Genetic parameters for urination traits and their relationships with production traits of dairy cattle grazing temperate ryegrass and white-clover pastures

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Abstract

The objective of this study was to quantify genetic parameters of daily urination traits; urinary nitrogen (UN), urination volume (Uvol), urination number (Unum), average UN concentration (UNconc) and volume per urination event (VolEvent) including their genetic correlations with milk urea nitrogen concentration (MUN) and other production traits in New Zealand dairy cattle. Heritability estimates for urination traits measured on four consecutive days ranged from 0.17 to 0.35. Repeatability estimates ranged from 0.31 to 0.58, indicating that reliable prediction of urination measures would benefit from repeated measurement over a number of days. The heritability estimate for MUN was 0.29, similar to reports in the literature, whilst the estimate of repeatability (0.73) was greater than previously published values. Estimates of the genetic correlations between MUN and urination traits ranged from -0.39 to 0.41, however, due to the small number of animals in the current study, the confidence intervals were wide. The low genetic correlations may result from variation in voluntary feed intake from cow to cow that is a feature of pasture-based grazing systems. The results suggest that urination traits may be changed through selection. The low estimates of genetic correlations between MUN and urination traits suggest that MUN may not be a useful estimator of individual UN excretion in pasture-fed dairy cattle.

Keywords: urine traits; milk urea

Introduction

Temperate pasture-based (perennial ryegrass/white clover) dairy cattle typically consume forages that contain a high concentration of protein relative to energy, in excess of their daily requirements (Kolver & Muller 1998). Unless supplemented with a high-energy low-protein feed, these cows will excrete surplus nitrogen (N) principally in their urine (Selbie et al. 2015). One method to reduce N losses at a farm level would be a strategy that reduces the total daily quantity of urinary nitrogen (UN) excreted. An alternative N-mitigation strategy would be to identify animals that have more frequent, smaller urinations, as this would lead to a more uniform and dilute spread of UN deposited on pasture at a lower UN application rate (Kennett et al. 2020).

The direct measurement of UN, urine volume and number of urinations from grazing dairy cattle is difficult and expensive. It has been suggested that milk urea nitrogen concentration (MUN) estimated from routine herd tests could be used as an estimator of UN excretion per cow (Beatson et al. 2019).

The objective of this study was to quantify genetic parameters of urination traits and their genetic correlation with MUN and other production traits in New Zealand dairy cattle grazing temperate perennial ryegrass/white clover pastures.

Materials and methods

This experiment was conducted over two years and comprised various experimental runs in mid-late lactation (summer and autumn). Measurements of daily urination traits spanned Monday to Friday and comprised of groups of 30 cows split evenly into two cohorts of 15 cows per group.

Year One was conducted with the approval of the Ruakura Ethics Committee (#14634) on three dairy farms; Massey University's Dairy 4, situated near Palmerston North (40.2333° S, 175.3634° E), DairyNZ's Scott farm, situated near Hamilton (37.772° S 175.378° E) and AgResearch's Tokanui Dairy Research farm, situated near Te Awamutu, Waikato (38.0719° S 175.3273° E). Six experimental runs were completed between January and March 2019. Runs 1, 3 and 5 were completed at Dairy 4, Runs 4 and 6 were completed at Tokanui and Run 2 was completed at Scott farm. One cow was measured in two runs so records were on a total of 179 Holstein-Friesian-Jersey crossbred cows milked twice-a-day.

Year Two was conducted at Ashley Dene, situated near Burnham, Canterbury, New Zealand (43.6468° S 172.34679° E) between January and August 2020 with the approval of the Lincoln University Animal Ethics Committee (#2019-46). Six experimental runs were completed on a total of 180 Holstein-Friesian-Jersey crossbred cows milked twice a day.

In Year Two, animals were selected based on divergence in their parent average MUN breeding value (MUNBV; mg/dL), supplied by CRV (Beatson et al. 2019). The parent average MUNBV was estimated without including the cows own MUN phenotypes. The parent average is the most accurate estimate of genetic merit on the individual that is not confounded by environmental effects on the cow that may otherwise influence the repeatability of subsequent measurements.

