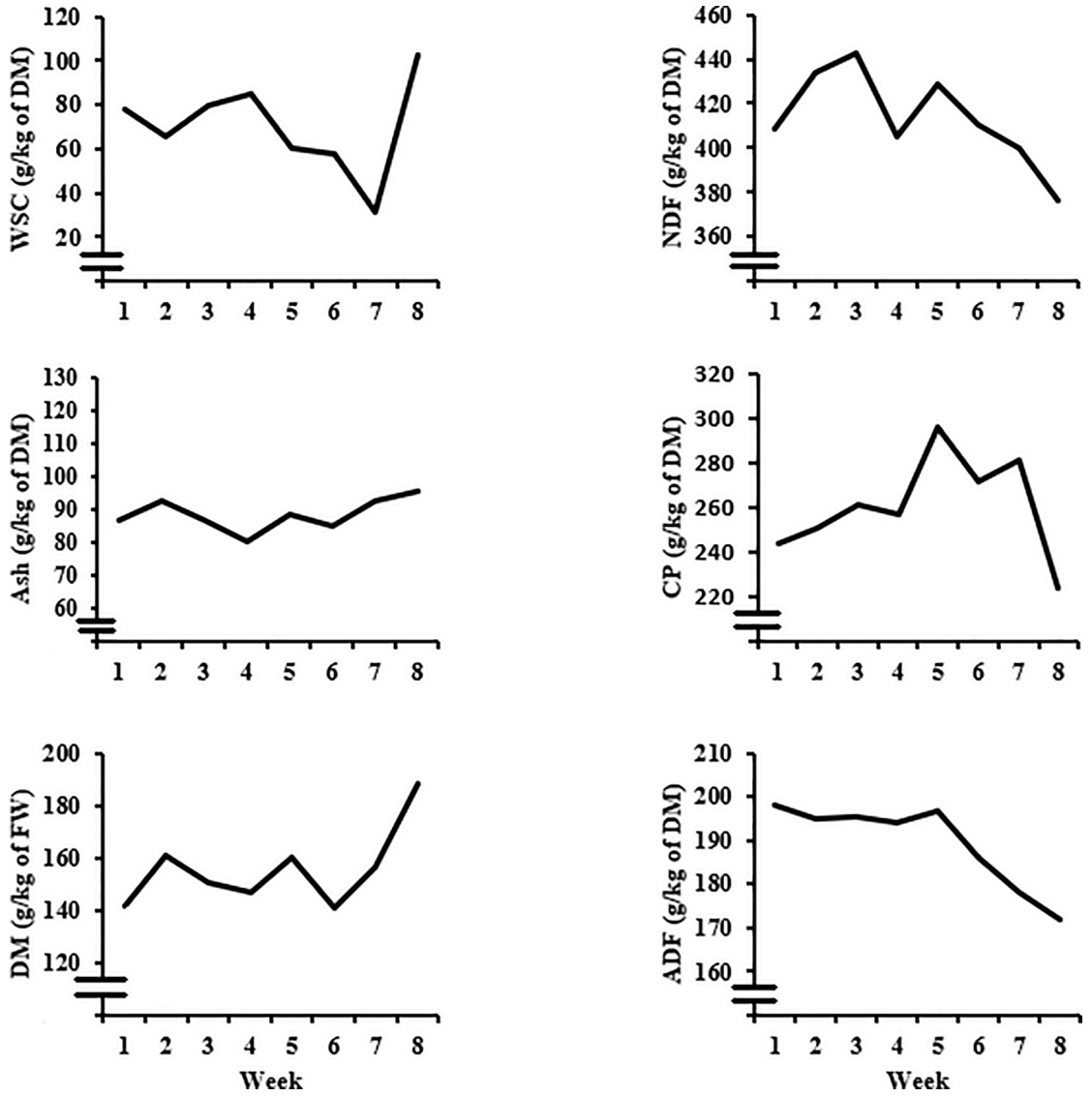

Fig. 1. Changes in autumn pasture quality offered to dairy cows during the experiment. Standard deviations across the 52-d study were; ± 21.4 g WSC (water soluble carbohydrate)/kg DM; ± 21.3 g NDF (neutral detergent fibre)/kg DM; ± 4.9 g ash/kg DM; ± 22.7 g CP (crude protein)/kg DM; ± 15.3 g DM/kg of fresh weight; and ± 62.2 g ADF (acid detergent fibre)/kg DM.

