Short communication: Individual cow variation in urinary excretion of phosphorus

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ABSTRACT

Some dairy cows excrete large amounts of P through their urine; thus, it was speculated that a genetic defect related to their efficiency in uptake of P or recirculation of P could cause such an effect. This speculation was pursued in a cross sectional study on 139 cows (103 Holstein and 36 Jersey) from an experimental herd using repeated sampling of urine (301 samples) to investigate sources of variation in urinary P concentration (Pu). Urine samples were taken on 6 testing sessions spread over 2 mo. Each sample was obtained by mild manual stimulation of the rear udder escutcheon area. The samples were immediately assayed for pH, stored frozen, and assayed for inorganic P and creatinine. Concentrations of P and creatinine in urine, the ratio of Pu to creatinine, and pH were analyzed using a linear mixed model. The model included fixed effects of breed, parity number, and sampling session. Stage of lactation was fitted as Wilmink-type lactation curves. Random effects included additive polygenic ancestry, permanent animal effects, and residual. The distribution of Pu approximated normality except for a single sample with very high Pu and very low pH. This sample came from a cow diagnosed independently with ketosis. For the remaining samples, it was shown that Pu has low to moderate heritability (0.12) and is only moderately repeatable (0.21). Based on a small data set, it is tentatively concluded that individual differences between cows exist in their Pu, and individual differences presumably result from genetic differences. However, it remains unclear if cows with genetically lower or higher Pu will perform better on a low-P diet. **Key words:** phosphorus retention, urine samples, dairy cows

Short Communication

The main P excretion route for adult cattle is through feces, but excretion of aberrantly high amounts of P through urine in individual animals has been reported in the scientific literature (reviewed by Breves and Schröder, 1991). However, reports on phenotypic and genetic variation in urinary P excretion are scarce; therefore, the current paper focuses on phenotypic variation between individual dairy cows in urinary excretion of P.

Urinary excretion of P in ruminants is usually insignificant due to efficient renal reabsorption of filtered P. Model estimates of the urinary P excretion from lactating dairy cows fed dietary P close to their requirement are 0.002 g/kg of BW (NRC, 2001) or 1% of absorbed P (Hill et al., 2008) corresponding to 1.0 to 1.4 g of P/d. Lower and higher urinary P excretions have been measured with lower respectively higher quantities of dietary P. Dietary P concentrations from 2.3 to 3.4 g/kg of DM did not influence the urinary P excretion in a long-term study with lactating dairy cows (0.038 g/d; Puggaard et al., 2014). In a short-term study with lactating dairy cows fed varying feed forage particle size and dietary urea at a low dietary P concentration (2.5 g/kg of DM) urinary P excretion was 0.035 g/d (Puggaard et al., 2013). Urinary excretion was 0.27 to 0.43 g of P/d in dairy cows 3 to 11 wk in lactation when fed 0.34% dietary P, 0.58 to 1.63 g of P/d when cows were fed 0.51% dietary P, and 2.26 to 6.08 g of P/d when cows were fed 0.67% dietary P (Knowlton and Herbein, 2002). Similar effects of dietary P on mean urinary P excretion were observed in more studies (Morse et al., 1992; Wu et al., 2001), although a lower excretion was found in other studies (Odongo et al., 2007; Ferris et al., 2010).

Urinary P excretion can become quantitatively significant in case of high plasma P concentrations (approximately 2 mmol/L) or if saliva secretion is inhibited by feeding diets low in physical fiber (Scott et al., 1985; Scott, 1988; Scott and Buchan, 1988; Knowlton

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and Herbein, 2002; Bravo et al., 2003; Wu, 2005; Hill et al., 2008). Several studies have reported the occurrence of one or a few experimental animals with exceptionally high urinary P excretion not related to any of the mentioned causes (reviewed by Breves and Schröder, 1991). Manston and Vagg (1970) concluded that a small proportion of cows have a tendency to excrete relatively large amounts of phosphate in the urine, and similar observations were reported in newer studies with high-yielding dairy cows (Wu et al., 2000). Genetic variation in urinary P was detected in an experiment with 4 sets of triplet lambs (Field et al., 1984; Field and Woolliams, 1984) and individual differences were reported by Sato (1981) in sheep and goats. A genetic predisposition to deviating high urinary P might be caused by single gene mutations or be caused by polygenic quantitative genetic variation. In either case, individual animals will have urine P concentrations that are less variable compared with randomly taken samples, and concentrations are therefore repeatable.

