Applied Animal Behaviour Science 242 (2021) 105429

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Dairy cows with different milk urea nitrogen breeding values display different grazing behaviours

C.J. Marshall *, M.R. Beck [1], K. Garrett, A.E. Fleming, G.K. Barrell, O. Al-Marashdeh, P. Gregorini

Faculty of Agriculture and Life Sciences, P.O Box 85084, Lincoln University, Lincoln, 7647, Christchurch, New Zealand

ARTICLE INFO

Keywords:
Grazing behaviour
Ruminating behaviour
Milk urea nitrogen
Jaw movement recorder

ABSTRACT

The objective of this study was to describe the diurnal pattern of grazing behaviour of multi-parous dairy cows divergent for milk urea N breeding values (MUNBV) and the consistency of these differences across different sward compositions (perennial ryegrass [RG; *Lolium perenne* L.] or ryegrass with plantain [*Plantago lanceolata* L.; RGPL]) and stages of lactation (early and late lactation). Jaw movement recorders were fitted to 24 animals in early lactation and 16 cows in late lactation, in early lactation 12 cows were assigned to the ryegrass diet and 12 cows assigned to the plantain diet, in late lactation 8 cows were assigned to ryegrass with the remaining 8 cows assigned to the plantain diet. For each diet half of the animals during both stages of lactation were classified as high for MUNBV and the other half as low for MUNBV. Low MUNBV animals had more mastications per bite over the day ($P < 0.01$) with a one-unit decrease in MUNBV resulting in 0.07 more bites per mastication during the first two grazing bouts, as well as differences in the temporal distribution of grazing bouts compared with high MUNBV cows. A one-unit decrease in MUNBV resulted in a 0.11 ± 0.02 increase in mastications per bite during the first grazing bout across both stages of lactation and sward composition. Ingestive behaviour has a large impact on the physical features of ingesta and thereby rumen function. The results of this study indicate that dairy cows divergent for MUNBV grazing the same forage apply different grazing strategies in terms of oral processing of ingesta and diurnal meal pattern. These results present potential explanatory variables for phenotypical differences observed in dairy cows divergent for MUNBV.

1. Introduction

Dairy cows that are genetically divergent for milk urea N breeding values (MUNBV mg/dL) have been shown to have differences in milk composition, rumen function and urination behaviour (Marshall et al., 2020, 2021). The potential for a reduced environmental impact has been documented from low MUNBV cows (Marshall et al., 2020, 2021) which has generated commercial interest in breeding for low MUNBV values to reduce environmental impact. The mechanism causing different environmental outcomes based on MUNBV is not fully understood. Grazing behaviour has been demonstrated to affect ingestive and digestive processes (Fleming et al., 2021) which in turn influences rumen fermentation characteristics (Dufreneix et al., 2019), grazing behaviour could therefore be viewed as a key determinate of many animal production and excretion characteristics.

Grazing involves a number of nested animal decisions at a meal (e.g. bite and ingestion rates, oral processing and searching) and the cluster of meals level (e.g. meal pattern, duration and intensity, and rumination behaviour – i.e. chewing rate per bolus) (Gibb et al., 1997; Woodward, 1997). Oral processing is the first step of digestion through mastication and salivation, where the animal mechanically releases soluble plant cell contents and determines the physical characteristics of the ingestive boli and therefore the ingesta particle size flowing into the rumen (Prinz and Lucas., 1997; Poppi et al., 1999; Gregorini et al., 2017). Therefore, the pattern of grazing not only determines characteristics of ingesta flowing to the rumen but also the daily level of herbage and nutrient intake and partitioning to the host animal (Martz and Belyea., 1986; Gregorini, 2012; Gregorini et al., 2017).

Several studies have reported differences in the grazing pattern of cattle selected for a particular genetic parameter (Sheahan et al., 2011; Gregorini et al., 2015; Pierce et al., 2018). For example, Gregorini et al. (2015) reported different grazing patterns of lactating dairy cows

* Corresponding author at: Lincoln University; Faculty of Agriculture and Life Sciences, P.O Box 85084, Lincoln University, Lincoln, 7647, Christchurch, New Zealand.

E-mail address: Cameron.Marshall@lincolnuni.ac.nz (C.J. Marshall).

[1] Current Address: USDA-ARS, Conservation and Production Research Laboratory, Bushland, Texas, USA.

https://doi.org/10.1016/j.applanim.2021.105429
Received 4 May 2021; Received in revised form 8 July 2021; Accepted 23 August 2021
Available online 24 August 2021

selected for divergent residual feed intake (i.e. feed conversion efficiency), where cows with low residual feed intake grazed more efficiently by varying grazing intensity, decreasing mastication jaw movements, increasing chewing jaw movements, and altering meal patterns during the day. No information, however, is available on the grazing patterns of dairy cows divergent in MUNBV. The question therefore arises, would the grazing pattern of dairy cows genetically divergent for MUNBV graze differently? and if so, would differences in grazing behaviour help to explain the differences in N partitioning reported previously (Marshall et al., 2020, 2021)? Therefore, the objectives of this study are to describe the diurnal pattern of grazing behaviour of multiparous dairy cows divergent for MUNBV and the consistency of these differences across different sward compositions of perennial ryegrass (*Lolium perenne* L) and white clover (*Trifolium* repens) or ryegrass with plantain [*Plantago lanceolata* L) across early and late lactation.

2. Materials and methods

All animal manipulations were conducted with approval from the Lincoln University Animal Ethics Committee (AEC 2018-36).

2.1. Research site, animals and treatments

The study was conducted at Lincoln University's Ashley Dene Research and Development Station in Canterbury, New Zealand (-43.65 °North, 172.33 °East) during early (24th of November to 7th of December 2018) and late (April 2019) lactation as previously described in Marshall et al. (2020). Briefly, 48 lactating multiparous Holstein--Friesian × Jersey cows were selected based on their estimated breeding value for MUNBV as described by Beatson et al. (2019) which ranged from -2 to +3. Cows had an average live weight of 483.3 ± 47.5 kg with a body condition score of 3.70 ± 0.32 out of 10 in early lactation and 4.29 ± 0.32 in late lactation. Cows were 72 ± 18 days in milk for early lactation and 223 ± 18 for late lactation (mean calving date 13/9/18 ± 18 days). For both early and late lactation, a 10-day acclimation period was used to adjust cows to their respective diets followed by a 7-day measurement period; all cows had previously been grazing a perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium* repens) sward.

A 2 × 2 factorial design with repeated measures was implemented incorporating stage of lactation (early and late) and diet type (perennial ryegrass and perennial ryegrass and plantain sward) with cows being grouped as either high (50 % highest MUNBV) or low (50 % lowest MUNBV) for MUNBV. Twenty-four cows were assigned to a perennial ryegrass -plantain (*Plantago lanceolata*) diet (RGPL) with the remaining 24 cows assigned to a perennial ryegrass (RG) only diet. Within each dietary group, there were 3 replicates of herds grazing independently for both high and low MUNBV, resulting in a total of 12 replicates. Replicates were found to have no significant effects or interactions and were therefore removed from the final statistical models used for analysis. Each dietary treatment had similar distribution and standard error for MUNBV, the same grouping of animals were used in both stages of lactation. All cows were offered an above ground (3 cm) herbage allowance of 17 kg DM/cow per day. Cows were milked twice daily at approximately 08:00 and 16:00 h and had free access to water at all times except when in the milking shed.

2.2. Pasture and grazing management

Three-year-old established swards receiving 150 kg N/ha annually were used. Daily herbage allowance was estimated with a rising plate meter (Jenquip: Feilding, New Zealand) to determine pre- and post-grazing herbage mass and calibrated as per methodology described in Marshall et al. (2020).

Herbage nutritive characteristics were assessed by taking randomly collected grab samples hand-clipped above 3 cm from both pre (n = 36) and post (n = 36) grazing breaks at the start, middle and end of the experimental period for both early and late lactation. Herbage samples had an average fresh weight of 300 g, samples were split into 3 equal representative sub-samples and were either used for DM analysis by drying in a 60 °C oven for 72 h, used to assess botanical composition or used for chemical analysis. Botanical composition was determined by separating individual sub-samples into the sown constituent components for each diet and measuring the dry weight as a percentage of the total subsample dry weight. The remaining grab samples were then lyophilized and ground using a centrifugal mill fitted with a 1 mm screen (ZM200 Retsch). Samples were analysed for organic matter (OM), water-soluble carbohydrates (WSC), neutral and acid detergent fibre (NDF, ADF), crude protein (CP), dry matter digestibility (DMD), organic matter digestibility (OMD) and digestible organic matter in the dry matter (DOMD) by near-infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000, Maryland, USA) with metabolizable energy (ME) calculated as 0.16 × DOMD (Primary Industries Standing Committee., 2007). Calibration equations for NIRS were conducted before sample analysis and all had R^2 values greater than 0.90 and were within the calibration range.

