

QUANTIFYING GENETIC VARIATION IN URINATION TRAITS OF GRAZING DAIRY CATTLE

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SUMMARY

This research quantified genetic variation in urination traits using sensors attached to grazing lactating dairy cattle that are designed to record timing, volume and nitrogen concentration of every urination event. The records from individual events were used to generate phenotypic traits that included daily urinary nitrogen (UN), urinary volume (Uvol), number of urination events (Unum) and average volume per urination event (VolEvent). Heritability estimates for these daily traits ranged from 0.20 to 0.37, showing these urination traits are heritable. Repeatability estimates ranged from 0.27 to 0.59, indicating there is considerable residual variation and sensor observations would need to be repeated over a number of days to get reliable phenotypic measures. Phenotypic and genetic correlations have been estimated, however due to the small number of animals in the current study, these preliminary estimates should only be viewed as indications. Overall, these results suggest there is potential for urination traits to be changed through selection however, these traits are difficult and expensive to measure and more cows need to be phenotyped in order to provide more reliable estimates of genetic parameters.

INTRODUCTION

Pasture-based dairy cows in New Zealand predominately consume a sward containing perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). These forages contain a high concentration of protein-nitrogen (N) relative to energy that is in excess of dairy cow requirements (Kolver and Muller 1998). Unless supplemented with a high-energy low-protein feed, these cows will excrete most of this surplus nitrogen in their urine (Selbie *et al.* 2015). Excreted N is concentrated in patches where it can be surplus to pasture requirements. Additionally the soil is unable to retain excess N which can lead to leaching through the soil and hence become a major source of N in waterways (Woods *et al.* 2016).

One method to reduce N losses at the farm level would be to reduce the total amount of urinary nitrogen (UN) excreted per cow per day. Another method would be to alter the urine patch dynamics, i.e. the UN load per urination event (Kennett *et al.* 2020). At a given daily UN load, a greater total urinary volume (Uvol) and smaller volume per urination event (VolEvent) would typically be favourable as it would lead to a more uniform spread of urine across the paddock and dilute the concentration of UN deposited in urine patches on pasture (Kennett *et al.* 2020).

The objective of this study was to quantify genetic variation in urination traits of grazing dairy cattle.

MATERIALS AND METHODS

This experiment 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. Each run was comprised of 30 cows split evenly into two grazing groups of 15 cows per group. Urine sensors (Mk II) developed by AgResearch (Betteridge *et al.* 2013; Shepherd *et al.* 2017) were attached to the

cows between 8am and 9am on a Monday and removed around 6am on Friday. For data analysis, day was defined as the 24 hours from 9am to 8:59am the following day, run-group-day was defined as the contemporary group made up of experimental run (1-6), grazing group (1 or 2) and day of the experiment (1-4).

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).

Over the four days, there were periods from time-to-time where the urine sensor was not functioning for the measurement of N concentration or was not functioning at all. 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 the known 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. Cow-days where the urine sensor was functioning for less than 50% of the day were not included in the final dataset (n=187 cow-days) for any of the urine traits. Similar edits to remove cow-days for UN were applied when the sensor recording N concentration was not functioning.

Two cows that were having extended lactations (>500 days) atypical of New Zealand pasture-based dairy cattle were removed from the dataset.

Genotypes. Cattle were genotyped by Weatherbys (www.WeatherbysScientific.com) 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 for the urination traits 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 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:

$$y = \text{RGD} + \text{age} + \text{DIM} + \text{pJ} + \text{het} + \text{AnimPerm} + \text{SNPs} + e$$

where y is the daily measurement on the trait of interest: UN (n=483 records on 164 cows), Uvol, Unum and VolEvent (n=517 records on 168 cows); RGD was the fixed class effect of run-group-day the animal was grazing; age was the fixed class effect of age of the cow in years from birth to most recent parturition; DIM was the fixed linear covariate of days in milk on day 1 of RGD; pJ was the linear covariate of Jersey breed proportion; het was the linear covariate of 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_a^2 ; and e is the residual effects independently and identically normally distributed with variance σ_e^2 .

Co(variance) components for UN, Uvol, Unum and VolEvent were estimated by fitting the model equation pairwise using six 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 AND DISCUSSION

Descriptive statistics for the final dataset are given in Table 1. Daily Uvol and UN (Table 1) were greater than that reported for Friesian-Jersey crossbred cows fitted with the same sensors as

used in the current study (Bryant *et al.* 2018). Another study using similar urine sensors reported a daily Uvol of 42.2 L (Mangwe *et al.* 2019), comparable to the current study. The mean number of urinations per day was similar to that reported by Bryant *et al.* (2018) and Mangwe *et al.* (2019).

