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Nitrogen Use Efficiency and Excretion in Grazing Cows with High and Low Milk Urea Nitrogen Breeding Values

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Abstract: Milk urea nitrogen content is moderately heritable and is phenotypically related to urine nitrogen (UN). Based on this relationship, it has been suggested that genetic selection for lower milk urea nitrogen in grazing dairy cows could decrease UN concentration thereby reducing nitrogen excretions into the ground. The objective of this study was to compare the nitrogen use efficiency (NUE) and excretion in grazing cows with high and low milk urea nitrogen breeding values (MUNBV) in two farms of contrasting farming intensity. On the high-intensity farm (HIF) 68 and 70 cows with low and high MUNBV, respectively, were fed higher levels of supplementation and milked twice-daily, while on the low-intensity farm (LIF) 82 and 86 cows with low and high MUNBV, respectively, were fed lower levels of supplementation milked once-daily. Nitrogen use efficiency (g/g) was calculated as the ratio of daily milk N to daily N intake. Daily N intake (g/day) was derived from feed intake estimates based on energy requirements. The UN (g/day) was estimated by back-calculation from dietary N and subtracting milk N, faecal N, and N retained in body tissues. Irrespective of farm, cows with low MUNBV had significantly lower MY and milk urea nitrogen ($p < 0.001$) but this was not linked to significantly less UN. In the LIF, cows with low MUNBV had lower NUE ($p < 0.001$) than cows with high MUNBV, and this was explained by the reduced protein yield ($p < 0.001$). Selecting cows for low MUNBV was not an effective tool to reduce N losses and to increase the NUE in two dairy farms of contrasting farming intensity.

Keywords: milk urea nitrogen; nitrogen use efficiency; nitrogen excreta; genetic selection; dairy cows

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1. Introduction

New Zealand dairy systems are predominantly grass based, although the proportion of supplementary feed has increased in recent decades [1]. The positive response in milk production by grazing cows fed supplements [2] has resulted in farmers increasing supplement allocation to milking cows. Fresh grazed pastures are typically high in crude protein (CP) concentration in seasons corresponding to early and late lactation (early spring and late autumn, respectively), containing mainly rumen-degradable protein (RDP) at levels that regularly exceed milk production requirements [3]. Nitrogen use efficiency (NUE), defined as nitrogen (N) output in milk protein as a percentage of N in the diet of grazing cows, is generally low. Reduced NUE results in increases in excreted N, predominantly in urine [4–6]. Urine N, which is deposited in concentrated patches, is the main driver influencing N leaching from pastures [7–9]. According to Franklin [10], the reduction in oxygen concentration of freshwater in New Zealand rivers, which is endangering the long-term sustainability of fish populations and is associated with risks in human health, is intrinsically linked to the intensification of land use. As part of this intensification process, the increase in cows per hectare and N fertiliser rates has resulted in increased N leaching from dairy systems [9,11]. From these variables, N fertiliser application rates seem to have a bigger impact probably due to a small increase in N uptake by pastures relative

to the amounts of N applied as a consequence of the seasonal variation in N uptake by plants [12]. Moreover, supplements are typically higher in energy content and lower in CP than pasture [3] and their inclusion can improve the NUE of a cow by diluting the dietary CP and increasing milk production [3,4,13].

Metabolisable protein (MP) refers to the true protein absorbed from the small intestine supplied by dietary rumen-undegradable protein (RUP), and by microbial protein synthesized in the rumen supplied by dietary RDP [13–15]. A surplus in the MP balance (supply minus demand) occurs by feeding CP in excess of protein requirements. In turn, this leads to increases in N excreta through urea production, whereas milk protein yield increases only modestly [6]. On the contrary, decreasing the CP supply below cow requirements triggers mobilisation of body reserves in order to meet protein requirements [14,16].

The excess of ruminal N is rapidly converted to urea to avoid harm from the excess of ammonia. Urea is transported from the plasma and subsequently transported to other fluids such as saliva in order to be recycled, or to be excreted in urine but it can also be found in milk [17,18]. Hence, milk urea nitrogen (MUN) has been proposed as a non-invasive proxy to assess inefficiencies of N use and as a predictor of N excreted through urine into the environment [18,19]. It was suggested that selecting cows of low MUN breeding values (MUNBV) would reduce N leaching by 20% over 20 years as more N would be captured in milk true protein [20]. Recently, a study published by Marshall et al. [21] confirmed with field measurements of MUN and urinary behaviour the estimations by Beatson et al. [20] regarding modelled urinary N reductions by selecting cows for low MUNBV and reported a significant increase in milk protein content while maintaining milk volume in pastured cows of low MUNBV relative to high MUNBV cows in early and late lactation. The objective of this study was to compare the NUE and nitrogen excretion in grazing cows with high and low MUNBV in two farms of contrasting farming intensity throughout the full lactation.