2.3. Animal measurements

Twenty-four cows were fitted with jaw movement recorders (Ultra Sound Advice, London, UK) in early lactation and 16 cows in late lactation immediately after the morning milking during the start, middle and end of the experimental period of both phases. After fitting with jaw movement recorders, cows were then let into a new pasture break. Recorders were removed after the next morning's milking (24 h later). In both stages of lactation, half of the jaw movement recorders were placed on cows offered the RGPL diet and the other half on the RG diet, within each diet half of the cows wearing the jaw movement recorders were classified as 'high' and the other half classified as 'low' for MUNBV. Jaw movements were analysed using the GRAZE software (Rutter, 2000) to identify bites, mastications, chews during rumination, as well as bolus formed. The GRAZE software also indicates both grazing and rumination bouts and total grazing, ruminating and idle time. The periods of time during the day were classified as; morning, day, evening and night. Morning was considered to be the hours of 05:00 to 08:00 in early lactation and 06:00 to 08:00 in late lactation and represented the time from the start of nautical twilight to commencement of the morning milking. Day was classified as the hours of 09:00 to 16:00 for both early and late lactation and represented the time between morning and afternoon milkings. Evening was classified as the hours of 17:00 to 21:00 for early lactation and hours of 17:00 to 18:00 in late lactation and was the time from completion of the afternoon milking to the end of nautical twilight. Night was classified as the hours of 22:00 to 04:00 in early lactation and 19:00 to 05:00 in late lactation, being the time between the end and beginning of nautical twilight.

2.4. Statistical analysis

All statistical analyses were conducted using R (R Core Team., 2021; v. 3.6.1). Botanical and chemical compositions of the pastures were analysed using a generalized linear mixed effects model with the 'glmer' function from the 'lme4' package (Bates et al., 2015). A gamma distribution was used with diet (RG vs RGPL), and stage of lactation (early vs late) as the fixed effects. Date of pasture sampling was included as a random effect for the botanical and chemical composition data. The main effects of diet and stage of lactation, and their interaction was determined by an analysis of deviance table using a type II Wald Chi-square test with the 'Anova' function of the 'car' package (Fox and Weisberg., 2019). No interaction ($P > 0.05$) terms with MUNBV for botanical or chemical composition were detected and therefore are not reported. Apparent intake was analysed using linear mixed model regression using the 'lme4' package with diet (RG vs RGPL), stage of lactation (early vs late), and the MUNBV grouped as a categorical variable (50 % highest vs 50 % lowest MUNBV cows), plus all possible interactions as fixed effects with date as the random effect. Following significance of the analysis of deviance tables, means separation was performed and least-squares means were back-transformed from the inverse scale using the 'emmeans' function (Lenth, 2019).

Ordinary least-squares (OLS) regression was used to analyse grazing and rumination behavioural characteristics using MUNBV (continuous), diet, stage of lactation and bout and all possible interaction combinations were included as fixed effects in the full model. For behavioural measurements, a linear mixed effect model was used with high or low MUNBV, diet, hour of the day and their interactions as fixed effects and date as the random effect. The least-squares means were determined using the 'emmeans' function and the grazing and ruminating least-squares means were then plotted across hour of the day using the 'ggplot' function of the 'ggplot2' package (Wickham, 2016). Statistical significance was declared at $P \leq 0.05$ and tendencies are discussed at $0.05 < P \leq 0.10$.

3. Results

3.1. Herbage and intake measurements

Herbage nutritive and botanical values have been reported previously (Marshall et al., 2020) and are displayed in Table 1. Briefly, RGPL swards contained 56 % ryegrass, 7 % white clover and 21 % plantain across both early and late lactation. Ryegrass-plantain swards also contained 14 % reproductive stem and 2 % dead material and weed during early lactation and 1 % reproductive stem and 8 % dead material and weed in late lactation. Ryegrass swards contained 80 % ryegrass, 8 % white clover and 0 % plantain in both early and late lactation. Ryegrass swards also contained 2 % dead material and weed and 8 % reproductive stem in early lactation and 9 % dead material and weed and 0 % reproductive stem in late lactation.

An effect of diet ($P < 0.05$) was detected on several of the nutritional values between the RG and RGPL diet. The RG diet had a 13 % greater value for NDF % compared to the RGPL diet, RG also had a 3 % greater ADF %, a 1 % greater OM % and a 2 % greater ME (MJ/Kg/DM) content compared to RGPL. No differences ($P > 0.10$) were detected for CP %, DMD %, OMD % or WSC % between the RG and RGPL diets.

A stage of lactation effect ($P < 0.05$) was detected on dietary nutritive values with early lactation having a 0.4 % greater OM % compared to late lactation. Early lactation also had a 2% increase in DMD %, a 3 % increase in OMD %, a 56 % increase in WSC and a 3 % increase in ME (MJ/Kg/DM) content compared to late lactation. During late lactation, there was a 6 % increase in NDF % and a 15 % increase in CP % compared to early lactation. An interaction effect was detected between the stage of lactation and diet with the RGPL diet in early lactation having a 16 % reduction in DM % compared to RG in early lactation as well as RG and RGPL in late lactation ($P < 0.01$). Apparent DM intake, as measured by pre- and post-grazing herbage mass, was 15.7 ± 2.0 kg/DM per d and was unaffected by diet, stage of lactation or MUNBV.

3.2. Grazing behaviour

3.2.1. Daily level

Table 2 presents grazing behaviour characteristics expressed on a total daily level. Only a diet effect ($P < 0.05$) was detected for the total daily time grazing, cows consuming RGPL spent 7 % more time grazing than cows consuming RG. Only a stage of lactation effect was detected ($P < 0.01$) for rumination time, cows in late lactation spent 28 % less time ruminating compared with early lactation. A stage of lactation effect was also detected ($P < 0.01$) for total daily idle time. Cows in late lactation increased the time they spent idle by 41 % compared with early lactation. No effect from MUNBV, diet, stage of lactation or the interaction term was detected for the total number of events for both grazing and ruminating; however, a tendency ($P = 0.10$) was detected for cows to have fewer ruminating events in late lactation. A similar relationship was detected for the inter-bout duration, with no main or interaction effects detected for either grazing or ruminating, but a tendency ($P = 0.10$) was detected for lower ruminating inter-bout durations in late lactation.

A stage of lactation effect was detected ($P < 0.01$) for total jaw movements, cows had 15 % fewer jaw movements in late lactation compared with early lactation. A diet effect was also detected ($P = 0.03$), cows consuming RGPL had 9 % more jaw movements than cows consuming RG. A stage by diet effect ($P < 0.01$) was detected for total daily mastications, cows consuming RG had 59 % fewer mastications compared with cows consuming RGPL in early lactation, and RG and RGPL in late lactation. A MUNBV effect ($P < 0.01$) was detected with cows having a $1{,}723.5 \pm 527.8$ decrease in total daily mastications per unit increase in MUNBV. Only a stage of lactation by diet effect was detected ($P < 0.01$) for total daily bites, cows in late lactation consuming RG had 28 % fewer total daily bites than cows consuming RGPL in late lactation and RG and RGPL in early lactation. A stage of lactation effect was detected for the total number of chews, cows in late lactation had 36 % fewer chews compared with cows in early lactation. Only a stage of lactation effect was detected for the total number of boli formed per day ($P < 0.01$), cows in late lactation formed 26 % fewer boli than cows in early lactation. A tendency (0.06) was detected for an effect of MUNBV on the number of boli formed per day, with high MUNBV cows forming more boli than low MUNBV cows.

A stage of lactation by diet effect was detected ($P < 0.01$) for total daily mastications per bite, cows consuming RG in late lactation had 311

Table 1
Herbage chemical composition of RG (ryegrass-based swards) and RGPL (RG plus plantain) swards grazed by cows differing in MUNBV (milk urea N breeding value) during stage of lactation (early or late). No significant ($P > 0.05$) interaction terms with MUNBV were detected and therefore are not reported.

Item[1]	Early lactation			Late lactation			P-value		
	RGPL	RG	SE	RGPL	RG	SE	Diet	Lactation	Diet × Stage of lactation
DM, %	13.2[b]	17.2[a]	0.95	15.62[a]	16.2[a]	1.05	≤0.01	0.02	≤0.01
OM, %	90.4	91.1	0.11	89.8	90.9	0.13	≤0.01	≤0.01	0.08
NDF, %	34.9	40.1	0.74	37.5	41.8	0.96	≤0.01	0.01	0.25
ADF, %	22.4	23.2	0.39	23.0	23.7	0.49	0.04	0.32	0.83
CP, %	20.6	21.2	0.47	23.9	24.0	0.65	0.42	0.01	0.62
DMD, %	78.4	79.1	0.47	76.9	77.2	0.55	0.13	0.04	0.61
OMD, %	82.9	83.9	0.57	80.9	81.4	0.67	0.06	0.02	0.64
WSC, %	16.0	17.0	0.70	10.1	11.1	0.44	0.06	≤0.01	0.25
ME, MJ/kg DM	12.2	12.4	0.09	11.8	12	0.10	≤0.01	≤0.01	0.99

[a–b]Mean values in the same row with differing superscripts differ ($P \leq 0.05$) for the interaction between diet and stage of lactation.
[1] DM, Dry Matter; OM, Organic Matter; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; CP, Crude Protein; DMD, Dry Matter Digestibility; OMD, Organic Matter Digestibility; WSC, Water Soluble Carbohydrates; ME, Metabolizable Energy.