2.5.2. Cow measurements analysis

Concentrations of milk fat, CP, lactose, casein, milk urea N and somatic cell count (SCC) were determined in a commercial milk laboratory (National Milk Laboratories Ltd.) using mid-infrared spectrometry (MilkoScan FT6000, Foss Analytical A/S; Soyeurt et al., 2006). The energy-corrected milk (ECM) yield was calculated as follows: ECM = [(0.327 × milk yield kg) + (7.2 × milk protein kg) + (12.95 × milk fat kg)] (Eslamizad et al., 2010).

Ruminal fluid was given time to thaw in the refrigerator for 16 h at 4 °C and was centrifuged at 2,100 × g for 10 minutes (min) at 4 °C. One mL of supernatant was diluted 1 in 5 with distilled H_2O and then centrifuged at 1,600 × g for 15 min at 4 °C. Next, 200 μL of supernatant was combined with three reagents and used to determine ruminal fluid NH_3-N concentrations using a spectrophotometer (UVmini-1240, Shimadzu). Ruminal fluid was prepared for VFA analysis by mixing 250 μL of ruminal fluid with 3.75 mL of distilled H_2O; to this 1 mL of internal standard solution (0.5 g 3-methylvaleric acid in 1,000 mL of 0.15 molar mass oxalic acid) was added. The resulting solution was centrifuged at 1,600 × g and filtered through a syringe-tip filter (PTFE, 13 mm diameter, 0.45 μm) into 2 mL gas chromatography (GC) vials. Concentrations of VFA were determined using GC (Varian 3800 GCL, Varian Inc.) fitted with a 15 m capillary column with an internal diameter of 0.53 mm coated with 1.20 μm acid-modified polyethylene glycol (EC-1000, Grace Davison Discovery Sciences).

2.5.3. Nitrogen partitioning study

Pasture DMI was determined by extracting *n*-alkanes from feed and faecal samples according to the method of Dove and Mayes (2006). Following extraction, samples were analysed for concentrations of *n*-alkanes by GC using a Scion 456-GC (Scion Instruments) fitted with a 30 m capillary column with an internal diameter of 0.53 mm coated with 1.5 μm of dimethyl polysiloxane (Agilent Technologies Ireland Ltd.). These data were then applied to the following modified equation to calculate pasture DMI (PDMI)/cow per d (Mayes et al., 1986): PDMI = [(*Fi/Fj*)(*Dj*+*IcCj*)-*IcCi*]/[*Hi*-(*Fi/FjHj*)], where *Fi* and *Fj* are the concentrations of naturally occurring odd-chain (feed derived) and even-chain (dosed *n*-dotriacontane *n*-alkane in faeces, respectively (mg/kg); *Hi* and *Hj* are the concentrations of natural odd-chain and even-chain *n*-alkanes in pasture, respectively (mg/kg); *Dj* is the daily dose rate of the even-chain *n*-alkanes (mg/kg); *Ic* is the daily concentrate intake (kg/d); and *Ci* and *Cj* are the concentrations of natural odd-chain and even-chain *n*-alkanes in concentrate feed (mg/kg), respectively. Nitrogen

partitioning was then calculated according to Whelan et al. (2012b) as follows: N intake (g) = [(kg of PDMI × g N/kg of DM pasture) + (kg of concentrate DMI × g of N/kg of DM concentrate)]; Faecal N (g) = (kg of faecal DM excretion × g of N/kg of DM faeces); Milk N = (kg of milk yield × g of N/kg milk); and Urinary N (g) = (N intake (g) – faecal N (g) – Milk N (g).