2. Materials and Methods

2.1. Animals and Their Management

The current study was carried out in the lower North Island of New Zealand (longitude 175°, latitude −40°) from June 2016 to May 2017 on two research farms of contrasting management, as previously described in Correa-Luna et al. [22]. Briefly, in the high-intensity farm (HIF), cows received 429 kg pasture silage and 1695 kg of grain-based concentrate per cow during the lactation and were milked twice-daily and in the low-intensity farm (LIF) cows received 304 kg pasture silage per cow during the lactation with no addition of concentrate and were milked once-daily. Cows of each farm were sorted into low, mid, and high MUNBV weighting each group by the number of lactations and breed in each farm. Only cows of high and low MUNBV were considered for this study.

The HIF was comprised of 68 and 70 cows of low and high MUNBV, respectively and the LIF was comprised of 82 and 86 cows of low and high MUNBV, respectively. In each farm, the planned start of calving commenced in the second half of July and continued up to the first week of October. In this study, stages of the lactation were defined as early (days in milk (DIM) < 100), mid (100 < DIM < 200), and late (200 < DIM).

Fresh ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) mixed pasture was the main feed ingredient in both farms. In HIF, maize silage (*Zea mays*) and grain-based concentrate were fed during the lactation at a rate of approximately 4.5 and 3 kg DM per cow per day, respectively, before the afternoon milking. Pasture silage was fed directly on the paddock during the mid-lactation stage at an approximate rate of 3.5 kg of DM per cow per day. In late lactation, the dried distillers grain was fed at a rate of 1 kg DM per cow during the morning milking and turnip crop was grazed in strips at an allowance of 1.7 kg DM per cow per day. In LIF, cows had daily access to an herb crop (mix of plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), and red clover (*Trifolium pratense*)) at an allowance of approximately 3.5 and 1.4 kg dry matter (DM) per cow during mid- and late-lactation, respectively. During late-lactation, lucerne (*Medicago sativa*) was grazed at

an offered allowance of approximately 3 kg DM per cow per day. Turnip crop (*Brassica campestris* ssp. *Rapifera*) was fed at an allowance of 0.5 kg DM per cow during late-lactation. Pasture silage was fed directly on the paddock in early- and late-lactation at a rate of 1.3 and 2.4 kg of DM per cow per day, respectively.

2.2. Herbage Measurements and Samplings

In each farm, cows had access as a single group to the mixed pasture after each milking. Herbage mass measurements of pasture and crops were assessed with a rising-plate meter following a 'W' pattern across the grazing area before and after each grazing event. Three quadrat cuts (0.1 m^2) were taken both before and after grazing to quantify pre- and post-grazing herbage mass (kg DM per ha) of the grazed crops. These measurements enabled the calculation of apparent pasture and crop utilisation, along with the proportion of herbage allocated to cows before each herd test. The same grazing management decision rules of pasture following the guidelines of DairyNZ [23] were utilised for both research farms during the lactation. Rotational grazing was practiced, and swards were strip grazed using temporary electric fences.

Samples (approximately 1500 g of wet weight) of fresh pastures and crops were taken by hand-plucking [24], and these, along with samples of silage and concentrate, were freeze-dried and ground (Wiley mill) to pass through a 1.0 mm screen. All samples were analysed by the near infrared reflectance spectroscopy (NIRS) technique [25] to evaluate neutral detergent fibre (NDF), acid detergent fibre, metabolisable energy (ME), and CP. Calibrations for each component had been previously developed (Massey University Nutrition Laboratory, Palmerston North, New Zealand) using NIRS after scanning finely-ground pasture samples in the range of 400 to 2500 nm. A Bruker MPA NIRS (Ettlingen, Germany) was used to scan the samples, and the resulting NIRS spectra were analysed using the Optic user software, version 5.0 (Ettlingen, Germany). The resulting NIRS calibration typically had a correlation of 0.90 when compared to the wet chemistry results for each component. The dietary chemical composition (DM basis) was computed from the chemical composition and proportion of ingredients in dietary DM (Table 1).

Table 1. Proportion of ingredients (DM basis) and dietary chemical composition in two contrasting pasture-based dairy farms (means ± standard deviations) in early, mid, and late lactation.

Farm	High Intensity			Low Intensity		
Stage of Lactation [1]	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**
Ingredients (kg DM/day)						
Pasture [2]	11.1 ±1.3	7.9 ± 1.4	9.7 ± 2.7	14.9 ± 3.3	12.1 ± 2.6	9.1 ± 1.6
Herb-mix crop [3]					3.5 ± 2.1	1.4 ± 1.5
Turnips crop			1.7 ± 0.8			0.5 ± 1.1
Pasture silage		3.5 ± 1.3		1.3 ± 2.2		2.4 ± 1.2
Lucerne crop						3.1 ± 3.2
Maize silage	4.4 ± 0.5	4.1 ± 0.6	5.1 ± 0.7			
Concentrate [4]	3.0 ± 0.3	3.3 ± 0.5	2.5 ± 0.3			
Dried distillers grains			1.0 ± 0.1			
Measured chemical composition (% DM unless otherwise stated)						
Energy, MJ ME [5]/kg DM	11.3 ± 0.1	11.2 ± 0.2	10.6 ± 0.3	11.5 ± 0.2	11.1 ± 0.6	10.5 ± 0.8
Crude protein	14.5 ± 0.1	14.7 ± 0.6	16.2 ± 1.0	18.6 ± 2.1	18.9 ± 2.1	20.4 ± 1.4
Energy: CP ratio, MJ ME/kg DM to CP%	0.78	0.76	0.65	0.62	0.59	0.51
Neutral detergent fibre	35.4 ± 0.2	35.8 ± 1.3	39.7 ± 1.9	43.8 ± 3.3	42.5 ± 4.0	42.4 ± 2.8
Acid detergent fibre	19.3 ± 0.3	19.5 ± 0.5	21.2 ± 0.6	22.0 ± 2.1	22.0 ± 2.0	23.9 ± 1.9
Rumen-undegradable protein [6], CP%	28.4	29	28.2	29.3	31.4	27.9
Rumen-degradable protein, CP%	71.6	71	71.8	70.7	68.6	72.1