Table 2
Daily grazing characteristics for cows of differing milk urea nitrogen breeding values (MUNBV) across a RG (ryegrass-based sward) and RGPL (RG plus plantain) during stage of lactation (early or late). Unless stated otherwise all three-way interactions between stage of lactation, diet and MUNBV were non-significant ($P > 0.05$) and therefore are not reported.

Item[1]	Early			Late			Slope			*P* -Value			
	RGPL	RG	SE	RGPL	RG	SE	MUNBV	SE	*P*-Val	Stage	Diet	Stage x Diet	R^2
Daily Time Budget, min/d													
Grazing	638	594	18.3	638	595	21.7	−14.18	9.94	0.16	0.97	0.05	0.14	0.08
Ruminating	492	490	20.5	355	353	24.3	2.61	11.13	0.82	<0.01	0.92	0.15	0.47
Idle	310	356	23.5	447	492	27.8	11.57	12.75	0.37	<0.01	0.11	0.91	0.39
Events, count/d													
Grazing	7.27	7.16	0.43	8.10	7.98	0.51	−0.30	0.24	0.22	0.12	0.83	0.44	0.04
Ruminating	15.2	14.7	0.82	13.6	13.1	0.97	−0.49	0.45	0.28	0.10	0.60	0.32	0.03
Interbout, min/d													
Grazing	63.7	58.3	4.10	62.4	57.0	4.86	−0.72	2.23	0.75	0.79	0.28	0.35	0.04
Ruminating	15.2	14.7	0.82	13.6	13.1	0.97	−0.49	0.45	0.28	0.10	0.60	0.32	0.03
Oral Processing, count													
Jaw Movements	80,623	74,264	2,556	68,829	62,471	2,669	−758.1	1,314.4	0.60	<0.01	0.03	0.33	0.32
Mastications	7,588^{a}	3,619^{b}	1,109	8,421^{a}	10,280^{a}	1,4798	−1,723.5	527.8	<0.01	0.59	0.01	0.02	0.46
Bites	40,101^{a}	40,925^{a}	1,842	39,341^{a}	28,739^{b}	2486	171.8	891.1	0.85	0.77	0.87	0.01	0.30
Chews	30,463	27,721	1,835	20,056	17,314	2,173	187.9	996.5	0.85	<0.01	0.22	0.15	0.37
Boli	614	655	41.0	472	512	48.7	43.38	22.27	0.06	0.01	0.42	0.21	0.25
Oral Processing, ratios													
Mast./Prehen.	0.21^{b}	0.09^{c}	0.04	0.22^{b}	0.37^{a}	0.05	−0.05	0.02	0.01	0.85	0.02	<0.01	0.46
Chews/Bolus[2]	52.1	41.9	4.24	42.0	37.1	6.08	−4.75	4.10	0.26	0.10	0.08	0.52	0.22

$^{a-c}$Mean values in the same row with differing superscripts differ ($P \leq 0.05$) for the interaction between diet and stage of lactation.
[2] A significant three-way interaction was detected ($P < 0.01$) for stage of lactation x diet x MUNBV, with a reduction in chews/boli of 25.29 for cows grazing a ryegrass diet in late lactation per unit increase in MUNBV.

% more mastications per bite than cows consuming RG in early lactation. Further, the cows consuming RG in late lactation had 72 % more mastications per bite compared with cows consuming RGPL in both stages of lactation. An effect of MUNBV was also detected ($P = 0.01$) with cows having a 0.05 ± 0.02 reduction in the total mastication to bite ratio per unit increase in MUNBV. A three-way interaction term ($P < 0.01$) was detected for chews per bolus between diet, stage of lactation and MUNBV. A 25.29 reduction in the total daily ratio of chews per

Table 3
Grazing and ingestive behaviour of the first and second grazing bouts for cows of differing milk urea nitrogen breeding values (MUNBV) that grazed either a RG (ryegrass-based sward) or RGPL (RG plus plantain) sward during early or late lactation. Unless stated otherwise all four-way interactions between stage of lactation, diet and MUNBV and bout were non-significant ($P > 0.05$) and therefore are not reported.

Item	Early			Late			Slope			*P* -Value				
	RGPL	RG	SE	RGPL	RG	SE	MUNBV	SE	*P*-Val	Bout	Stage	Diet	Stage x Diet	R^2
Grazing Duration, min														
Bout 1	144.2	162.2	15.38	178.5	139.0	19.74	−3.9	6.61	0.56	<0.01	0.08	0.34	0.06	0.31
Bout 2	65.9	83.9	15.38	100.2	60.7	19.74								
Grazing, % total														
Bout 1	24.6	25.1	2.30	26.3	26.7	2.63	0.01	1.08	0.99	<0.01	0.50	0.86	0.15	0.28
Bout 2	12.2	12.6	2.30	13.8	14.2	2.30								
Grazing Interbout time, min														
Bout 1	34.8	56.3	10.35	26.23	47.70	11.82	3.8	4.83	0.44	<0.01	0.78	0.02	0.18	0.21
Bout 2	73.7	95.2	10.35	65.10	86.57	11.82								
Ruminating time, min														
Bout 1	23.3	26.6	6.30	0.52	3.8	7.20	1.9	2.95	0.51	<0.01	<0.01	0.62	0.26	0.27
Bout 2	47.0	50.2	6.30	24.2	27.4	7.20								
Ruminating, % total														
Bout 1	4.5	5.1	1.47	0.5	1.1	1.67	0.2	0.69	0.81	<0.01	0.01	0.69	0.26	0.21
Bout 2	9.9	10.5	1.47	6.0	6.6	1.67								
Bite														
Bout 1	8,428.8^{ab}	10,263.0^{a}	1,076.82	10,721.8^{a}	6,737.5^{b}	1,382.12	1,120.1^{x}	631.17	0.08	<0.01	0.09	0.17	<0.01	0.30
Bout 2	4,148.9^{ab}	5983.1^{a}	1,076.82	6,442.0^{a}	2,457.6^{b}	1,382.12	−879.2^{y}	631.17	0.08					
Bite, % total														
Bout 1	20.9	25.3	2.6	26.7	22.2	3.35	2.7^{x}	1.58	0.07	<0.01	0.08	0.09	0.08	0.26
Bout 2	10.3	14.7	2.6	16.1	11.6	3.35	−2.6^{y}	1.58	0.07					
Mastications														
Bout 1	2,551.8^{a}	1,772.2^{b}	316.93	2,648.9^{a}	3,082.7^{a}	406.79	−444.4	136.30	<0.01	<0.01	0.81	0.05	0.05	0.44
Bout 2	838.6^{a}	59.0^{b}	316.93	935.7^{a}	1,369.5^{a}	406.79								
Mastications, % total														
Bout 1	33.8	37.3	2.65	32.7	36.3	3.01	−0.68	1.11	0.54	<0.01	0.68	0.30	0.20	0.62
Bout 2	11.8	9.1	2.65	10.8	8.1	3.01								
Mastications/Bite														
Bout 1	0.37^{b}	0.20^{c}	0.05	0.32^{bc}	0.55^{a}	0.06	−0.07	0.02	<0.01	<0.01	0.48	<0.01	<0.01	0.41
Bout 2	0.22^{b}	0.06^{c}	0.05	0.18^{bc}	0.41^{a}	0.06								

$^{a-c}$Mean values between rows with differing superscripts are considered different ($P < 0.05$), differing superscripts between columns for MUNBV mean values are considered different ($P < 0.05$).

bolus occurred per unit increase in MUNBV during late lactation for cows grazing the RG sward.

3.2.2. Grazing bout level

Table 3 presents grazing and ruminating durations for the first 2 bouts during a 24 h period. The 1st grazing bout starts at 11:00 and 10:30 h in early and late lactation, respectively and finishes at 13:00 h for both stages of lactation. The 2nd bout starts at 14:00 and 14:30 h for early and late lactation respectively and finishes at 16:00 and 15:30 h for early and late lactation respectively. An effect of bout ($P < 0.01$) was detected for grazing duration, cows spent 50 % less time grazing during the second bout compared with the first. A tendency ($P = 0.06$) was detected for a stage of lactation by diet interaction, with cows spending a longer time grazing RGPL in late lactation in both bouts. Only an effect of bout was detected ($P < 0.01$) for the duration of time spent grazing as a percentage of total daily time, cows spent 49 % less of the total daily time grazing in the second bout compared with the first bout. An effect of bout ($P < 0.01$) was also detected for grazing inter-bout time, cows increased the interbout time by 94 % between bouts 2 and 3 compared with bouts 1 and 2. An effect of diet was also detected ($P = 0.02$) for inter-bout time, cows had a 30 % reduction when consuming RG compared with RGPL.