2.6. Statistical Analysis

Residuals of data were checked for normality and homogeneity of variance by histograms, QQ-plots and formal statistical tests as part of the UNIVARIATE procedure of SAS (version 9.1.3; SAS Institute, 2013). Somatic cell count data were not normally distributed and were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS (Fahey et al., 2007). The transformed SCC data were used to calculate *P*-values. The corresponding least square means and SE of the non-transformed SCC data are presented in the results for clarity. The relationships between feed N intake, milk N output, urinary N excretion and faecal N excretion were tested for associations using the REG procedure of SAS. Milk production, milk composition, ruminal fermentation, BCS, DMI and N excretion parameters were analysed as a 2 × 2 factorial arrangement with repeated measures using the MIXED procedure of SAS. The fixed effects in the model were genotype (LM vs. HM), CS (PO vs. CS), wk and their interaction, and cow was considered the random effect. Week of experiment was the repeated unit. Heterogenous compound symmetry, unstructured, autoregressive, heterogeneous 1st order autoregressive, toeplitz and heterogenous toeplitz were (co)variance structures considered. The model with the lowest Bayesian information criterion value was selected. A Tukey adjustment was used for multiple comparisons. A probability of $P < 0.05$ was selected as the level of significance and statistical tendencies were reported when $P \geq 0.05$ but < 0.10.

3. Results

3.1. Dry matter intake, milk production and milk composition

Effects of CS and genotype on DMI, BCS, milk production and milk composition are shown in Table 3. Cows offered CS had a decreased daily PDMI (-1.41 kg; $P < 0.01$) but an increased daily TDMI (+1.29 kg; $P < 0.01$) compared to cows offered PO. Cows offered CS had an increased daily fat + protein kg (+0.18 kg; $P < 0.05$), lactose kg (+0.13 kg; $P < 0.01$) and ECM yield (+2.46 kg; $P < 0.01$) compared to cows offered PO. The HM cows had an increased daily lactose kg (+0.1 kg; $P < 0.05$) compared to the LM cows. Cows offered CS had a decreased daily milk protein concentration (-0.14%; $P < 0.05$) and tended to have decreased milk fat (-0.18%; $P = 0.08$) and casein (-0.11%; $P = 0.06$) concentrations, but increased lactose concentration (+0.1%; $P < 0.05$) compared to cows offered PO. The HM cows had decreased daily milk fat (-0.2%; $P < 0.05$), protein (-0.16%; $P < 0.05$) and casein (-0.14%; $P < 0.05$) concentrations compared to LM cows. Cows offered CS had an increased daily ECM (+2.46 kg; $P < 0.01$).

3.2. Ruminal fermentation

Effects of CS and genotype on ruminal fermentation parameters are shown in Table 4. Cows offered CS tended to have a decreased ruminal pH (-0.06 pH; $P = 0.07$) at 1600 h compared to cows offered PO. Furthermore, cows offered CS had increased total VFA (+6.03 mmol/L; $P < 0.01$), acetic (+3.72 mmol/L; $P < 0.01$), propionic (+1.04 mmol/L; $P < 0.01$) and butyric (+0.78 mmol/L; $P < 0.01$) acid concentrations compared to cows offered PO. The HM cows had increased propionic (+0.84 mmol/L; $P < 0.05$) and isovaleric (+0.32 mmol/L; $P < 0.05$) acid concentrations compared to the LM cows.