[1] Stages of the lactation were defined as early (days in milk (DIM) < 100), mid (100 < DIM < 200), and late (200 < DIM). [2] Perennial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) pasture. [3] Herb-mix crop comprised of plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), and red clover (*Trifolium pratense*). [4] Grain-based concentrate. [5] Megajoules of metabolisable energy. [6] Computed using the actual composition of feeds, least squares means of actual feed intake, milk yield, live weight and milk composition for each diet, and period on the nutrition model software Rumen8 based on Feed into Milk equations [26].

2.3. Live Weight, Milk Yield, and Composition

Daily live weight (LW) measurements were available and body condition scores (BCS) were estimated at each herd test by a single trained research technician using a 10-point scale [27]. Live weight loss (LW_{loss}) and BCS loss (BCS_{loss}) were calculated as the sum of LW (or BCS) loss between the day of reference and the previous day in the first 100 days of lactation. Total yields of milk (MY), fat (FY), and protein (PY) (full lactation) were calculated from the accumulated daily yields collected monthly using mechanical milk meters (Tru-Test Field Collection meter WB HI/Pullout) provided by the Livestock Improvement Corporation (Hamilton, New Zealand). Somatic cell count was obtained from monthly herd test records. In addition, milk samples from each cow were taken in early, mid, and late lactation using the same milk meters and analysed by MilkTestNZ (Hamilton, NZ) using the CombiFoss technique [28] for MUN (mg/dL) content and lactose percentage. Each MUN record was converted into MUN yield (MUNY) (g MUN/cow/day) using the milk yield of the MUN sampling date.

2.4. Estimation of Breeding Values for Milk Urea Nitrogen

Each cow's MUNBV was estimated from the dataset of this study using a single-trait repeatability animal model as described by López-Villalobos et al. [29]. The model included the fixed effects of herd test date, lactation number, stage of lactation, and covariables for deviation from mean calving date by herd, stage of lactation, the proportion of Holstein-Friesian and heterosis coefficients of Holstein-Friesian × Jersey, as well as the random effects of cow permanent environment, animal, and residual error. Breeding values and estimates of (co)variance components were obtained using the restricted maximum likelihood procedure as implemented in the ASReml package [30] of VSN International Ltd. The PROC RANK procedure of SAS was used independently in each farm to obtain three MUNBV categories within cows of the same age and breed in each herd: Low, intermediate, and high.

2.5. Metabolisable Energy and Protein Analyses

Total net energy requirements for maintenance, pregnancy, production, and daily LW variation were calculated using the equations provided by Berry et al. [2]. Apparent DM intake (DMI) (kg DM/cow/day) was estimated by dividing the daily ME expenditure by the ME content of any feed offered on the day of the herd test after multiplying total net energy requirements by 11.85 MJ ME.

Compared to N concentration in urine, the concentration of N in faeces is constant relative to DMI in lactating cows [31,32]. Considering this, faecal N (FN; g faecal N per day) was estimated by employing a multivariate equation which included dietary characteristics, feed intake, and stage of lactation [32]:

$$FN = 72.7 - 11.8 \times ME - 0.4 \times NDF + 3.5 \times CP + 0.2 \times \text{dietary forage ratio} + 9.3 \times DMI - 0.1 \times DIM$$

Urinary N (UN; g N in urine per day) was estimated by back-calculation, taking into account N intake (NI, g of N per day) estimated by multiplying diet N content (g N/100 g DM) by daily DMI, milk N (MN, g of N in milk per day) calculated assuming 95% of protein N in total N ((daily milk yield × milk protein percentage)/6.38)/0.95 [33], and the N retained (or mobilised from) body tissues according to the daily LW variation (RetN; 160 g of CP per kg of LW change; [17]):

$$UN = NI - FN - MN - RetN$$

Nitrogen use efficiency was calculated as the ratio of the daily MN over the daily NI. The MP balance estimations were undertaken using Rumen8 [34], a software designed as a decision support tool for dairy nutrition advisors and farmers as part of a collaborative project between Western Dairy and Dairy Australia. This software calculates the MP supply and demand using the equations provided by Givens et al. [26]. The a, b, and c values for

fractions of the protein degradability [35] for each of the feed ingredients were used from the reference values provided by the Rumen8 library.