A main effect of bout and stage of lactation was detected for ruminating time. Cows spent 174 % more time ruminating during the second bout compared with the first bout and spent 62 % less time ruminating in late lactation compared with early lactation. An effect of bout and stage of lactation ($P \leq 0.01$) was also detected for rumination time as a percentage of total daily rumination. Cows spent 195 % more of the total daily ruminating time in the second bout compared with the first and 53 % less time in late lactation compared with early lactation.

Table 3 presents the ingestive behaviours for the first and second grazing bouts during a 24 h period. An effect of bout ($P < 0.01$) as well as a stage of lactation by diet interaction ($P < 0.01$) were detected for bite count; cows had 47 % less bites during the second bout compared with the first bout. Cows consuming RG in early lactation and RGPL in both stages of lactation had 97 % more bites compared to cows consuming RG in late lactation. A bout by MUNBV interaction was also detected ($P < 0.05$) for bites, a one-unit increase in MUNBV resulted in a 1,201.1 $\pm$ 631.17 increase in bites during the first bout, but an 879.2 $\pm$ 631.17 reduction in the second bout. A bout effect was detected for the number of bites per bout as a percentage of total daily bites. Cows had 13 % fewer bites as a percentage of total daily bites in the second bout compared with the first, a tendency was detected for a stage by diet interaction ($P = 0.08$) with cows in late lactation on RG having lower values. An effect of MUNBV and bout was also detected ($P < 0.05$) for the number of bites per bout as a percentage of total daily bites with a one-unit increase in MUNBV resulting in a 2.7 $\pm$ 1.58 % increase during the first bout, and a 2.6 $\pm$ 1.58 % decrease during the second bout.

A bout ($P < 0.01$) and a stage of lactation by diet ($P \leq 0.05$) interaction was detected for the number of mastications. Cows had 68 % fewer mastications during the first bout compared with the second. Whilst cows consuming RG in early lactation had 52 % fewer mastications compared with cows consuming RGPL in early lactation and both diets in late lactation. An effect of MUNBV ($P < 0.01$) was detected for mastications, cows had 444.4 $\pm$ 136.3 fewer mastications per unit increase in MUNBV. Only an effect of bout ($P < 0.01$) was detected for mastications as a percentage of total daily mastications, cows had 72 % fewer mastications during the second bout compared with the first. An effect of both bout and stage of lactation by diet ($P < 0.01$) was detected for mastications per bite, cows had 39 % less mastication per bite in the second bout compared to the first. Cows grazing RG in early lactation had 73 % fewer mastications per bites compared, with cows grazing RG in late lactation and 56 % less than RGPL cows in early lactation. A MUNBV effect was also detected ($P < 0.01$) for the mastications per bite with the ratio decreasing 0.07 $\pm$ 0.02 per unit increase in MUNBV.

Table 4 presents the ingestive and rumination behaviour split into periods of the day. A stage of lactation by the period of day interaction was detected ($P < 0.01$) for the proportion of time spent grazing during a 24 h period. Cows in both early and late lactation spent the largest proportion of the 24 h period grazing during the ‘day’ period, which increased 17 % from early lactation to late lactation. For early lactation, the next largest period of time spent grazing was in the ‘evening’, which was 103 % greater than in late lactation. The second-largest period of time spent grazing for late lactation occurred during the ‘night’ period, which was 274 % greater than early lactation. Cows in early lactation spent 94 % more time grazing during the ‘morning’ period than cows during late lactation. A MUNBV by stage of lactation by the time of day interaction ($P = 0.04$) was detected for grazing proportion; during the ‘day’ a one-unit increase in MUNBV increased the proportion of time spent grazing by 3.08 $\pm$ 1.33 %.

A stage of lactation by period of day interaction ($P < 0.01$) was also detected for the proportion of time spent idle during a 24 h period. The largest proportion of time spent idle was over the ‘night’ period in both early and late lactation, with cows in late lactation spending 33 % more time idle than in early lactation. The second-largest proportion of time spent idle was during the ‘day’ period, which was 27 % and 45 % less than the time spent idle during the ‘night’ for early and late lactation respectively. No difference was detected for time spent idle during the ‘morning’ for the stage of lactation, or between ‘morning’ and ‘day’ during late lactation. The least amount of time idle was during the evening for both stages of lactation, with cows in late lactation spending 70 % less time idle than cows in early lactation. A three-way interaction ($P = 0.02$) between MUNBV, stage of lactation and period of the day was also detected for the proportion of time spent idle with animals in early lactation increasing the proportion of time idle by 2.8 % $\pm$ 1.55 during the ‘day’ and 3.47 % $\pm$1.55 less time during the ‘night’ per unit increase in MUNBV.

A stage of lactation by period of day interaction ($P < 0.01$) was also detected for the proportion of bites in a 24 h period. The greatest proportion of bites was during the ‘day’, with cows in late lactation having 15 % more than cows in early lactation. Cows in early lactation had the second largest proportion of bites during the ‘evening’; comparatively, late lactation cows did not differ in the proportion of bites they had between ‘evening’ and ‘night’. Early lactation cows had the lowest proportion of bites during the ‘night’, which was 74 % less than in late lactation. Late lactation had the lowest proportion of bites during the ‘morning’, which was 48 % less than in early lactation. A three-way interaction was detected ($P < 0.01$) between MUNBV, stage of lactation and period of the day with a one-unit increase in MUNBV resulting in a 4.88 $\pm$ 1.46 increase in the proportion of bites during the ‘day’ period.

A stage of lactation by the period of day effect was detected ($P < 0.01$) for the proportion of mastications in a 24 h period. Cows in both stages of lactation had the greatest proportion of mastications in the ‘day’ period, with cows in late lactation having 9 % more than cows in early lactation. Cows in early lactation had a 68 % lower proportion of mastications during the ‘night’ compared with late lactation cows, whilst cows in late lactation had a 41 % decrease in the proportion of mastications in the ‘morning’ compared with cows in early lactation.

A stage of lactation by diet effect was detected ($P < 0.01$) for the mastications to bite ratio in a 24 h period. Cows on the RG diet in late lactation had the greatest proportion of mastications per bite, 76 % and 319 % greater than cows on RGPL in both stages of lactation and cows on RG in early lactation respectively. An effect of period of the day was also detected ($P < 0.01$), with cows having 56 % more mastications per bite during the ‘day’ compared with any other time period. A consistent effect of MUNBV was detected ($P < 0.01$), with cows decreasing the number of mastications per bite by 0.04 $\pm$ 0.02 per unit increase in MUNBV across all time periods, stages of lactation and diet types.

A stage of lactation by period of day effect ($P < 0.01$) was detected for the proportion of time spent ruminating during a 24 h period. Cows in both stages of lactation spent the greatest proportion of time

Table 4
Proportion of time spent by cows of differing milk urea nitrogen breeding values (MUNBV) across a RG (ryegrass-based sward) or RGPL (RG plus plantain) sward during either early or late lactation exhibiting ingestive and rumination behaviour over different time periods during a 24 h period.