3.3. Nitrogen partitioning

The effects of CS and genotype on N partitioning parameters are shown in Table 5. Cows offered CS had an increased daily feed N intake (+0.085 kg; $P < 0.001$) compared to cows offered PO. Cows offered CS tended to have an increased daily urinary N excretion (+0.003 kg; $P = 0.08$) compared to cows offered PO. Cows offered CS had an increased daily proportion of N partitioned to urine (+0.1; $P < 0.05$) compared to cows offered PO. Cows offered CS had an increased daily percentage of N excreted (+0.1%; $P < 0.05$) and a decreased (-0.09%; $P < 0.05$) NUE compared to cows offered PO. Genotype had no effect ($P > 0.10$) on daily N partitioning parameters. We found linear relationships (Figs. 2 and 3) between daily feed N intake and estimated daily urinary N excretion (Eq. (1), $P < 0.001$, $R^2 = 0.76$) and between daily milk N output and estimated daily urinary N excretion (Eq. (2), $P < 0.05$, $R^2 = 0.12$).

[1] Estimated daily urinary N excretion (kg/d) = -0.1799 (±0.05982) + 0.937 (±0.06732) × Daily feed N intake (kg/d),

Table 3
Effects of concentrate supplement (CS) and genotype on dry matter intake, body condition score, milk production and milk composition [1].

Genotype	LM		HM			Significance		
CS	-	+	-	+	SEM	Genotype	CS	Interaction
Dry matter intake								
Pasture, kg/d	17.44[a]	16.21[b]	17.75[a]	16.16[b]	0.372	0.70	< 0.01	0.56
Total, kg/d	17.44[a]	18.91[b]	17.75[a]	18.86[b]	0.372	0.70	< 0.05	0.56
Body condition score	2.86	2.95	2.84	2.81	0.055	0.19	0.60	0.27
Milk production, kg/d								
Energy-corrected milk	18.01[a]	19.81[ab]	18.39[ab]	21.51[b]	0.890	0.22	<0.01	0.43
Fat + protein	1.26[a]	1.37[a]	1.32[a]	1.57[b]	0.068	0.06	< 0.05	0.29
Lactose	0.58[a]	0.67[a]	0.64[a]	0.81[b]	0.039	< 0.05	< 0.01	0.40
Milk composition, %								
Fat	4.98[a]	4.77[ab]	4.75[ab]	4.60[b]	0.101	< 0.05	0.08	0.78
Protein	4.12[a]	4.00[ab]	3.98[ab]	3.82[b]	0.065	< 0.05	< 0.05	0.74
Casein	3.28[a]	3.19[ab]	3.16[ab]	3.03[b]	0.058	< 0.05	0.06	0.70
Lactose	4.17	4.27	4.20	4.27	0.031	0.62	< 0.05	0.58
Milk urea nitrogen, g/100mL milk	0.043	0.043	0.042	0.043	0.0012	0.81	0.88	0.48
Somatic cell count, x10^3 cells/mL	104.13	103.45	146.89	239.31	63.465	0.70	0.59	0.84

[1] LM = lower milk genotype cows; HM = higher milk genotype cows; - = cows fed pasture-only; and + = cows fed pasture and 2.7 kg dry matter CS.

Table 4
Effects of concentrate supplement (CS) and genotype on ruminal fermentation parameters [1].

Genotype	LM		HM			Significance		
CS	-	+	-	+	SEM	Genotype	CS	Interaction
Ruminal pH	6.61^{x}	6.50^{y}	6.52^{xy}	6.51^{xy}	0.033	0.32	0.07	0.14
Ruminal NH_3-N, mmol/L	6.81	6.48	6.77	6.86	0.185	0.37	0.53	0.26
Volatile fatty acids, mmol/L								
Total volatile fatty acids	124.75^{a}	132.11^{b}	129.14^{ab}	133.84^{b}	1.930	0.14	< 0.01	0.45
Acetate	88.72^{a}	93.11^{ab}	91.28^{ab}	94.32^{b}	1.430	0.23	< 0.01	0.60
Propionate	16.88^{a}	17.98^{ab}	17.78^{ab}	18.76^{b}	0.356	< 0.05	< 0.01	0.87
Acetate: propionate	5.35	5.19	5.19	5.11	0.099	0.22	0.24	0.66
Butyrate	11.55	12.41	11.53	12.22	0.268	0.69	< 0.01	0.76
Isobutyrate	1.50	1.51	1.54	1.50	0.038	0.63	0.68	0.50
Valerate	1.74	1.86	1.76	1.78	0.048	0.48	0.10	0.25
Isovalerate	4.92^{xy}	4.79^{x}	5.23^{y}	5.13^{xy}	0.122	< 0.05	0.26	0.90