The estimates of heritability for the four urination traits were moderate (Table 1). Repeatability for VolEvent was greater than for UN (0.59 vs 0.27). Estimates of repeatability were similar to those observed for lactation test-day traits spread monthly or alternate monthly throughout a lactation. For example, estimates of repeatability were 0.52 for milk yield, 0.43 for fat yield and 0.44 for protein yield with approximately 80,000 multibreed cows and an average of two test-day records per cow (Lembeye *et al.* 2016).

Heritability and repeatability estimates of urination traits in cattle are scarce, although a Danish study reported a heritability of 0.12 for concentration of phosphorus in urine from random spot samples and a repeatability of 0.21 (Løvendahl and Sehested 2016). The same study reported a heritability of 0.05 and a repeatability of 0.38 for urinary creatinine, a nitrogen containing compound in urine.

Table 1. Unadjusted phenotypic mean and standard deviation for daily urinary nitrogen (UN; g/d), urination volume (Uvol; L/d), urination number (Unum; count) and mean volume per urination event (VolEvent; L/event). Posterior means with lower and upper 95% credibility intervals (presented in brackets) of the genetic variance, heritability and repeatability

Trait	Mean	Standard deviation	Genetic Variance	Heritability	Repeatability
UN	238	80	868 (441, 1,311)	0.20 (0.10, 0.30)	0.27 (0.18, 0.36)
Uvol	36.8	12.4	50.2 (22.3, 78.9)	0.36 (0.17, 0.51)	0.50 (0.41, 0.58)
Unum	13.0	4.4	3.9 (0.8, 7.5)	0.24 (0.05, 0.44)	0.46 (0.36, 0.55)
VolEvent	2.9	0.7	0.16 (0.08, 0.26)	0.37 (0.18, 0.55)	0.59 (0.51, 0.66)

Genetic and phenotypic correlations among the four urination traits are in Table 2. The phenotypic correlations among UN, Uvol and Unum were moderately high and positive, suggesting that cows that excreted a high volume of urine per day would be doing so with more urination events per day and at a greater daily UN load. The posterior means for the genetic correlations between Uvol and UN, Unum and VolEvent were moderate to moderately high and positive (Table 2). The genetic correlation between daily UN and VolEvent was near zero.

Due to the small numbers of animals in this study, the 95% credibility intervals around the posterior means of genetic parameters were wide, thus these preliminary estimates of genetic parameters should only be viewed as indications. Subsequent studies with larger cohorts of cattle are required to increase the reliability of the genetic parameters for urination traits. Nevertheless, based on this study, the genetic correlations of Uvol with Unum and VolEvent are likely to be positive.

Phenotyping cows for urination traits is expensive and logistically challenging, especially when cows are lactating and are outdoors grazing pasture. For this reason, there are few studies that have summarised whole day urination traits in grazing dairy cattle (Shepherd *et al.* 2017; Bryant *et al.* 2018; Mangwe *et al.* 2019), and none that have quantified genetic variation in the same traits. Comparing the square root of the estimated genetic variance to the raw mean shows that there is opportunity for urination traits to be included in the national breeding objective to ultimately reduce N losses to waterways, however, a cheaper and easier measurement to predict the urination traits

would be advantageous to enable faster and more accurate selection over the national dairy herd.

Table 2. Posterior means of the phenotypic (above the diagonal) and genetic (below the diagonal) correlations between daily urinary nitrogen (UN; g/d), urination volume (Uvol; L/d), urination number (Unum; count) and mean volume per urination event (VolEvent; L/event) with lower and upper 95% credibility intervals (presented in brackets)

Trait	UN	Uvol	Unum	VolEvent
UN	-	0.68	0.60	0.09
	-	(0.62, 0.73)	(0.53, 0.67)	(-0.01, 0.19)
Uvol	0.59	-	0.75	0.31
	(0.29, 0.78)	-	(0.70, 0.80)	(0.21, 0.41)
Unum	0.58	0.75	-	-0.29
	(-0.03, 0.83)	(0.44, 0.91)	-	(-0.39, -0.18)
VolEvent	-0.09	0.47	-0.17	-
	(-0.49, 0.36)	(0.04, 0.76)	(-0.64, 0.41)	-

CONCLUSIONS

This study shows that there is genetic variation in the urination traits UN, Uvol, Unum and VolEvent. This suggests there is potential for urination traits to be changed through selection however, these traits are difficult and expensive to measure and more cows would need to be phenotyped in order to provide more reliable estimates of genetic parameters among the urination traits in addition to other important traits such as lactation and fertility.

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