We hypothesized that urinary P concentrations (**Pu**) would be normally distributed except if some individual animals have aberrantly high Pu. The objectives of the present study were to estimate individual and genetic cow variation in Pu, and to detect cows with aberrant Pu.

This experiment used repeated sampling of urine from a cross section of cows at the Danish Cattle Research Centre (Foulum, Denmark; Table 1). The cows were sampled during 6 testing sessions (January to March 2009, 2-wk intervals) with the aim of obtaining on average 2 samples per cow to allow for studying repeatability.

Each sample only took few minutes to obtain and each testing session lasted less than 2 h, during which as many cows as possible were sampled. The lactating cow herd (5–391 DIM) contained 2 groups of Holsteins (103 sampled cows) and 1 group of Jerseys (36 cows sampled). Each group was assigned to an automated milking system (VMS, DeLaval, Tumba, Sweden). All cows were fed a partially mixed ration, which was fed ad libitum (DM composition: 36.7% maize silage, 35.5% grass silage, 11.4% rolled barley, 8.9% soy expeller, 5.8% canola expeller, 1.7% mineral and salt mix), and supplementary concentrates during milking restricted to total 3 kg/d. The total feed intake from the mixed ration (Insentec, RIC-system, Marknesse, the Netherlands) and of concentrates provided to cows was 19.4 ± 5.9 kg of DM/d (mean $\pm$ SD for all cows across breeds) containing 4.04 g of P/kg of DM. The P content in feed was determined from composition and assayed content in ingredients. Daily milk yield on each test day was used as a reference trait to compare with urine-based traits.

Urine samples from 139 cows were used for the present study, covering all stages of lactation and parities from 1 to 5 (Table 1). Urine samples (100 mL) were obtained following mild manual stimulation of the rear udder escutcheon area, or in some cases without stimulation. Sampling sessions lasted up to 2 h. Urinary pH was measured immediately after sampling (HI-98127, Hanna Instruments Inc., Woonsocket, RI), before aliquots were transferred to 5-mL tubes (Sarstedt AG & Co, Nümbrecht, Germany), and stored frozen (-25°C) until assayed.

Table 1. Data overview and estimates of fixed effects and random variance parameters for concentrations of P and creatinine in urine of dairy cows

Item	Grouping	n	Mean $\pm$ SD	Fixed effects ¹					Sampling date	Repeatability $t \pm \text{SE}$	Heritability $h^2 \pm \text{SE}$
				Breed	Parity	β_1	β_2	β_3			
Cows	All	139									
	Holstein	103									
	Jersey	36									
DIM	All	300	174 $\pm$ 110								
Live weight (kg)	All	300	599 $\pm$ 100								
	Holstein	233	640 $\pm$ 69								
	Jersey	67	456 $\pm$ 41								
Phosphorus (mmol/L)	All	300	0.069 $\pm$ 0.029	NS	NS	NS	NS	*	NS	0.21 $\pm$ 0.07	0.12 $\pm$ 0.17
Creatinine (mmol/L)	All	300	5.56 $\pm$ 2.18	***	**	***	*	NS	***	0.38 $\pm$ 0.07	0.05 $\pm$ 0.17
P-to-Creatinine ratio	All	300	1.37 $\pm$ 0.71	NS	***	NS	NS	NS	NS	0.13 $\pm$ 0.08	0.11 $\pm$ 0.16
pH	All	297	8.03 $\pm$ 0.13	***	NS	NS	NS	NS	***	0.16 $\pm$ 0.07	0.08 $\pm$ 0.14
Milk yield (kg/d)	All	300	32.6 $\pm$ 11.9	***	***	***	***	**	**	0.49 $\pm$ 0.07	0.36 $\pm$ 0.29
		233	35.4 $\pm$ 11.4								
		67	22.8 $\pm$ 7.9								