Urination measures

Urine sensors (Betteridge et al. 2013; Shepherd et al. 2017a) of the same kind (Mk II, developed by AgResearch) were attached to the cows on a Monday after the morning milking (between 8 am and 10 am) and removed on Friday before the morning milking (between 5 am and 7 am). For data analysis, day was defined as the 24 hours from 9 am one day to 8:59 am the following day.

The urine sensors measure refractive index (RI), pressure, duration and time of each urination event. These data are used to estimate the urinary N concentration and volume of each urination event. The urinary nitrogen (UN) yield of each urination event (g N/event) was calculated as UN concentration (g/dL) x 10 x volume of the event (L). Mid-stream urine samples were collected at the start and end of each experimental run to calibrate the RI to urinary N concentration, as described by Shepherd et al. (2017b)

Over the four days, there were occasional periods when the urine sensor was not operational for the measurement of N concentration or was not functioning at all (due to being dislodged or blocked by faeces). For each cow-day, the number of urination events (Unum), cumulative urinary volume (Uvol) and cumulative UN was known for the total elapsed time that the urine sensor was functioning. These cumulative measures were divided by their respective total elapsed time of valid observations to give a per-minute value. The per-minute value was multiplied by the number of minutes in the day to get a cumulative measure representing daily Unum, Uvol and UN. For each cow-day the average volume per urination event (VolEvent) was calculated by dividing Uvol by Unum. Likewise, the average UN concentration (UNconc) was calculated by dividing UN by Uvol. Cow-days when the urine sensor was functioning for less than 50% of the day were excluded from the analysis (n=85 cow-days for Year One and n=187 cow-days for Year Two).

Milk production

In Year One, individual cow milk yields (MY) were recorded during each milking using individual test buckets and collecting the weight of milk in each bucket at Tokanui, using DeLaval milkmeters (FloMaster Pro) and its associated AlproTM herd and parlour management software (DeLaval International AB. Tumba, Sweden) for Scott farm and for Dairy 4. Milk samples were also collected from each milking starting Monday afternoon and ceasing Friday morning. Milk samples were transported daily to DairyNZ Hamilton for proportional milk composition (milk fat percentage, milk protein percentage and milk urea (MU) concentration; mg/dL) using the FOSS MilkoScan FT1 machines.

In Year Two, individual cow MY were automatically recorded each milking using the inbuilt Afimilk system with Afikim Afiflo 2000 milk meters. Milk samples were collected using the in-line fat sampler which is included with the Afikim Afiflo 2000 milk meters and then sub-sampled before being transported daily to MilkTestNZ Hamilton for proportional milk composition on the FOSS MilkoScan FT and 7 RM machines that use mid-infrared technique to measure milk components. The machines in both years were operated according to the ISO 9622:2013 guidelines (https://www.iso.org/standard/56874.html).

In both years MU concentration (mg/dL) was converted to MUN concentration (mg/dL) by multiplying by 0.466. Milk urea nitrogen yield (MUNY; grams) at each milking was calculated as MUN concentration (mg/dL) divided by 100 and multiplied by MY (L). Milk fat yield (FY), and milk protein yield (PY) at each milking were calculated by multiplying the milk fat percentage and MY or milk protein percentage and MY, respectively. The daily MY, MUNY, FY and PY were calculated as the sum of the afternoon and following morning yields to correspond to the 9 am to 8:59 am defined day for the urine sensors.

Two cows that were having extended lactations (>500 days) atypical of New Zealand pasture-based dairy cattle were removed from the dataset. Only cows that had data on urination traits had their milk traits included.

Genotypes

Cattle were genotyped by Weatherbys on an Illumina 50,000 SNP bovine panel. The small proportion of SNPs that were missing on any animal were imputed using the methodology of FImpute (Sargolzaei et al. 2014). Only mapped SNPs assigned to chromosomes 1 through 29 were included in the analysis (n=42,231).

Statistical analysis

Bayesian univariate repeatability models were run using the Julia for Whole-genome Analyses Software (JWAS) package (Cheng et al. 2018) run in a Julia computing environment (julialang.org). Inference was based on Markov chain Monte Carlo (MCMC) chains of 90,000 samples, retaining every 10th sample, after a burn-in of 10,000 samples which had been discarded.