Item	Early Lactation			Late Lactation				MUNBV Slope							R^2
								Early			Late				
	RGPL	RG	SE	RGPL	RG	SE		RGPL	RG	SE	RGPL	RG	SE		
Ingestive Behaviour															
Grazing proportion															
Morning	13.73^{e}	13.73^{e}	1.34	7.06^{f}	7.06^{f}	1.62		0.16^{AB}	0.16^{AB}	1.33	−0.87^{A}	−0.87^{A}	1.33		0.91
Day	47.60^{b}	47.60^{b}	1.34	55.71^{a}	55.71^{a}	1.62		−1.42^{B}	−1.42^{B}	1.33	3.08^{A*}	3.08^{A*}	1.33		0.91
Evening	33.07^{c}	33.07^{c}	1.34	16.27^{e}	16.27^{e}	1.62		1.03^{AB}	1.03^{AB}	1.33	−0.44^{AB}	−0.44^{AB}	1.33		0.91
Night	5.61^{f}	5.61^{f}	1.34	20.96^{d}	20.96^{d}	1.62		0.23^{AB}	0.23^{AB}	1.33	−1.77^{A}	−1.77^{A}	1.33		0.91
Idle proportion															
Morning	23.08^{d}	23.08^{d}	1.45	21.52^{d}	21.52^{d}	1.74		0.67^{AB}	0.67^{AB}	1.55	0.19^{ABC}	0.19^{ABC}	1.55		0.78
Day	27.7^{c}	27.7^{c}	1.45	26.08^{cd}	26.08^{cd}	1.74		2.83^{A*}	2.83^{A*}	1.55	−1.68^{BC}	−1.68^{BC}	1.55		0.78
Evening	12.35^{e}	12.35^{e}	1.45	3.74^{f}	3.74^{f}	1.74		−0.03^{ABC}	−0.03^{ABC}	1.55	0.24^{ABC}	0.24^{ABC}	1.55		0.78
Night	36.87^{b}	36.87^{b}	1.45	48.86^{a}	48.86^{a}	1.74		−3.47^{C*}	−3.47^{C*}	1.55	1.23^{AB}	1.23^{AB}	1.55		0.78
Bite proportion															
Morning	13.16^{e}	13.16^{e}	1.46	6.83^{f}	6.83^{f}	1.77		0.25^{B}	0.25^{B}	1.46	−1.32^{B}	−1.32^{B}	1.46		0.88
Day	45.77^{b}	45.77^{b}	1.46	52.71^{a}	52.71^{a}	1.77		−1.35^{B}	−1.35^{B}	1.46	4.88^{B*}	4.88^{B*}	1.46		0.88
Evening	35.45^{c}	35.45^{c}	1.46	18.55^{d}	18.55^{d}	1.77		0.88^{B}	0.88^{B}	1.46	−1.35^{B}	−1.35^{B}	1.46		0.88
Night	5.63^{f}	5.63^{f}	1.46	21.91^{d}	21.91^{d}	1.77		0.23^{B}	0.23^{B}	1.46	−2.21^{A}	−2.21^{A}	1.46		0.88
Mastication proportion															
Morning	11.11^{de}	11.11^{de}	1.72	6.54^{ef}	6.54^{ef}	2.08		0.07	0.07	1.72	−0.27	−0.27	1.72		0.90
Day	58.85^{b}	58.85^{b}	1.72	64.27^{a}	64.27^{a}	2.08		−0.71	−0.71	1.72	−0.26	−0.26	1.72		0.90
Evening	24.99^{c}	24.99^{c}	1.72	13.48^{d}	13.48^{d}	2.08		−0.27	−0.27	1.72	0.45	0.45	1.72		0.90
Night	5.04^{f}	5.04^{f}	1.72	15.72^{d}	15.72^{d}	2.08		0.90	0.90	1.72	0.08	0.08	1.72		0.90
Mastication per bite															
Morning	0.20^{b}	0.09^{c}	0.03	0.20^{b}	0.34^{a}	0.04		−0.04*	−0.04*	0.01	−0.04*	−0.04*	0.01		0.32
Day	0.27^{b}	0.16^{c}	0.03	0.27^{b}	0.41^{a}	0.04		−0.04*	−0.04*	0.01	−0.04*	−0.04*	0.01		0.32
Evening	0.15^{b}	0.04^{c}	0.03	0.15^{b}	0.29^{a}	0.04		−0.04*	−0.04*	0.01	−0.04*	−0.04*	0.01		0.32
Night	0.15^{b}	0.04^{c}	0.03	0.15^{b}	0.30^{a}	0.04		−0.04*	−0.04*	0.01	−0.04*	−0.04*	0.01		0.32
Rumination behaviour															
Rumination proportion															
Morning	15.38^{d}	15.38^{d}	1.17	8.40^{e}	8.40^{e}	1.42		−0.67	−0.67	1.01	−0.04	−0.04	1.17		0.96
Day	19.70^{c}	19.70^{c}	1.17	7.84^{e}	7.84^{e}	1.42		−0.18	−0.18	1.01	−1.56	−1.56	1.17		0.96
Evening	12.52^{d}	12.52^{d}	1.17	1.76^{f}	1.76^{f}	1.42		−0.28	−0.28	1.01	1.09	1.09	1.17		0.96
Night	54.40^{b}	54.40^{b}	1.17	82.00^{a}	82.00^{a}	1.42		1.15	1.15	1.01	0.50	0.50	1.17		0.96
Chew proportion															
Morning	15.37^{d}	15.37^{d}	1.18	8.05^{e}	8.05^{e}	1.43		−0.65	−0.65	1.02	−0.17	−0.17	1.18		0.96
Day	19.20^{c}	19.20^{c}	1.18	7.43^{e}	7.43^{e}	1.43		−0.29	−0.29	1.02	−1.73	−1.73	1.18		0.96
Evening	12.38^{d}	12.38^{d}	1.18	1.68^{f}	1.68^{f}	1.43		−0.18	−0.18	1.02	1.07	1.07	1.18		0.96
Night	53.05^{b}	53.05^{b}	1.18	82.85^{a}	82.85^{a}	1.43		1.12	1.12	1.02	0.84	0.84	1.18		0.96
Boli proportion															
Morning	14.88^{d}	14.88^{d}	1.19	9.40^{e}	9.40^{e}	1.58		0.36	−1.80	1.56	−0.56	2.53	2.96		0.95
Day	21.04^{c}	21.04^{c}	1.19	9.81^{e}	9.81^{e}	1.58		0.24	−0.47	1.56	−2.59	2.76	2.96		0.95
Evening	14.06^{d}	14.06^{d}	1.19	1.64^{f}	1.64^{f}	1.58		−1.51	0.07	1.56	1.60	−0.43	2.96		0.95
Night	50.03^{b}	50.03^{b}	1.19	79.15^{a}	79.15^{a}	1.58		0.91	2.20	1.56	1.55	−4.86	2.96		0.95
Chews per boli															
Morning	50.1^{a}	45.5^{a}	1.90	38.7^{b}	35.2^{b}	3.22		−5.77^{B*}	−0.40^{A}	1.79	−3.05^{AB}	−25.50^{D*}	3.71		0.36
Day	50.1^{a}	45.5^{a}	1.90	38.7^{b}	35.2^{b}	3.22		−5.77^{B*}	−0.40^{A}	1.79	−3.05^{AB}	−25.50^{D*}	3.71		0.36
Evening	50.1^{a}	45.5^{a}	1.90	38.7^{b}	35.2^{b}	3.22		−5.77^{B*}	−0.40^{A}	1.79	−3.05^{AB}	−25.50^{D*}	3.71		0.36
Night	50.1^{a}	45.5^{a}	1.90	38.7^{b}	35.2^{b}	3.22		−5.77^{B*}	−0.40^{A}	1.79	−3.05^{AB}	−25.50^{D*}	3.71		0.36
P - values															
	M	S	P	D	M:S	M:P	S:P	M:D	S:D	P:D	M:S:P	M:S:D	M:P:D	S:P:D	M:S:P:D
Ingestive behaviour															
Grazing	1.00	1.00	<0.01	1.00	1.00	0.39	<0.01	1.00	1.00	0.19	0.04	1.00	0.68	0.40	0.33
Idle	1.00	1.00	<0.01	1.00	1.00	0.21	<0.01	1.00	1.00	0.08	0.02	1.00	0.23	0.21	0.54
Bite	1.00	1.00	<0.01	1.00	1.00	0.30	<0.01	1.00	1.00	0.10	<0.01	1.00	0.61	0.75	0.21
Mastications	1.00	1.00	<0.01	1.00	1.00	0.90	<0.01	1.00	1.00	0.60	0.96	1.00	0.98	0.78	0.80
Mastications /Bite	<0.01	<0.01	<0.01	0.58	0.72	0.36	0.24	0.84	<0.01	0.96	0.46	0.56	0.60	0.46	0.36
Rumination behaviour															
Rumination	1.00	1.00	<0.01	1.00	1.00	0.77	<0.01	1.00	1.00	0.41	0.59	1.00	0.47	0.38	0.11
Chews	1.00	1.00	<0.01	1.00	1.00	0.71	<0.01	1.00	1.00	0.48	0.66	1.00	0.31	0.34	0.24
Boli	1.00	1.00	<0.01	1.00	1.00	0.89	<0.01	1.00	1.00	0.31	0.37	1.00	0.84	0.54	0.04
Chew/Boli	<0.01	<0.01	0.06	0.93	0.02	0.79	0.98	0.78	0.07	0.98	0.93	<0.01	0.85	0.37	0.74

$^{a-f}$ Differing superscript between columns and rows per item indicate a difference ($P < 0.05$), an * next to a superscript in MUNBV slope indicates the value does not include 0 in its confidence interval.
M, MUNBV; S, Stage of lactation; P, Period of the day; D, Diet.

ruminating during the 'night', with cows in late lactation spending 51 % more time ruminating compared with cows in early lactation. The next largest proportion of rumination occurred during the 'day' period, with cows in late lactation spending 19 % less time ruminating compared with early lactation, whilst cows in early lactation had an 83 % greater proportion of rumination in the 'morning' compared with late lactation cows.