¹F-test for fixed effects.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Urine was assayed for concentration of inorganic phosphorus (mmol/L) using an ammonium molybdate-based kit (Cobas PHOS kit, Roche Diagnostics GmbH, Mannheim, Germany; on a Roche-Hitachi 912 auto-analyzer). Urine was also assayed for concentration of creatinine (**CREATu**; mmol/L) using an enzymatic colorimetric kit. Assays for Pu and CREATu both had total coefficient of variation below 2.5% based on 2 reference samples.

The Pu, CREATu, the ratio between Pu and CREATu, and urine pH value were used as response variables. The variables were approximately normally distributed and transformation was not imposed before ANOVA. One sample deviating strongly in both pH and Pu was detected and omitted from further analysis because the cow was independently diagnosed with ketosis on that day.

A linear mixed model was fitted to each variable to estimate fixed effects of stage of lactation, breed, sampling day, parity, and random variance belonging to individual cows (σ_{cow}^2). For the genetic analysis, ancestral relationships between animals were obtained by tracing their parentage at least 3 generations back through the national database. The between-cow variance (σ_{cow}^2) component was further partitioned into additive genetic background (σ_a^2) and the permanent environment (σ_{pe}^2) of that particular animal:

$$y_{ijklm} = \alpha + \beta_1 e^{-0.05t} + \beta_2 t + \beta_3 t + \text{DATE}_t \\ + \text{BREED}_j + \text{PARITY}_k + \text{COW}_{jkl} + \varepsilon_{ijklm},$$

where y was one of the response variables at a time and α is the intercept. The systematic factors were sampling session (DATE_i , 6 levels), breed (BREED_j , Holstein or Jersey), parity (PARITY_k , 1, 2, and ≥ 3), cow (COW_{jkl}), and sample (m), and ε_{ijklm} is the error term. Stage of lactation was modeled as a continuous curve with 3 coefficients on DIM (t ; $\beta_1, \beta_2, \beta_3$) as also used for milk yield (Wilmink, 1987). Random variance from cows (σ_{cow}^2) and residual (σ_e^2) were assumed normally distributed. Variance components were used to calculate repeatability as the intraclass correlation for cows,

$$t = \sigma_{\text{cow}}^2 / (\sigma_{\text{cow}}^2 + \sigma_e^2),$$

being a measure of individuality. From the variance components the heritability coefficient was calculated as

$$h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2).$$

The ANOVA and variance component estimates were obtained using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), and the software package DMU (Madsen and Jensen, 2013).

One urine sample was omitted from further statistical analysis, as the cow was diagnosed as ketotic on the day of sampling due to highly elevated milk levels of BHB detected by the HerdNavigator herd management system (Lattec, Hillerød, Denmark). This sample also had very low pH (5.9) and extremely high values for Pu (2.05 mmol/L) and CREATu (2.98 mmol/L). Cattle on energy-rich diets low in fiber and high in starch have previously been reported to have disturbed rumen function leading to acidosis with low pH in urine and also with high Pu, as seen in steers (Reed et al., 1965). However, it is not known if the phosphate in urine has a specific role as buffer or if it is leaked for another purpose (Bravo et al., 2003). However, studies on anion-cation balance have also shown effects on Pu and on pH in urine (Ramos-Nieves et al., 2009; Grünberg et al., 2011); thus, pH and pU may be useful as indicators of mineral supply. For our study it was important to have the pH values of urine samples available to correctly classify this sample as caused by ketosis rather than confusing it with a genetic defect.

The distribution of Pu in Holstein and Jersey cows resembled normality (visual inspection of distribution plots), with a somewhat right-skewed distribution. It was also clear that some cows had very low Pu. Distributions for CREATu in Jersey and Holstein differed because of different mean values. The ratio of Pu to CREATu had skewed distributions, probably as a result of the way this ratio is calculated and the distributions of the 2 variables going into the ratio.