The repeatability model equation was:

$$\boldsymbol{y} = \mathrm{RGD} + \mathrm{MUNBV} + \mathrm{age} \times \mathrm{year} + \mathrm{DIM} + \mathrm{pJ} + \mathrm{het} + \mathrm{AnimPerm} + \mathrm{SNPs} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is the daily measurement on the trait of interest; RGD was the fixed class effect of run-group-day in which the animal was grazing; MUNBV was the fixed class effect of MUN BV group (Low, High or Year One); age × year was the interaction of the fixed class effects of age of the cow in years from birth to most recent parturition within year of the study; DIM was the fixed covariate of days in milk on day 1 of RGD; pJ was the Jersey breed proportion; het was the specific heterosis coefficient between Holstein-Friesian and Jersey (Dickerson 1973); AnimPerm is the random permanent effect of animal assumed to be independently and identically normally distributed with variance σ_c^2; SNPs are additive covariates for all of the 42,231 autosomal loci with effects independently and identically normally distributed with variance σ_α^2; and $\boldsymbol{e}$ is the residual effects independently and identically normally distributed with variance σ_e^2.

Co(variance) components were estimated by fitting the model equation pairwise using bivariate repeatability animal models.

The 95% credibility intervals were calculated by taking the 97.5th percentile of the MCMC samples as the upper bound and the 2.5th percentile as the lower bound.

Results

Descriptive statistics for the urination and milk traits are presented in Table 1. On average, after accounting for missing values, cows had approximately three days of urination traits included in the final dataset. The average daily UN excretion was 203.8 g with an average frequency of 12.3 urinations per day. On average, MUN concentration was 11.18 mg/dL.

The estimates of heritability for the five urination traits were moderate and ranged from 0.17 to 0.35 (Table 2). Repeatability estimates were greater than 0.3 for all traits studied, with milk traits tending to have higher repeatabilities than urination traits (Table 2). There were no discernible differences between the MUN BV groups (High minus Low) for any of the urination or milk traits, based on the 95% CI bordering zero (Table 2).

Genetic and phenotypic correlations among the five urination traits are in Table 3. Phenotypic correlations among three of those, namely UN, Uvol and Unum were moderately high and positive, suggesting that cows that excreted a high daily UN load would be doing so with more urinations and with a greater total volume per day. Although phenotypic correlations between UN and other urination traits were positive, they were negative between UNconc and other urination traits. The genetic correlations of UN with UNconc, Uvol and Unum and between Uvol and Unum were moderately high and positive (Table 3). The genetic correlation between UNconc and Uvol was moderately high and negative (Table 3).

The posterior mean of the genetic correlation between UN and MUN was 0.09, between UNconc and MUN was 0.41, whereas between MUN and the other three urination traits the genetic correlations were negative (-0.27 to -0.39; Table 4). Genetic correlations of MUNY with UN (0.24) and MUNY with UNconc (0.35) were low and positive (Table 4). Phenotypic correlations between MUN and urination traits were near zero, between -0.09 and 0.11 (Table 4). Likewise, phenotypic correlations between MUNY and urination traits were low and near zero (0.01 to 0.09; Table 4).

Estimates of the genetic correlations of milk production traits (MY, FY and PY) with urination traits were between -0.20 and 0.43; the phenotypic correlations were low and near zero for these same traits (Table 4).

Table 1 Number (N) of records, N of cows, unadjusted phenotypic mean and standard deviation (SD) of urination and milk traits in grazing dairy cows.

Trait	N records	N cows	Mean	SD
UN (g/d)	1,075	339	203.8	78.9
UNconc (g/L)	1,075	339	6.07	1.71
Uvol (L/d)	1,121	343	34.4	11.9
Unum (count)	1,121	343	12.3	4.2
VolEvent (L/event)	1,121	343	2.87	0.70
MY (L/d)	1,090	343	18.8	4.9
FY (kg/d)	1,089	343	0.91	0.22
PY (kg/d)	1,079	340	0.70	0.17
MUNY (g/d)	1,079	340	2.09	0.74
MUN (mg/dL)	1,079	340	11.18	3.12

daily urinary nitrogen (UN), mean UN concentration (UNconc), urination volume (Uvol), urination number (Unum), mean volume per urination event (VolEvent), milk yield (MY), milk fat yield (FY), milk protein yield (PY), milk urea nitrogen yield (MUNY) and milk urea nitrogen concentration (MUN).