2.6. Statistical Analyses

The PROC RANK procedure of SAS was employed independently in each farm to obtain the three MUNBV categories (i.e., low, intermediate, and high) within cows of the same age and breed. Analyses of variance of the dependent variables were performed using the MIXED procedure of the statistical package SAS 9.4 (SAS Institute Inc. 2013, Cary, NC, USA) within each farm with a mixed model that included the fixed effects of lactation number, MUNBV category, and as co-variables deviation from the median calving date, proportion of Holstein-Friesian and heterosis effect between Holstein-Friesian and Jersey, and the random effect of cow. Least-squares means for the different classes of the independent variables included in the model were obtained and used for multiple mean comparisons using Fisher's least significant difference test. Significant differences were declared at $p < 0.05$.

3. Results

The proportion of ingredients and chemical composition of both diets corresponding to each farm in the three stages of lactation are presented in Table 1. Whereas, the contrasting difference among dietary ingredients was reflected in the CP levels, the soluble and insoluble potentially degradable fractions did not differ between diets. The diets employed in each farm were comparable in terms of energy content but, given the contrasting CP between diets, the energy:protein ratio was higher in the HIF diet throughout the lactation. The rate of CP degradation (RDP) tended to be slightly higher when alfalfa was included at a rate of approximately 18% in late lactation in LIF. The fibre proportion in the diet during the lactation was higher in LIF reflecting the higher proportion of forage relative to HIF.

Table 2 presents least squares means of total milk production performance observed in cows with low and high MUNBV in each farm (HIF and LIF). Lactation length was the same for low and high MUNBV on both HIF and LIF. Irrespective of farm, MY was significantly higher for high MUNBV cows when compared to low MUNBV cows. The difference in MY between high versus low MUNBV was 7% and 11% for HIF and LIF, respectively. With respect to total milk solids yield (MSY = FY + PY), the same trend was observed as in the case of MY but the differences remained at a significant level only in LIF with lower MSY in low MUNBV cows.

There were no significant differences of LW between cows with low versus high MUNBV in either farm. Irrespective of the management practices employed in each farm, BCS was higher in low MUNBV cows compared to high MUNBV cows, and this was accompanied by a minor BCS_{loss} in the first 100 days of lactation in each instance ($p < 0.001$).

Regardless of farm, cows with low MUNBV had lower MUN when compared to high MUNBV cows. The difference in MUN between low and high MUNBV cows was 3.2 and 4.4 mg/dL in HIF and LIF along the lactation, respectively ($p < 0.001$) and this difference was also seen in the total MUNY along the lactation in each farm.

In line with the similar levels of DMI and PY observed in cows of both low- and high-MUNBV of HIF, the N intake and milk N were the same ($p > 0.05$) (Table 3). In LIF, the DMI of low MUNBV cows was 3% less when compared to high MUNBV cows (Table 2) ($p < 0.001$) and this difference was also reflected in NI ($p = 0.03$). No improvements in NUE were observed with either of the farms by selecting cows of lower MUNBV (Table 3 and Figure 1). While the NUE of low and high MUNBV in HIF was the same in the three measuring periods (Figure 1a), in LIF the mean of NUE was one percentage unit of NUE lower for the low MUNBV when compared to high MUNBV ($p < 0.001$) (Table 3) and the differences were originated in early and late lactation (Figure 1b). Regardless of management practices employed in each farm, cows with low MUNBV did not significantly reduce N partitioned to urine (Table 3 and Figure 2).

Table 2. Animal characteristics and least squares means (± standard errors) of full-lactation milk production and animal performance of grazing dairy cows with low and high milk urea nitrogen breeding values (MUNBV) in two contrasting pasture-based dairy farms.

Farm	High Intensity		Low Intensity	
Trait	**Low MUNBV**	**High MUNBV**	**Low MUNBV**	**High MUNBV**
N	68	70	82	86
Mean cow genetic merit ($BW) [1]	78.09 ± 32.69	84.40 ± 35.75	88.32 ± 40.68	90.31 ± 38.27
Mean MUN [2] breeding value	0.12 ± 1.09	3.08 ± 0.84	-0.57 ± 1.44	3.24 ± 1.32
Lactation length, days	269 ± 4	273 ± 4	274 ± 3	277 ± 3
Milk yield, kg	4934 ± 99 ^{b}	5282 ± 99 ^{a}	3900 ± 86 ^{y}	4369 ± 83 ^{x}
Milk solids yield, kg	420 ± 8	431 ± 8	368 ± 7 ^{y}	397 ± 7 ^{x}
Fat yield, kg	231 ± 5 ^{a}	236 ± 5 ^{b}	208 ± 4 ^{y}	222 ± 4 ^{x}
Protein yield, kg	188 ± 4	195 ± 4	160 ± 3 ^{y}	175 ± 3 ^{x}
Lactose yield, kg	280 ± 5	291 ± 5	198 ± 5 ^{y}	221 ± 5 ^{x}
Somatic cell score [3]	4.93 ± 0.15	5.2 ± 0.2	5.71 ± 0.13	5.65 ± 0.13
Live weight, kg	479 ± 7	473 ± 7	475 ± 6	479 ± 6
Live weight loss [4], kg	34 ± 4 ^{a}	30 ± 4 ^{b}	13 ± 4 ^{y}	19 ± 4 ^{x}
Body condition score	4.31 ± 0.04 ^{a}	4.19 ± 0.04 ^{b}	4.73 ± 0.03 ^{x}	4.53 ± 0.03 ^{y}
Body condition score loss [5]	0.42 ± 0.03 ^{b}	0.49 ± 0.03 ^{a}	0.28 ± 0.02 ^{y}	0.41 ± 0.02 ^{x}
Dry-matter intake, kg/day	18.12 ± 0.14	18.19 ± 0.14	15.67 ± 0.07 ^{y}	16.19 ± 0.07 ^{x}
MUN, mg/dL	8.20 ± 0.30 ^{b}	11.40 ± 0.29 ^{a}	11.11 ± 0.25 ^{y}	15.46 ± 0.24 ^{x}
MUN yield, g	429.97 ± 13.63 ^{b}	620.74 ± 13.57 ^{a}	425.61 ± 11.82 ^{y}	632.90 ± 11.44 ^{x}