A stage of lactation and period of day ($P < 0.01$) effect was also detected for the proportions of chews during a 24 h period. The greatest proportion of chews occurred during the 'night' for both stages of lactation with cows in late lactation having 56 % more chews than in early lactation. The second-largest proportion of chews occurred during the 'day' for early lactation and the 'morning' period for late lactation. Cows had 61 % fewer chews during the 'day' in late lactation compared with early lactation and 48 % fewer chews during the 'morning' in late lactation compared with early lactation. The 'evening' period had the lowest proportion of chews for both late and early lactation, with cows in late lactation having 86 % fewer chews as a proportion of the total chews in a 24 h period.

A stage of lactation by period of day effect ($P < 0.01$) was detected for the proportion of boli during a 24 h period. Cows regurgitated the largest proportion of boli during the 'night' period during both stages of lactation, with cows in late lactation having a 58 % greater proportion of boli as a total daily boli compared with cows in early lactation. During the 'day' period, animals during early lactation had a 58 % reduction in the proportion of boli formed compared with the 'night' period; cows in late lactation had a 91 % reduction during this same period. Cows during late lactation had a 61 % decrease in the proportion of boli formed during the 'day' relative to the proportion formed during early lactation. The proportion of boli formed in early lactation during the periods of 'morning' and 'evening' did not differ and were 31 % lower than in the 'day' period; cows in late lactation had a 35 % and 89 % reduction in the proportion of boli formed during the 'morning' and 'evening' respectively compared with early lactation.

An effect of stage of lactation ($P < 0.01$) was detected for the proportion of chews per bolus during a 24 h period. The proportion of chews per bolus decreased 27 % from early lactation to late lactation for all periods of the 24 h period. An interaction between MUNBV, stage of lactation and diet was also detected ($P < 0.01$). Cows during early lactation on RGPL had a 5.77 ± 1.79 reduction in the proportion of chews per bolus per unit increase in MUNBV, in late lactation animals had a 25.50 ± 3.71 reduction per unit increase in MUNBV whilst grazing the RG diet.

Fig. 1 presents the diurnal bites taken from cows divergent for MUNBV across both early and late lactation for both the RG and RGPL diet. An interaction term was detected for MUNBV by diet at hours 18:00 and 19:00. At 18:00 h, for a one-unit increase in MUNBV animals on RG had a 720.62 ± 286 increase in the number of bites taken, whilst cows on RGPL had 439.45 ± 222 fewer bites. At 19:00 h, only an effect from RG and MUNBV was detected with animals having a 580.44 ± 286 increase in the number of bites taken. This relationship was consistent across the stage of lactation.

Fig. 2 presents the diurnal pattern of the number of mastications to bites. A consistent effect was detected from MUNBV across both diet and stages of lactation. A one-unit increase in MUNBV resulted in a 0.12 ± 0.02 (10:00 h), 0.10 ± 0.02 (11:00 & 12:00 h) and 0.06 ± 0.02 (13:00 h) reduction in the number of mastications per bite.

Fig. 3 presents the diurnal pattern of chews per bolus. An effect of MUNBV, stage of lactation and diet was detected throughout the 24 h period. In early lactation at 07:00 h on RG, a one-unit increase in MUNBV reduced the ratio of chews per bolus by 17.17 ± 6.66. At 09:00 h, a one-unit increase in MUNBV resulted in a 22.56 ± 6.79 reduction in chews per bolus for cows on RGPL. Cows on RGPL also had a 23.02 ± 6.79, 13.50 ± 6.79, 14.37 ± 6.79 and 14.09 ± 6.79 reduction in chews per bolus per unit increase in MUNBV during 13:00, 16:00, 21:00 and 22:00 h respectively. Cows offered RG then had a 15.07 ± 6.66 reduction in the ratio of chews per bolus at 23:00 h during early lactation. In late lactation, cows on RG for every one-unit increase in MUNBV had a 65.10 ± 12.66, 84.22 ± 12.66, 37.48 ± 12.66, 29.33 ± 12.66 and 41.53 ± 12.66 reduction in the ratio of chews per bolus at 01:00, 02:00, 06:00, 08:00 and 16:00 h respectively. Cows on RGPL had a 41.53 ± 6.18 reduction in the ratio of chews per bolus at

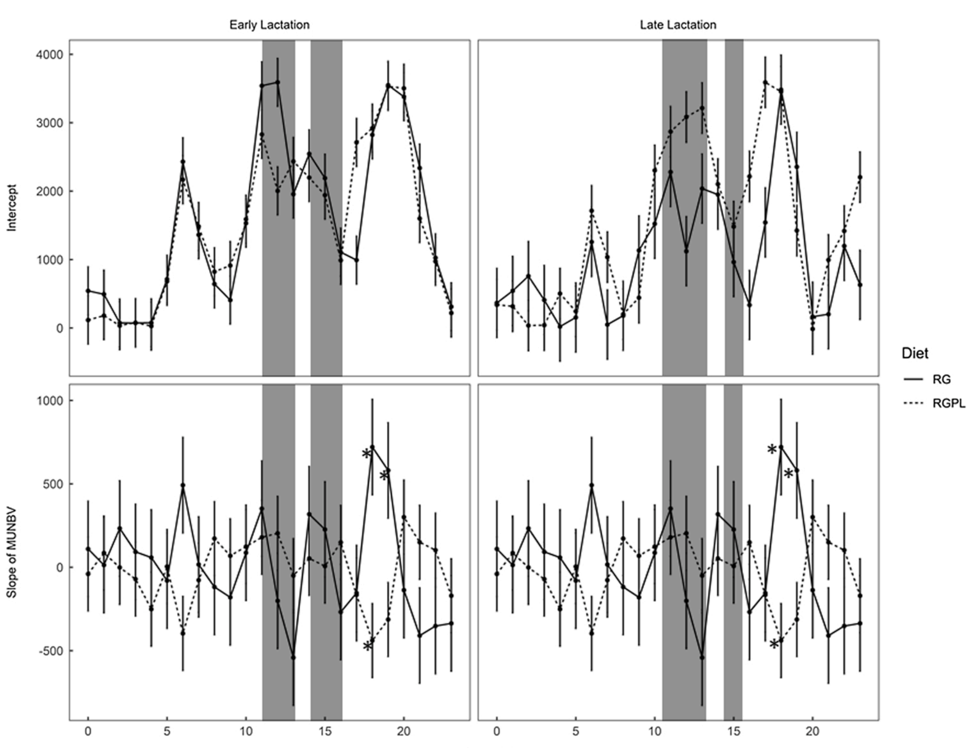

Fig. 1. The change in bites (count) taken throughout a 24 h period of cows fitted with jaw movement recorders which are divergent for milk urea N breeding value (MUNBV) grazing a ryegrass white clover (RG) or ryegrass white clover and plantain (RGPL) sward across both early and late lactation. The top graph presents the average bites taken by cows, the bottom graph presents the effect of a one unit increase in MUNBV and how it would shift the average value. The shaded area represents both the first and second grazing bouts taken during the measurement period. Points marked with * are considered statistically significant from 0.

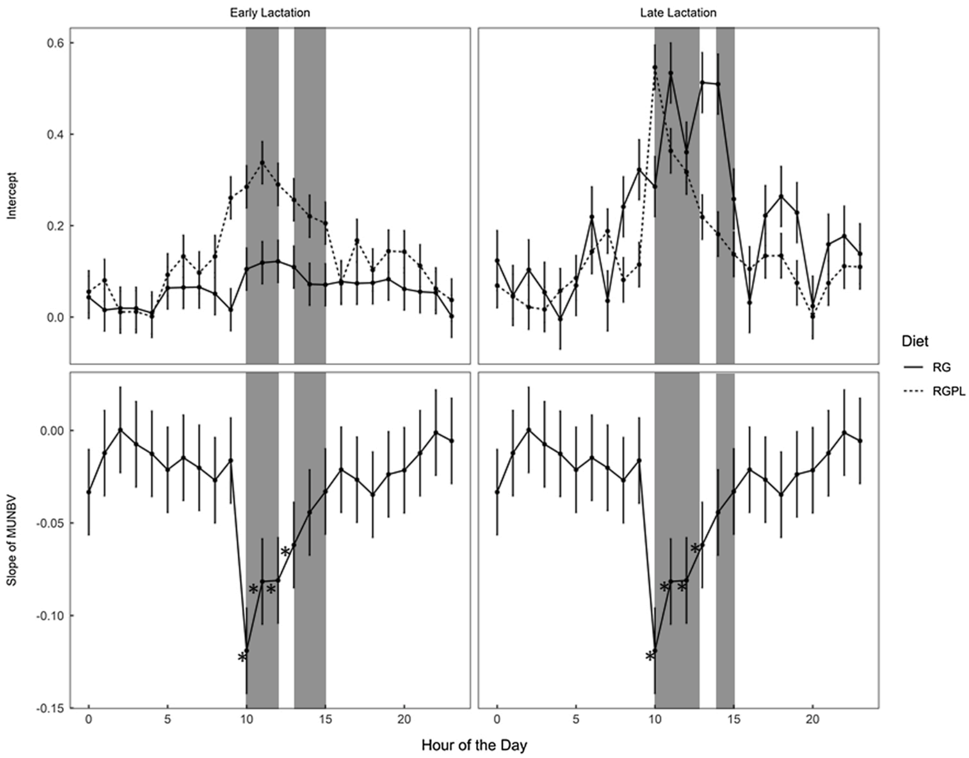

Fig. 2. The change in mastications to bites (ratio) throughout a 24 h period of cows fitted with jaw movement recorders which are divergent for milk urea N breeding value (MUNBV) grazing a ryegrass white clover (RG) or ryegrass white clover and plantain (RGPL) sward across both early and late lactation. The top graph presents the average mastication to bites ratio, the bottom graph presents the effect of a one unit increase in MUNBV and how it would shift the average value. The shaded area represents both the first and second grazing bouts taken during the measurement period. Points marked with * are considered statistically significant from 0.