[1] LM = lower milk genotype cows; HM = higher milk genotype cows; - = cows fed pasture-only; and + = cows fed pasture and 2.7 kg dry matter CS.

Table 5
Effects of concentrate supplement (CS) and genotype on nitrogen partitioning [1].

Genotype	LM		HM			Significance		
CS	-	+	-	+	SEM	Genotype	CS	Interaction
Nitrogen intake, kg/d								
Total feed nitrogen	0.724^{a}	0.809^{b}	0.724^{a}	0.809^{b}	0.00004	0.49	< 0.001	0.56
Nitrogen excreted, kg/d								
Milk	0.111	0.111	0.111	0.110	0.0003	0.45	0.99	0.86
Faeces	0.117	0.118	0.121	0.119	0.0033	0.33	0.91	0.69
Urine	0.532	0.536	0.532	0.541	0.0036	0.38	0.08	0.49
Nitrogen proportions [2]								
Milk	0.145	0.144	0.147	0.145	0.0047	0.61	0.55	0.88
Faeces	0.153	0.151	0.158	0.152	0.0047	0.45	0.42	0.65
Urine	0.695	0.703	0.698	0.710	0.0046	0.29	< 0.05	0.63
Nitrogen excreted [3], %	85.36	85.46	85.38	85.47	0.040	0.70	< 0.05	0.88
NUE [4], %	14.64	14.54	14.62	13.53	0.040	0.70	< 0.05	0.88

[1] LM = lower milk genotype cows; HM = higher milk genotype cows; - = cows fed pasture-only; and + = cows fed pasture and 2.7 kg dry matter CS.
[2] Nitrogen proportions = Nitrogen out [faeces, urine, milk (kg/d)]/N intake (kg/d).
[3] Nitrogen excreted = Nitrogen out [(faeces + urine output (kg/d))/ N intake (kg/d)] × 100.
[4] Nitrogen utilisation efficiency = Nitrogen out [(milk output (kg/d))/ N intake (kg/d)] × 100.

[2] Estimated daily urinary N excretion (kg/d) = 0.6362 (±0.03963) – 0.8814 (±0.35165) × Daily milk N output (kg/d).

4. Discussion

Offering CS to dairy cows is common during the autumn within grazing systems as pasture growth is reduced at this time (Patton et al., 2016). Concentrate supplementation aids in maintaining the quality of the diet and maximising TDMI. Within grazing systems, fixed rate supplementation is most common as it simplifies the milk production system.

4.1. Dry matter intake, substitution rate, milk production and milk composition

In this study, cows that were offered CS had a decreased PDMI but an increased TDMI, concurring with Kellaway and Harrington (2004) and McKay et al. (2019). Substitution rate in our study was 0.52 kg pasture/kg CS DM offered, similar to the SR in the study of McKay et al. (2019), where pasture allowance was also 17 kg DM/cow per d. Substitution rate is known to have an inverse relationship with MR (calculated as the difference in milk produced between unsupplemented and supplemented treatments divided by the CS DM offered; Bargo et al., 2002). Our findings showed that the MR for fat + protein kg was 0.04 kg fat + protein/kg CS DM offered to LM cows and was 0.09 kg fat + protein/kg CS DM offered to HM cows. The LM cows MR for fat + protein kg is similar to the MR reported by McKay et al. (2019) (+0.05 kg fat + protein/kg CS DM offered). The HM cows' MR may have been increased in our study compared to cows in McKay et al. (2019) study as milk fat and milk protein kg PTA was potentially greater in our study. However, milk fat kg and milk protein kg PTA values for cows in McKay et al. (2019) were not disclosed. The increased MR to CS for fat + protein kg in HM cows highlights the greater propensity of HM cows to respond to CS compared to LM cows.