[1] Breeding worth, $ in NZD (May 2017). [2] Milk urea nitrogen. [3] The somatic cell count records were log2-transformed to SCS. [4] Sum of live weight loss between day of reference with respect to a previous day in the first 100 days of lactation. [5] Sum of body condition score (on a 1–10 scale) loss between day of reference with respect to a previous day in the first 100 days of lactation. ^{a,b} Means with different superscripts within rows indicate they were significantly different for the high intensity farm ($p < 0.05$). ^{x,y} Means with different superscripts within rows indicate they were significantly different for the low intensity farm ($p < 0.05$).

Table 3. Least-squares means (± standard errors) of daily nitrogen (N) partitioning (in g N/day) and N use efficiency (NUE) of grazing dairy cows with low and high milk urea breeding values (MUNBV) in two contrasting pasture-based dairy farms.

Farm	High Intensity		Low Intensity	
Item [1]	**Low MUNBV**	**High MUNBV**	**Low MUNBV**	**High MUNBV**
N intake	423.2 ± 6.3	427.2 ± 6.2	476.5 ± 3.5 ^{x}	493.0 ± 3.4 ^{y}
N milk	111.2 ± 1.7	113.8 ± 1.7	90.7 ± 1.4 ^{x}	98.5 ± 1.3 ^{y}
N retained	1.2 ± 0.6 ^{b}	2.1 ± 0.6 ^{a}	2.7 ± 0.5	1.9 ± 0.5
N in faeces	153.1 ± 1.9	154.0 ± 1.8	146.4 ± 1.1 ^{x}	151.4 ± 1.1 ^{y}
N in urine	149.0 ± 4.6	150.4 ± 4.5	237.1 ± 2.4	241.1 ± 2.3
NUE, g/g	0.26 ± 0.03	0.27 ± 0.02	0.19 ± 0.02 ^{y}	0.20 ± 0.01 ^{x}

[1] In g N/day unless otherwise stated. ^{a,b} Means with different superscripts within rows indicate they were significantly different for the high intensity farm ($p < 0.05$). ^{x,y} Means with different superscripts within rows indicate they were significantly different for the low intensity farm ($p < 0.05$).

The modelling of requirements, supplies, and balances of ME and MP did not show significant differences between MUNBV cows for each farm. In line with the estimated N balance in Table 3, the greater difference was observed between farms rather than in the genetic merit for MUN but these differences were not statistically compared. While cows of HIF showed a positive energy balance in the three lactation stages, cows of LIF had a negative ME balance at the beginning of the lactation and, as lactation progressed, the ME balance became positive. Neither farm showed any evident difference in terms of MP balance when comparing cows of low versus high MUNBV. Although not statistically tested, the greater contrast was observed in the MP balance between farms, presumably due to the contrasting dietary CP levels resulting from the different diets employed in each farm.