Discussion

Measuring urination phenotypes of lactating cattle grazing outdoors is logistically challenging and expensive and, therefore, restricted for research purposes. Few studies have summarised whole-day urination traits in grazing dairy cattle (Shepherd et al. 2017; Bryant et al. 2018; Mangwe et al. 2019), and none have quantified genetic variation. Our estimates of heritability for the urination traits were moderate and greater than the heritability of urinary creatinine (0.05) or phosphorus concentrations (0.12) in a Danish study (Løvendahl & Sehested 2016). That same study reported repeatabilities for urinary creatinine (0.38) and phosphorus concentrations (0.21), comparable to the

Table 2 Posterior means with 95% credibility intervals (95% CI; presented in brackets) of the heritability, repeatability and the difference between cows that had High or Low MUN breeding values for urination and milk traits in grazing dairy cows.

Trait	Heritability	95% CI	Repeatability	95% CI	High – Low MUNBV	95% CI
UN (g/d)	0.17	(0.07, 0.26)	0.31	(0.24, 0.38)	6.6	(-9.3, 22.4)
UNconc (g/L)	0.35	(0.18, 0.49)	0.58	(0.53, 0.64)	0.24	(-0.17, 0.66)
Uvol (L/d)	0.32	(0.14, 0.45)	0.50	(0.43, 0.55)	0.4	(-2.7, 3.5)
Unum (count)	0.28	(0.11, 0.44)	0.50	(0.44, 0.56)	0.2	(-0.9, 1.3)
VolEvent (L/event)	0.18	(0.08, 0.32)	0.56	(0.49, 0.61)	0.03	(-0.18, 0.23)
MY (L/d)	0.51	(0.31, 0.67)	0.75	(0.71, 0.78)	-0.6	(-1.5, 0.3)
FY (kg/d)	0.27	(0.10, 0.49)	0.56	(0.49, 0.62)	0.00	(-0.04, 0.04)
PY (kg/d)	0.44	(0.21, 0.59)	0.66	(0.61, 0.71)	-0.01	(-0.04, 0.02)
MUNY (g/d)	0.17	(0.06, 0.35)	0.72	(0.67, 0.76)	-0.04	(-0.19, 0.12)
MUN (mg/dL)	0.29	(0.11, 0.52)	0.73	(0.68, 0.77)	0.17	(-0.49, 0.81)

daily urinary nitrogen (UN), mean UN concentration (UNconc), urination volume (Uvol), urination number (Unum), mean volume per urination event (VolEvent), milk yield (MY), milk fat yield (FY), milk protein yield (PY), milk urea nitrogen yield (MUNY) and milk urea nitrogen concentration (MUN).

Table 3 Posterior means of the phenotypic (above the diagonal) and genetic (below the diagonal) correlations among urination traits in grazing dairy cows with 95% credibility intervals (presented in brackets).

Trait	UN	UNconc	Uvol	Unum	VolEvent
UN	-	0.28	0.66	0.55	0.14
	-	(0.21, 0.35)	(0.61, 0.70)	(0.49, 0.60)	(0.07, 0.22)
UNconc	0.32	-	-0.45	-0.24	-0.29
	(-0.07, 0.62)	-	(-0.51, -0.39)	(-0.32, -0.17)	(-0.36, -0.21)
Uvol	0.43	-0.67	-	0.73	0.33
	(-0.19, 0.75)	(-0.81, -0.41)	-	(0.69, 0.77)	(0.26, 0.40)
Unum	0.67	-0.29	0.79	-	-0.33
	(0.44, 0.84)	(-0.55, 0.22)	(0.64, 0.89)	-	(-0.40, -0.26)
VolEvent	-0.29	-0.51	0.40	-0.21	-
	(-0.61, 0.24)	(-0.74, -0.19)	(0.03, 0.68)	(-0.63, 0.12)	-

daily urinary nitrogen (UN), mean UN concentration (UNconc), urination volume (Uvol), urination number (Unum), mean volume per urination event (VolEvent)

Table 4 Posterior means of the genetic and phenotypic correlations between milk traits and urination traits in grazing dairy cows with 95% credibility intervals (presented in brackets).