Offering 2.7 kg DM CS/cow per d increased milk fat + protein kg, lactose kg and ECM kg and is similar to the findings of Kennedy et al. (2003). The aforementioned study was conducted when cows were 200 DIM and are representative of cows advancing in lactation stage, which is similar to the cows used in our study (208 DIM). We can attribute the increase in milk production to the additional TDMI that was achieved through offering CS. Milk protein concentration was decreased by offering CS to cows in our findings and this result is contrary to the literature where offering CS to cows maintained milk protein concentration (Reid et al., 2015; McKay et al., 2019). O'Brien et al. (1999) found that offering cows 3 kg fresh weight of CS increased the milk protein (+0.15%) and casein (+0.11%) concentration. The reduction in milk protein concentration observed with offering CS to cows in our study may have been due to dilution because of the high level of MR for

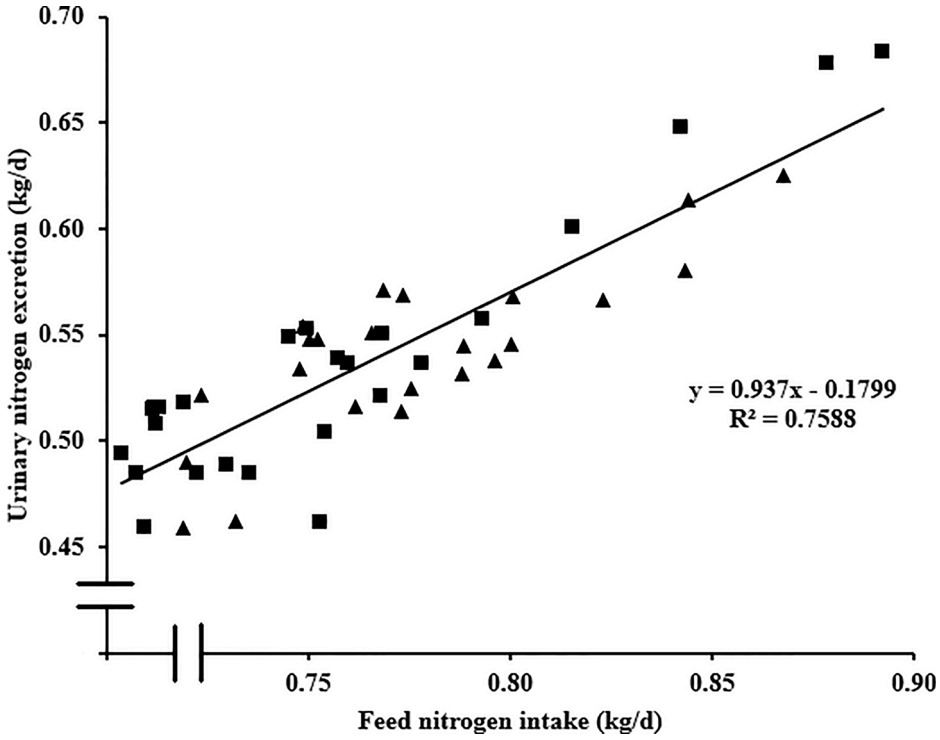

Fig. 2. Pattern of estimated urinary nitrogen excretion plotted over the range of feed nitrogen intake observed for cows during the nitrogen partitioning study (urinary nitrogen excretion was measured by a difference calculation as stated in the text).