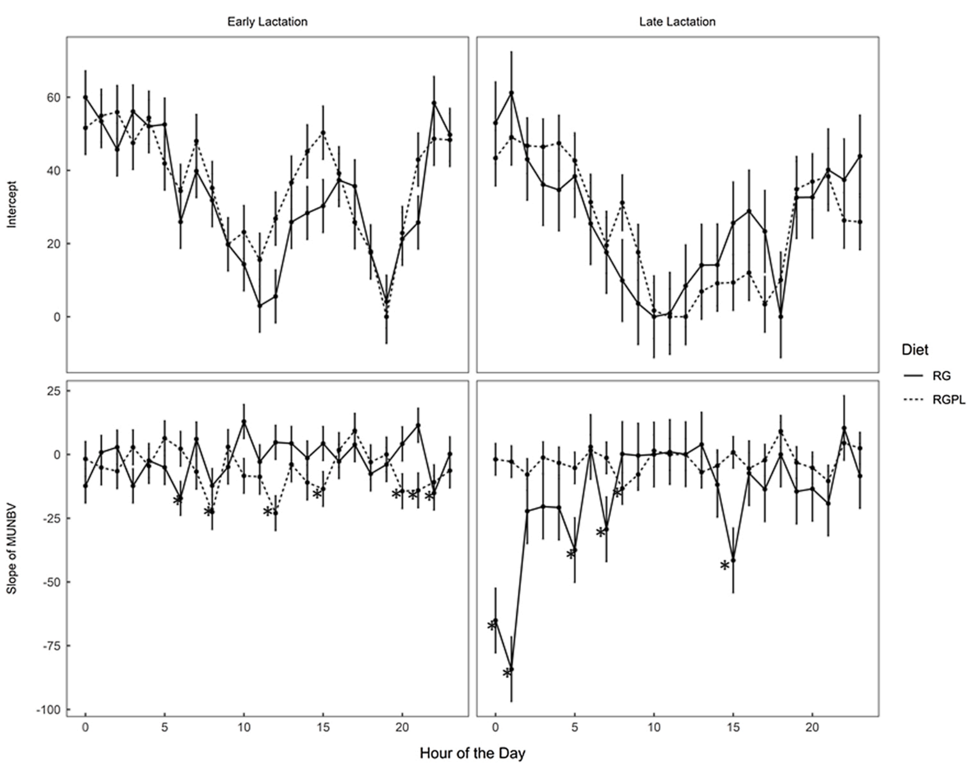

Fig. 3. The change in chews per bolus (ratio) throughout a 24 h period of cows fitted with jaw movement recorders which are divergent for milk urea N breeding value (MUNBV) grazing a ryegrass white clover (RG) or ryegrass white clover and plantain (RGPL) sward across both early and late lactation. The top graph presents the average chews per bolus ratio, the bottom graph presents the effect of a one unit increase in MUNBV and how it would shift the average value. Points marked with * are considered statistically significant from 0.

16:00 h in late lactation.

4. Discussion

The objective of this experiment was to describe the diurnal grazing pattern and assess differences in oral processing (mastication and chewing) behaviour of dairy cows divergent for MUNBV at different stages of lactation grazing different swards. Cows divergent for MUNBV displayed consistent differences in grazing pattern and oral processing behaviour across both stages of lactation and diet type. The effects of stage of lactation from an animal physiological perspective and its relationships with sward characteristics and diet type on diurnal grazing pattern and ingestive behaviours have been well documented and described (Gibb et al., 1998; Gregorini et al., 2009; Gregorini, 2012; Larson-Praplan et al., 2015). Therefore, we focus the discussion of the present results on the effect of genetics, i.e. MUNBV, on grazing pattern and oral processing of ingesta and digesta as potential explanatory variables for the previously reported differences in phenotype based on MUNBV.

A consistent relationship was documented between MUNBV and mastications, with low MUNBV cows having more mastications and more mastications per bite compared with high MUNBV cows. A similar relationship was reported in Gregorini et al. (2015) where the number of mastications was found to be different based on the animal's genetics. Importantly, the relationship found in this study is most highly defined during the first grazing bout, as illustrated in Fig. 1. The first grazing bout represents cows being released into a new and fresh pasture break after morning milking. Several studies have reported that the first bout has the greatest herbage dry matter intake and defines the intensity of the following bouts and ultimately the daily level of herbage intake (Gregorini et al., 2009). The first bout can therefore be considered as one of the most important grazing bouts of the day in a pasture-based temperate dairy production system. This is due to the cows: 1) grazing a fresh and ungrazed, excretion free pasture break; 2), cows have spent the previous night ruminating and evacuating their rumen, and 3) they have been in the dairy shed off feed; the culmination of 2 and 3 is likely to be an elevated level of hunger. Hungry animals have been reported to have increased intake rates (Baile and McLaughlin., 1987; Allen, 2014).

In this study, we use bite counts/rate as a proxy for ingestion rate, assuming a constant bite mass. The greatest herbage dry matter intake occurred during the first grazing bout of the morning and the one just before dusk, which are both periods of time known to induce intense periods of grazing in ruminants (Gregorini, 2012). The difference in mastications per bite during one of the most important grazing bouts of the day (Table 3) highlights a significant difference in the approach that dairy cows divergent in MUNBV have to grazing, i.e. the acquisition of nutrients. The lower level of mastication from high MUNBV cows can be seen on one hand, as a grazing efficiency, potentially resulting in greater intake rates by reducing the mastication to bite ratio, thus having more herbage harvesting bites per oral processing bite (Laca et al., 1994). On the other hand, minimization of oral processing can lead to longer rumen retention times and therefore facilitating non-glucogenic and methanogenic fermentation, which have negative environmental impacts (Gregorini et al., 2017; Ungerfeld, 2020). Conversely, a potentially steadier bite rate — ingestion rate — and more mastications from low MUNBV cows at the bite and meal level may have reduced the particle size of the ingesta and increased salivary secretion and flow to the rumen. Steadier swallowing of boli with smaller particle size distribution would lead to a greater surface area for microbial degradation, enhancing rumen glucogenic fermentation patterns and accelerating passage of digesta through the rumen and flow of microbial protein to the duodenum. Marshall et al. (2021) reported greater microbial protein flow for low MUNBV cows compared with high MUNBV cows. The greater number of mastications from low MUNBV at both the bite and meal level seen in this study may help explain results relating to microbial crude protein flow observed from animals divergent for MUNBV reported previously (Marshall et al., 2021). More controlled 'cause and effect' studies into rumen fermentation patterns and digesta outflow from cattle divergent for MUNBV are required to test this premise.

At the same herbage allocation, the MUNBV difference in bite parameters during the first two grazing bouts of the day may suggest that MUNBV divergent cows perceive and conform a different grazing environment, which may have implications for strategic grazing management. Several studies have indicated similar results where the genetics and breed of the animal have influenced the animals grazing behaviour and therefore perception of the environment (Dumont et al., 2007; Sheahan et al., 2011; Pierce et al., 2018).

Greater levels of herbage intake rate during the first grazing bout (Table 3), as a product of low oral processing (Laca et al., 1994) may result in longer rumen retention time (Gregorini et al., 2017) from high MUNBV cows, negatively affecting intake at subsequent grazing bouts (e.g. bout 2) and consequently reducing daily herbage intake. There was a reduction of chews per rumination bolus with the increase of MUNBV across all periods of the day. In other words, as MUNBV increased, cows displayed fewer chews per rumination bolus. Cows of high MUNBV may actively be compensating for lower ingestive oral processing by increasing the number of boli at the same total rumination time. An alternative explanation is that high MUNBV animals may be pseudo ruminating. Pseudo rumination is described as short and less intensive chewing per rumination bolus and is indicative of fewer chews per boli (Okamoto, 2000). Pseudo rumination is known to increase with poorly masticated forages resulting in longer flexible particle size of ingesta which constrain effective regurgitation and rumination boli formation (Deswysen and Ehrlein., 1979). This may be occurring in high MUNBV cows with reduced ingestive oral processing. The strong tendency for more boli formation by high MUNBV cows also supports our argument. Due to a potentially less masticated and interwoven rumen mat of large particles, high MUNBV cows may be unable to regurgitate effectively and instead regurgitate smaller boli more often. As rumination is one of the key processes of digestion, further study is required to investigate bolus formation characteristics and pseudo rumination events in cattle divergent for MUNBV.