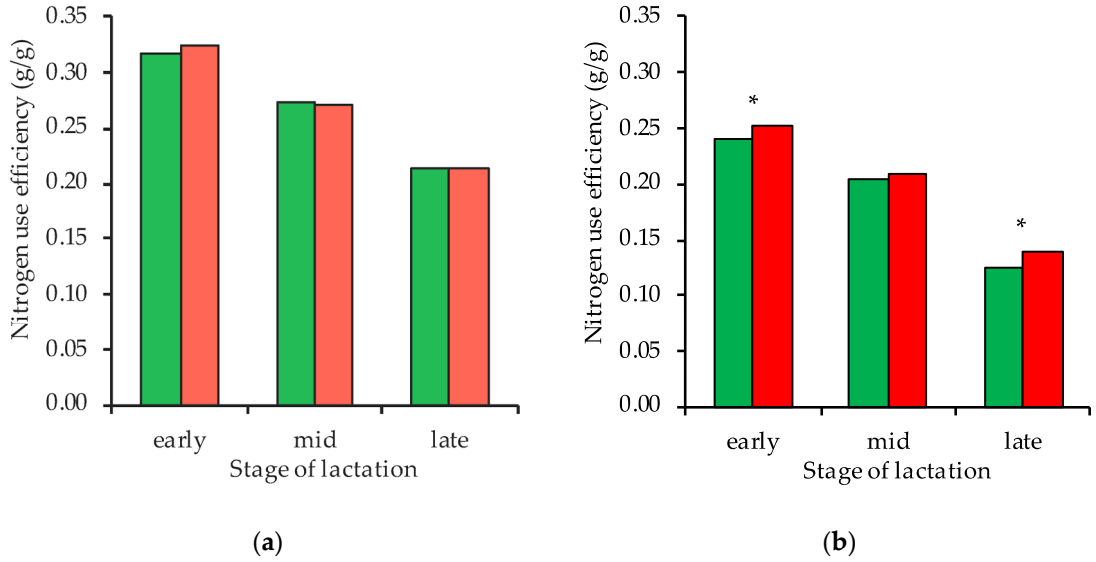

Figure 1. Nitrogen use efficiency in grazing dairy cows of low (green) and high (red) milk urea nitrogen breeding values (MUNBV) during early, mid, and late lactation in two grazing dairy farms of contrasting intensification: (**a**) High intensity and (**b**) low intensity. * Indicates significant differences for MUNBV ($p < 0.05$) within the same farm and stage of lactation: Early (days in milk (DIM) < 100), mid (100 < DIM < 200), and late (200 < DIM).

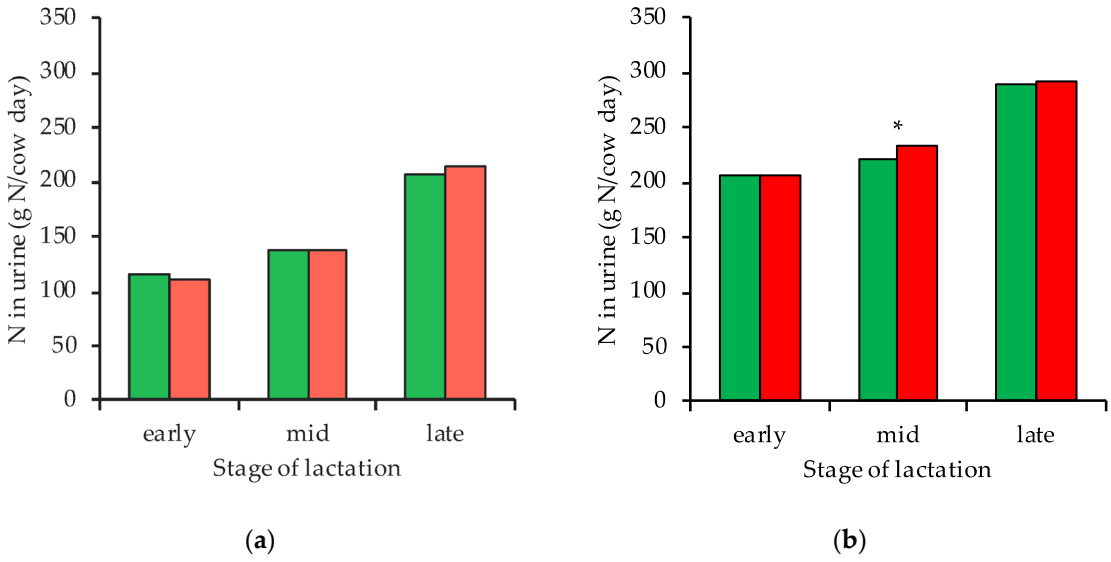

Figure 2. Daily urinary nitrogen (N) excreta in grazing dairy cows of low (green) and high (red) milk urea nitrogen breeding values (MUNBV) during early, mid, and late lactation in two grazing dairy farms of contrasting intensification: (**a**) High intensity and (**b**) low intensity. * Indicates significant differences for MUNBV ($p < 0.05$) within the same farm and stage of lactation: Early (days in milk (DIM) < 100), mid (100 < DIM < 200), and late (200 < DIM).

4. Discussion

This research compared the milk production performance along with NUE and urinary N excreta of cows with low and high BV for MUN in two dairy farms of contrasting intensification level. The present experimental setup limited the comparison of cows with low versus high MUNBV between farms, so it was not possible to evaluate statistically how diets divergent in CP concentration affected the N accretion in cows milked once- or twice-daily separately. It was confirmed that cows of low MUNBV produced milk with reduced concentrations of MUN in both farms. Milk yield was lower in low MUNBV cows regardless of farm and this significantly reduced the NUE in the farm where cows were

milked once-daily and fed a high CP diet throughout the lactation. Under the conditions of this study, selecting cows for genetically less MUN secretion only reduced the daily urinary N excreta by about 1.4 and 4 g per cow per day in HIF and LIF, respectively. On the other hand, manipulating the dietary CP represented a reduction of approximately 88.10 and 90.70 g per cow per day of urinary N in HIF and LIF, respectively.