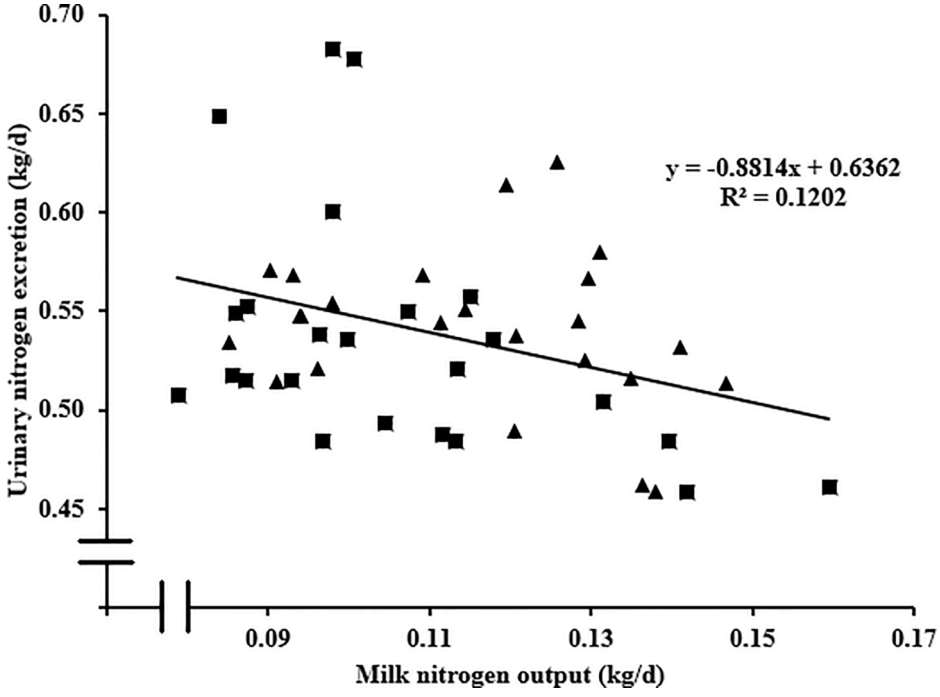

Fig. 3. Pattern of estimated urinary nitrogen excretion plotted over the range of milk nitrogen output observed for cows during the nitrogen partitioning study (urinary nitrogen excretion was measured by a difference calculation as stated in the text).

milk yield that was achieved (Doran et al., 2020).

The diet that is fed to dairy cows is unlikely to affect milk lactose concentration as milk lactose does not respond predictably to dietary changes, but it has been observed to increase as energy intake increases (Looper, 2012). The increased TDMI observed in cows offered CS supports the fact that cows offered CS had increased energy intakes and this was likely responsible for the increased milk lactose concentration. Offering CS increased milk lactose concentration to above the 4.2% threshold as is generally required by Irish milk processors (Glanbia, 2016).

4.2. Ruminal fermentation

Normally, ruminal pH is decreased with offering cows CS (Ørskov, 1986) and with increasing levels of concentrate inclusion (Condren et al., 2019) because of the additional starch digestion that results from grain ingestion. However, ruminal pH was not significantly decreased by offering cows CS in our study. This result is in contrast to McKay et al. (2019). The starch content of the barley and maize-based supplements in McKay et al. (2019) were higher (34.9 and 41.3% respectively) than the starch content of our supplement (23.6%), likely explaining why no significant decrease in ruminal pH with CS was observed. Total VFA concentration was increased where cows were offered CS, corroborating the increased energy supply associated with offering CS to cows, and is consistent with Bargo et al. (2002). Cows that were offered CS had increased milk yield and lactose concentration. The increased milk yield and lactose concentration is supported by the increase in ruminal propionic acid.