If oral processing is a key influencer in the observed phenotypical differences in cows divergent for MUNBV then plant morphology may explain the documented MUNBV by diet interactions seen previously (Marshall et al., 2021). A study conducted by Gregorini et al. (2013) demonstrated an increase in mastication efficiency from grazing cows consuming plantain compared to ryegrass. This relationship might explain why the observed effects of MUNBV documented previously on ryegrass diets were not observed in Marshall et al. (2021) when cows were given a whole diet of plantain. Potentially the increased efficiency of oral processing of a low MUNBV cow is comparable to the less efficient high MUNBV cow on a diet that is easily masticated, thus resulting in comparable particle sizes of ingesta flowing into the rumen. The lack of dietary interaction in Marshall et al. (2020) may simply be a result of the low prevalence of plantain in the diet (<30 %) which may not have been sufficient to elicit a difference in mastication efficiency compared to a ryegrass diet based on MUNBV.

If increased oral efficiency from low MUNBV cows is the mechanism behind the environmental and production benefits associated with low MUNBV cows, it could be hypothesized that these effects may become more pronounced on diets requiring more oral processing. Further studies should be conducted to test the interaction between MUNBV and diets that induce different oral processing, with a particular reference to mastications and boli formation dynamics.

5. Conclusion

During this study cows divergent for MUNBV exhibited different grazing patterns and oral processing of ingesta and digesta through differentiated mastication and chewing rates, respectively. A greater number of mastications and chews in low MUNBV animals may result in a steadier inflow of more fermentable ingesta and digesta to the rumen, respectively. This in turn may add to and help explain differential rumen function and nutrient supply to the host animal, which could help elucidate different observations in phenotypes previously reported for grazing dairy cows divergent for MUNBV.

Declaration of Competing Interest

None.

Acknowledgements

The authors acknowledge and thank the Lincoln University Doctoral Scholarship for providing funding for this project and the first author's overall thesis programme. The authors also thank the Lincoln University Open Access Fund for helping partially fund the publication cost of this article.

References

Allen, M.S., 2014. Drives and limits to feed intake in ruminants. Anim. Prod. Sci. 54, 1513–1524. https://doi.org/10.1071/AN14478.

Baile, C.A., McLaughlin, C.L., 1987. Mechanisms controlling feed intake in ruminants: a review. J. Anim. 64, 915–922. https://doi.org/10.2527/jas1987.643915x.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 1–48.

Deswysen, A., Ehrlein, H.J., 1979. Radiography of intake and (pseudo) rumination behaviour. Ann. Rech. Vet. 10, 208–210.

Dufreneix, F., Faverdin, P., Peyraud, J.-L., 2019. Influence of particle size and density on mean retention time in the rumen of dairy cows. J. Dairy Sci. 102, 3010–3022. https://doi.org/10.3168/jds.2018-15926.

Dumont, B., Rook, A.J., Coran, C., Röver, K.-U., 2007. Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. Grass Forage Sci. 62, 159–171. https://doi.org/10.1111/j.1365-2494.2007.00571.x.

Fleming, A.E., Garrett, K., Froehlich, K., Beck, M.R., Mangwe, M.C., Bryant, R.H., Edwards, G., Gregorini, P., 2021. Rumen function and grazing behavior of early-lactation dairy cows supplemented with fodder beet. J. Dairy Sci. 104, 7696–7710. https://doi.org/10.3168/jds.2020-19324. Available from: https://doi.org/10.3168/jds.2020-19324.

Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third. Sage, Thousand Oaks.

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass Forage Sci. 53, 41–46. https://doi.org/10.1046/j.1365-2494.1998.00102.x.

Gregorini, P., 2012. Diurnal grazing pattern: its physiological basis and strategic management. Anim. Prod. Sci. 52, 416. https://doi.org/10.1071/an11250.

Gregorini, P., Clark, C.E.F., Jago, J.G., Glassey, C.B., Mcleod, K.L.M., Romera, A.J., 2009. Restricting time at pasture: effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. J. Dairy Sci. 92, 4572–4580. https://doi.org/10.3168/jds.2009-2322. Available from: https://doi.org/10.3168/jds.2009-2322.

Gregorini, P., Minnee, E.M.K., Griffiths, W., Lee, J.M., 2013. Dairy cows increase ingestive mastication and reduce ruminative chewing when grazing chicory and plantain. J. Dairy Sci. 96, 7798–7805. https://doi.org/10.3168/jds.2013-6953. Available from: https://doi.org/10.3168/jds.2013-6953.

Gregorini, P., Waghorn, G.C., Kuhn-Sherlock, B., Romera, A.J., Macdonald, K.A., 2015. Short communication: grazing pattern of dairy cows that were selected for divergent residual feed intake as calves. J. Dairy Sci. 98, 6486–6491. https://doi.org/10.3168/jds.2015-9614. Available from: https://doi.org/10.3168/jds.2015-9614.

Gregorini, P., Villalba, J., Chilibroste, P., Provenza, F., 2017. Grazing management: setting the table, designing the menu and influencing the diner. Anim. Prod. Sci. 57, 1248. https://doi.org/10.1071/an16637.

Laca, E.A., Ungar, E.D., Demment, M.W., 1994. Mechanisms of handling time and intake rate of a large mammalian grazer. Appl. Anim. Behav. Sci. 39, 3–19. https://doi.org/10.1016/0168-1591(94)90011-6.

Larson-Praplan, S., George, M.R., Buckhouse, J.C., Laca, E.A., 2015. Spatial and temporal domains of scale of grazing cattle. Anim. Prod. Sci. 55, 284–297. https://doi.org/10.1071/AN14641.

Lenth, R., 2019. Emmeans: Estimated Marginal Means, Aka Least-squares Means. R Package Version 1.4.1. Available from: https://cran.r-project.org/package=emmeans.

Marshall, C.J., Beck, M.R., Garrett, K., Barrell, G.K., Al-Marashdeh, O., Gregorini, P., 2020. Grazing dairy cows with low milk urea nitrogen breeding values excrete less urinary urea nitrogen. Sci. Total Environ. 739, 1–8. https://doi.org/10.1016/j.scitotenv.2020.139994. Available from: https://doi.org/10.1016/j.scitotenv.2020.139994.

Marshall, C.J., Beck, M.R., Garrett, K., Barrell, G.K., Al-Marashdeh, O., Gregorini, P., 2021. Nitrogen balance of dairy cows divergent for milk urea nitrogen breeding values consuming either plantain or perennial ryegrass. Animals 11. https://doi.org/10.3390/ani11082464.

Martz, F.A., Belyea, R.L., 1986. Role of particle size and forage quality in digestion and passage by cattle and sheep. J. Dairy Sci. 69, 1996–2008. https://doi.org/10.3168/jds.S0022-0302(86)80626-9.

Okamoto, M., 2000. Comparison of rumination activities of dairy herds fed relatively high and low concentrate diet. Nihon Chikusan Gakkaiho 71, 50–56. https://doi.org/10.2508/chikusan.71.50.

Pierce, C., Bailey, D., Medrano, J., Canovas, A., Speidel, S., Coleman, S., Enns, R., Thomas, M., 2018. Validation of quantitative trait loci associated with grazing distribution traits in beef cattle using bayes C. J. Anim. Sci. 96, 105. Available from: https://search-proquest-com.idpproxy.reading.ac.uk/docview/1915881733?accountid=13460.

Primary Industries Standing Committee, 2007. Nutrient Requirements of Domesticated Ruminants. CSIRO Publishing, Victoria.

Prinz, J.F., Lucas, P.W., 1997. An optimization model for mastication and swallowing in mammals. Proc. R. Soc. B Biol. Sci. 264, 1715–1721. https://doi.org/10.1098/rspb.1997.0238.

R Core Team, 2021. R: a Language and Environment for Statistical Computing. Available from:. R Foundation for Statistical Computing, Vienna https://www.r-project.org/.

Rutter, S.M., 2000. Graze: a program to analyze recordings of the jaw movements of ruminants. Behav. Res. Methods Instrum. Comput. 32, 86–92. https://doi.org/10.3758/BF03200791.

Sheahan, A.J., Kolver, E.S., Roche, J.R., 2011. Genetic strain and diet effects on grazing behavior, pasture intake, and milk production. J. Dairy Sci. 94, 3583–3591. https://doi.org/10.3168/jds.2010-4089. Available from: https://doi.org/10.3168/jds.2010-4089.

Ungerfeld, E.M., 2020. Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. Front. Microbiol. 11 https://doi.org/10.3389/fmicb.2020.00589.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.