No improvements in NUE were observed with cows of low MUNBV in either of the two farms (Table 3 and Figure 1). On the contrary, in LIF, a marginal improvement was observed in the NUE in cows of high MUNBV, but this was explained by higher increases in milk protein than the increase observed in the NI, when compared to cows of low MUNBV. On the contrary, Marshall et al. [21] observed an increase in milk protein percentage of 0.09 when MUNBV decreased one unit in early and late lactation at higher levels of CP relative to our high CP treatment. The difference with our study relies on the fact that we observed higher milk yield in cows of high MUNBV and this might reduce the milk protein percentage relative to the study by Marshall et al. [21]. In the study by Marshall et al. [21], the CP levels were 21% and 24% in early and late lactation and in our study, we measured 18.6% and 20.4% in early and late lactation, respectively. Probably, the excessive dietary CP in their study had a negative impact in the energy metabolism and this could have impacted the milk production performance [3,36] in the cows of their study as they also reported a negative correlation of MUNBV with milk solids. Moreover, it needs to be considered that the study by Marshall et al. [21] analysed milk production performance in early and late lactation and our results are drawn from early, mid, and late lactation stages and this, along with the different CP in diets and milking frequency, might lead to the differing results.

In line with the current study, Sebek et al. [37] reported the absence of a relationship between MUNBV and NUE by analysing more than 15,700 records from 723 cows in 26 experiments. In a study by Wood et al. [38], the heritability for MUN ranged from 0.44 to 0.59, indicating that MUN can be included in a genetic selection plan. Selection for low MUNBV might result in unfavourable effects on other traits, such as the reduction in milk yield observed in both HIF and LIF farms in the current study. Berry et al. [2] corroborated a moderate heritability of energy balance and energy partition towards milk production by selecting cows with high overall genetic merit. In the current study, the modelled energy balance and the BCS and the BCS_{loss} kinetics in both HIF and LIF dietary groups was for low MUNBV cows in the first 100 days of lactation and this is a consequence of lower milk yield when compared to high MUNBV cows. Only in LIF, a lower LW_{loss} for low MUNBV cows was accompanied by a lower milk yield, compared to high MUNBV cows. Cows in HIF diet had no differences in LW_{loss} irrespective of MUNBV, but milk yield was higher for the high MUNBV cows. There might be an interaction between the energy balance of cows with different milking frequencies [39] and energy content of the different diets offered on both HIF and LIF farms [3].

In agreement with the current study, a significant reduction in milk production was reported when comparing cows milked once-daily versus twice-daily [40]. By increasing the milking interval in cows of LIF, a suppression of nutrients partitioned towards the mammary gland would lead to a reduction in milk production [39]. Irrespective of the lower DMI of cows in LIF, NI was greater due to a larger proportion of pasture allocation with higher degradable N and this, along with the reduced MN, resulted in a lower NUE for these groups of cows relative to cows in HIF. A negative and strong relationship between dietary CP and NUE has been previously reported [4,18,32]. Diets comprised mainly of ryegrass pasture of good quality are moderate in ME and high in CP, and the lower inclusion of concentrate on such diets would reduce the total ME intake, which was the case for the LIF herd. The reduced energy:protein ratio in the diet would unbalance the energy and protein proportions in the rumen, interfering with the uptake of CP towards microbial protein synthesis [3]. Additionally, as the diet provided in HIF was more energy-dense and had less CP, this lowered (diluted) the NI and resulted in higher NUE with less N partitioned towards urine.

Metabolisable Protein Balance and Nitrogen Partitioning

Neither HIF nor LIF reflected larger differences in MP surplus or energy balance regardless of MUNBV (Table 4). Although not tested, the higher NI observed in LIF resulted in a bigger MP surplus which led to a lower NUE and in larger N excreta losses relative to HIF. Reducing the MF to once-daily over short periods was demonstrated as a tool to alleviate the negative energy balance that cows undergo during the early peripartum period [39]. Nevertheless, a more pronounced negative energy balance was observed throughout the season in LIF particularly, in early lactation. Cows in LIF had an excess of effective RDP which resulted in insufficient fermentable metabolisable energy for the rumen microbes. All the excess RDP would have resulted in elevated levels of ammonia which is converted into urea and mainly excreted in urine [35]. Compared to the HIF herd, the lower energy balance of the LIF herd was probably due to the extra energetic cost spent in eliminating the excess N [36], which also explained the higher measured MUN and the higher predicted UN (Table 3). In turn, these higher N losses and MUN observed in both low and high MUNBV cows fed with LIF were in line with the higher MP surplus and with the inferior NUE when compared to their counterpart low and high MUNBV cows in the HIF dietary herd.

Table 4. Modelled balances of metabolisable energy (ME) and protein (MP) in grazing dairy cows with low and high milk urea breeding values (MUNBV) in two dairy production systems of contrasting intensification level (milking frequency and supplementation). Requirements and supply were calculated from the actual feed chemical composition along with least squares means of estimated feed intake, milk yield and composition, and live weight, as well as its daily variation on the nutrition model software Rumen8 (Morris et al. [34]) based on Feed into Milk equations (Givens et al. [26]).