	UN	UNconc	Uvol	Unum	VolEvent
Genetic correlation					
MY	0.36 (0.01, 0.68)	-0.06 (-0.34, 0.25)	0.34 (0.02, 0.68)	0.32 (-0.03, 0.57)	-0.08 (-0.50, 0.33)
FY	0.43 (-0.29, 0.85)	0.12 (-0.37, 0.46)	0.24 (-0.31, 0.68)	0.32 (-0.04, 0.63)	-0.10(-0.69, 0.57)
PY	0.25 (-0.20, 0.63)	-0.12 (-0.39, 0.2)	0.40 (0.04, 0.69)	0.41 (0.05, 0.76)	-0.20 (-0.68, 0.29)
MUNY	0.24 (-0.29, 0.72)	0.35 (-0.01, 0.67)	-0.06 (-0.49, 0.42)	0.21 (-0.42, 0.69)	-0.23 (-0.64, 0.23)
MUN	0.09 (-0.64, 0.75)	0.41 (-0.02, 0.72)	-0.39 (-0.80, 0.22)	-0.31 (-0.68, 0.12)	-0.27 (-0.74, 0.36)
Phenotypic correlation					
MY	0.14 (0.06, 0.21)	-0.04 (-0.13, 0.04)	0.16 (0.08, 0.24)	0.10 (0.02, 0.18)	0.08 (0.00, 0.17)
FY	0.14 (0.07, 0.22)	-0.06 (-0.14, 0.02)	0.17 (0.10, 0.25)	0.08 (0.00, 0.16)	0.14 (0.06, 0.22)
PY	0.15 (0.08, 0.23)	-0.08 (-0.16, 0)	0.21 (0.13, 0.28)	0.14 (0.06, 0.22)	0.09 (0.01, 0.17)
MUNY	0.09 (0.01, 0.17)	0.04 (-0.05, 0.12)	0.04 (-0.04, 0.12)	0.06 (-0.02, 0.14)	0.01 (-0.08, 0.10)
MUN	0.01 (-0.07, 0.09)	0.11 (0.02, 0.20)	-0.09 (-0.17, -0.01)	-0.02 (-0.10, 0.06)	-0.08 (-0.16, 0.01)

daily urinary nitrogen (UN), mean UN concentration (UNconc), urination volume (Uvol), urination number (Unum), mean volume per urination event (VolEvent), milk yield (MY), milk fat yield (FY), milk protein yield (PY), milk urea nitrogen yield (MUNY) and milk urea nitrogen concentration (MUN).

repeatability estimate for UN in the current study, but less than for the other urination traits. Repeatability estimates for the urination traits measured across four consecutive days ranged from 0.31 to 0.58, as these estimates are not near unity, they are indicative that reliable prediction of urination traits would benefit from measurements being repeated over several days, potentially spread over lactations and stages of lactation.

The heritability estimate for MUN of 0.29 in the current study is in the range reported in New Zealand cattle of 0.22 to 0.49 (Lopez-Villalobos et al. 2018; Ariyarathne et al. 2019; Beatson et al. 2019) and in overseas Holsteins from 0.14 to 0.59 (Wood et al. 2003; Mitchell et al. 2005; Hossein-Zadeh & Ardalan 2011). The heritability of MUNY was comparable to the value of 0.19 reported by Lopez-Villalobos et al. (2018). The estimates of repeatability for MUN and MUNY were greater than the range of 0.35 to 0.55 reported in New Zealand studies (Lopez-Villalobos et al. 2018; Ariyarathne et al. 2019), likely due to the data in the present study being collected on any one cow over just four consecutive days rather than on four days spread over early, mid and late lactation.