4.3. Nitrogen partitioning

Our findings show that feed N intake was increased when CS was offered to cows. The increase in feed N intake was due to the increased TDMI that was achieved when cows were offered CS and this finding is consistent with Steinshamn et al. (2006). Cows that were offered CS had an increased proportion of N excreted in their urine compared to cows offered PO. Usually, increased urinary N excretion in dairy cows is the result of an oversupply of feed N (Colmenero and Broderick, 2006; Spek et al., 2013), and previously, urinary N and feed N intake parameters have been positively correlated with each other (Mulligan et al., 2004; Whelan et al., 2012b). Within the studies of Mulligan et al. (2004) and Whelan et al. (2012b), milk production was not increased with increasing feed N intake; however, urinary N excretion was increased. In this study, the increased proportion of N excreted in the urine occurred because of the increased total feed N intake that was associated with offering cows CS (Eq. (1)).

Castillo (2001) reported that as DIM increases, NUE decreases, and this is due to a naturally decreasing milk yield and an increase in the proportion of N excreted in the urine and faeces. Approximately 25% of ingested N is retained in early lactation (Mulligan et al., 2004; Whelan et al., 2012b), decreasing to 17% in mid lactation (Whelan et al., 2017). Studies report NUE levels as low as 12% in late lactation (Reid et al., 2015). Late lactation studies have shown that NUE can be improved by offering cows a low level of CS (3 and 2.65 kg DM/cow per d; Reid et al., 2015a and McKay et al., 2019, respectively). The study of McKay et al. (2019) found that the improvement in NUE with offering cows a barley-based supplement was due to an increase in milk N output. However, in the current study, there was no increase in NUE when cows were offered CS. This result was due to no difference in the proportion of N excreted in the milk and a concurrent increase in the proportion of N excreted in the urine when cows were offered a low level of CS. In the study of McKay et al. (2019), cows that were offered a barley-based supplement excreted 14.1% of their total feed N intake in the milk. In comparison, we found that the proportion of feed N intake excreted in the milk of cows offered CS was 14.6%. Therefore, it may not be achievable to further increase this proportion with grazing dairy cows that are greater than 200 DIM.

Previous studies have demonstrated the importance of cow genotype in improving cow NUE (Ferris et al., 1999; Cheng et al., 2014). In the aforementioned studies, higher genetic merit cows partitioned an increased proportion of feed N intake to milk, resulting in improved NUE. The HM cows in our study did not partition an increased proportion of feed N intake to milk production and consequently did not have increased NUE compared to LM cows. The LM cows in this study were in the top 5% nationally for milk protein concentration (Table 1), with the HM cows in the top 30%.

Overall, results of this study show that offering cows 2.7 kg CS DM/cow per d can increase milk production at a time when pasture availability is reduced. However, the potential environmental impact of offering cows CS with a high CP concentration on reducing cow NUE should also be considered. This study highlights the importance of milk, fat and protein kg PTA within the EBI and how it can impact MR when cows are in late lactation and are pasture-based.

5. Conclusion

Offering HM cows CS increased milk fat + protein kg compared to offering HM cows PO whilst offering LM cows CS did not increase milk fat + protein kg compared to offering LM cows PO. Furthermore, cows that were offered CS had increased TDMI compared to cows that were offered PO. However, cows that were offered CS had a decreased NUE compared to cows that were offered PO, likely due to no difference in the proportion of milk N output, in combination with an increase in the proportion of urinary N excretion in cows that were offered CS. This study demonstrates the potential of offering cows 2.7 kg DM CS to increase TDMI and milk production when cows are in late lactation and when pasture availability is reduced. However, the reduction in cow NUE with offering a high CP CS should also be considered. The study also demonstrates the propensity of higher milk production PTA cows (>200 DIM) to have an increased MR for fat + protein kg compared to lower milk production PTA cows in pasture-based grazing systems.

CRediT authorship contribution statement

M.J. Doran: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **F.J. Mulligan:** Methodology, Validation. **M.B. Lynch:** Funding acquisition, Project administration, Resources. **A.G. Fahey:** Data curation, Formal analysis, Software, Validation. **G. Rajauria:** Resources. **E.L. Brady:** Conceptualization, Visualization. **K.M. Pierce:** Investigation, Methodology, Writing – review & editing.

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