Farm	**High Intensity**						**Low Intensity**					
Item	**Low MUNBV**			**High MUNBV**			**Low MUNBV**			**High MUNBV**		
Stage of Lactation [1]	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**
Metabolisable energy, MJ/day												
Requirements	209.2	216.3	219.2	209.8	216.8	225.6	185.4	178.4	174.0	194.0	184.0	182.0
Supply	209.7	233.8	233.1	210.6	234.0	241.2	180.2	185.5	187.2	185.9	192.4	194.9
Balance	0.5	17.5	13.9	0.8	17.2	15.6	−5.2	7.1	13.2	−8.1	8.4	12.9
Metabolisable protein, g/day												
Requirements	1792	1797	1700	1806	1805	1756	1525	1509	1344	1619	1494	1432
Supply	1739	1843	2115	1744	1857	2190	2000	1915	1967	2106	2013	2079
Balance	−53	46	415	−62	52	434	475	406	623	487	519	647

[1] Early (days in milk (DIM) < 100), mid (100 < DIM < 200), and late (200 < DIM).

The comparable level of ME in diets of both farms was not surprising. Well managed temperate pastures comprised mainly of perennial rye-grass and white clover, as in the case of New Zealand pastures, can achieve moderate to high levels of ME [3,21,23] and excessive dietary CP relative to requirements for milk production at some stages of the lactation [3,11,13]. Regardless of the higher, energy-dense, grain-based concentrate included in the HIF farm, the higher inclusion of high-quality pasture was sufficient to uphold the dietary ME content in the LIF farm. The contrast between diets was in the dietary CP. For instance, mean pasture CP was 15% higher when compared to the concentrate CP (data not shown). This, along with the contrasting diet composition from one farm to another resulted in lower dietary CP, and in a more energy:protein balanced diet in HIF throughout the season. Regardless of MUNBV, the observed negative MP balance in early lactation in cows of the HIF herd was attributed to the deliberately lower CP in the diet which led to a deficient NI. Nevertheless, cows were still able to achieve moderate levels of milk production thanks to the supply of protein through the mobilisation of body reserves [14,16].

In both farms, the relationship between MUN and CP was positive [18,19] and as a consequence of this, MUN was effectively diminished by reducing CP in the diet. Moreover,

MUN was also reported to be positively related to RDP [41] and RUP [14]. Ureagenesis occurs in the liver and is a vital mechanism to overcome poisoning from the excess of ammonia present in the systemic circulation. This labile N pool is highly influenced, amongst other factors, by the feeding management [19]. In turn, urea is transported from the plasma to other fluids such as saliva in order to be recycled, and urine to be excreted, but due to its molecular weight and neutral charge urea easily diffuses across cellular membranes where it is incorporated to milk. As such, the relationship between urine urea and MUN was previously recognised by Jonker et al. [18]. A problem is that these associations are from housed conditions with diets controlled (and reduced) in CP. This management incompatibility of grazing systems relative to housed systems along with physiological and animal related factors might be interfering with the more significant N partitioning effects in this study. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cows by Spek et al. [42] showed that among the number of aspects affecting this relationship, the diurnal variation between UN and MUN was substantial from one cow to another and this variation could be affected by the moment of milk sampling. In this review, it was recognised that sources of variations for the fluctuations in MUN are founded in feeding time, renal urea filtration, frequency of feeding and milking, etc. Importantly, one of the main sources of variation of MUN is the CP in diet and this, along with the effective individual DMI of grazing cows, is more difficult to determine in pasture-based systems relative to housed systems [43]. Whereas, MUN might have a strong correlation with dietary CP in individual observations [18,19] and from a group of cows [44,45], the set of physiological and managerial explanations presents a limitation to draw a solid conclusion about the effect of selecting genetically for low MUN to manipulate the N partitioning in grazing dairy cows. Nevertheless, this study showed that in order to reduce wastage of CP in both cows milked once- and twice-daily, it was suggested that the focus needs to be on matching the RDP supply with the fermentable metabolisable energy [35,46,47]. It has also been proposed that shifting the site of digestion from the rumen to the large intestine, by increasing the RUP, has the potential to reduce urinary N excretion [4]. In line with this, Aguilar et al. [45] suggested that if the herd is nutritionally well managed and has a balanced diet that does not exceed NRC requirements for protein with adequate energy, MUN could serve as an appropriate indicator of N partitioning in lactating cows.

5. Conclusions

Regardless of the genetic merit of cows to produce less MUN, the reductions of MUN observed during the lactation in two farms of contrasting management were not associated with improvements in NUE or with substantial reductions in N partitioned towards excreta, particularly UN. On the contrary, it was observed that in the farm with lower farming intensity (i.e., cows milked once-daily and fed less supplements), cows with higher MUNBV had higher NUE and higher MUN during the lactation. This research showed that selecting cows for low MUNBV in two contrasting dairy farms was not an effective tool to reduce N losses and to increase the NUE. Feeding a more energy:protein balanced diet was a more effective tool to reduce N losses and to increase the NUE in these two farms of contrasting management approach. Further validation with field measurements of N leaching should be conducted to confirm the results observed in this study.

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