It has been suggested that MUN measured using MIR from individual herd tests could be used as an estimator of per cow UN excretion (Beatson et al. 2019). The genetic correlations of daily MUN with UN was 0.09 and of MUNY with UN was 0.24, i.e., low and near zero. The 95% credibility intervals around the genetic correlations were wide, thus, these preliminary estimates based on 343 cows should be viewed as indicative, and collection of further data from a larger number of cows might be warranted.

The phenotypic correlations between MUN and UN was 0.01 and for MUNY and UN was 0.09. These near-zero phenotypic correlations indicate that cows that have lower MUN or MUNY are not likely to have lower UN excretion compared with cows that have higher MUN or MUNY. It has been reported internationally that MUN is related linearly to UN excretion at the phenotypic level (Kauffman & St-Pierre 2001; Spek et al. 2013). However, this relationship appears to be due to diet differences, i.e., if a cow is fed a higher-protein diet, their MUN and UN increases linearly (Kauffman & St-Pierre 2001; Spek et al. 2013). Based on the results from the current study it appears that the phenotypic relationship between MUN and UN from controlled feeding studies does not hold in a within-herd comparison of grazing cows with higher MUN compared to those with lower MUN when they were offered the same diet. Although grazing cattle may be offered the same quantity of pasture, their voluntary feed intake can differ considerably. Therefore, it is difficult to identify which cows are consuming more feed and, hence, more N than others and what impact this has on their subsequent MUN or UN excretion.

A 0.67 g/L decrease in urinary urea N concentration per unit decrease in MUNBV was reported in a study of 48 lactating Holstein-Friesian cattle divergently selected for MUNBV (Marshall et al. 2020). Urinary urea N was not measured in the current study, but it is a component of UN. There were small positive genetic and phenotypic correlations between MUN and UNconc, but the 95% credibility intervals for the genetic correlation were wide (-0.02, 0.72), leading to uncertainty whether or not selection for decreased MUN would result in cows that had markedly decreased UNconc. Furthermore, UNconc is only one factor influencing N leaching, with VolEvent and Unum being other important animal factors to consider (Kennett et al. 2020). The genetic and phenotypic correlations between UNconc and VolEvent were negative in the current study indicating that selection for lower UNconc would likely be associated with an increase in volume per urination event.

Marshall et al. (2020) reported an R^2 of 0.67 for the regression of MUN phenotype on MUNBV. The R^2 for the regression of phenotype on true breeding value is the definition of heritability (Bourdon 2000; Mrode 2005). The value of 0.67 reported by Marshall et al. (2020) is outside the 0.14 - 0.59 range of estimates for the heritability of MUN (Wood et al. 2003; Mitchell et al. 2005; Hossein-Zadeh & Ardalan 2011; Lopez-Villalobos et al. 2018; Ariyarathne et al. 2019; Beatson et al. 2019). The higher than expected R^2 indicates that the MUNBV was not a parent average estimate but was likely calculated by including individual MUN phenotypes of the cows studied by Marshall et al. (2020). Although using the cow's own MUN phenotype will increase the accuracy of the estimated MUNBV, it introduces a non-genetic covariance between MUNBV and any subsequent MUN phenotype measured on the individual, hence, it was not simply a genetic relationship being studied by Marshall et al. (2020) but a repeatability relationship. Therefore, their conclusion that selecting dairy cows with low MUNBV can reduce urinary urea N deposition onto pasture may not hold in practice and is not supported by the results from the current study on a larger dataset.

A recent study modelled the likely change in UN excretion when including MUN in the New Zealand national selection index (Breeding Worth; BW) and concluded that there was unlikely to be much environmental benefit from including selection for lower MUN (Ariyarathne et al. 2021). The authors attributed this to whole-herd N leaching being more a function of stocking rate rather than individual cow UN excretion. They predicted that after 10 years of selection for lower MUN with 20% emphasis in BW, UN excretion per hectare would be 154.5 kg, only 3.8 kg less than if MUN was not included in BW at all.

Overall, the results from the current study indicate that urination traits may be changed through direct selection. However, the low and near-zero estimates of genetic correlations between MUN and urination traits suggest that MUN may not be a useful estimator of individual UN excretion in pasture-fed dairy cattle that vary in voluntary feed intake from cow to cow.

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