The excretion of P through urine in dairy cows accounts for only a small fraction of the total P excreted, and Pu will be low (Scott et al., 1985; Scott and Buchan, 1988; Knowlton and Herbein, 2002; Bravo et al., 2003; Hill et al., 2008). However, many dairy herds are oversupplied with P (Powell et al., 2002; Rotz et al., 2002) as compared with recommended dietary P concentrations (NRC, 2001), leading to higher concentrations of P in plasma and urine (Scott et al., 1985; Challa and Braithwaite, 1988; Scott, 1988). Increased Pu has also been observed in animals where saliva secretion is inhibited by feeding diets low in physical fiber (Scott et al., 1985; Scott and Buchan, 1988; Knowlton and Herbein, 2002; Bravo et al., 2003; Hill et al., 2008).

The systematic change in Pu over the lactation was quantitatively small (Figure 1). However, a decrease in Pu was found in early lactation, followed by a slow and small incline until about 7 mo in lactation, when concentrations decreased again. Only the decrease in

late lactation was significant (Table 1). In contrast, CREATu decreased in early lactation to reach a nadir at around 50 DIM, followed by a slow increase over the rest of the lactation (Table 1). Consequently, the ratio between Pu to CREATu increased in early lactation to reach a plateau between 90 and 180 DIM before it decreased toward the end of lactation. The pH value was stable throughout lactation (data not shown). In other studies, CREATu has been used to adjust Pu concentrations by expressing those as ratios (Estermann et al., 2002). In the current study, CREATu was strongly influenced by stage of lactation effects, more than the Pu concentrations themselves, so that the ratio was also affected by stage of lactation. Consequently, the ratio between Pu to CREATu was not more useful for studying urinary P excretion than Pu concentrations themselves.

Holstein and Jersey cows did not differ significantly in Pu. Holsteins had significantly higher CREATu than Jerseys ($P < 0.001$; Table 1), and consequently the Pu-to-CREATu ratio was higher in Jersey than in Holstein. Jersey cows had 0.09 units higher urinary pH than Holstein cows (Jersey = 8.10 ± 0.02 ; Holstein = $8.01 \pm$

0.02 ; $P < 0.01$; Table 1). Parity only affected CREATu with the highest concentrations in young cows (parity 1) and no difference between second and later parities.

We found no indication of a specific subpopulation of samples with either high or low Pu values, except for the one sample from the ketotic cow. The effect of individuals on the concentration of Pu expressed as the repeatability was moderate ($t = 0.21$; Table 1); likewise, CREATu was moderately repeatable. The urinary pH had lower repeatability than Pu and CREATu; in comparison, milk yield had the highest repeatability ($t = 0.49$). The heritability estimate for milk yield was 0.36, which was higher than the estimates for Pu, CREATu, and pH (range = 0.05–0.12; Table 1). Standard errors of heritability estimates were in the range between 0.14 and 0.29 for all variables; thus, these preliminary heritability estimates should only be viewed as indications. The results of our study showed that cows had large individual variation in their Pu concentration, and that a large part of the individual variation was likely to have an additive genetic background. However, the estimated heritability of Pu concentration was lower than heritability estimates from sheep studies (Field

Figure 1. Concentrations of urinary P (Pu) from Holstein and Jersey cows at various stages of lactation (DIM), together with an overall fitted curve using model 1 (Pred_P). The outlier with 2.05 mmol/L of Pu from a ketotic cow as described in the text was excluded from this figure. Color version available online.

and Woolliams, 1984) and could not be compared with a study in goat and sheep that did not quantify individual or genetic variation (Sato, 1981). Heritability estimates for Pu based on cattle data are scarce, and the present results are in support of the previous findings in sheep, jointly indicating that Pu is a moderately repeatable trait and that genetic variation is the basis of the individual differences. However, we found no obvious indication of Pu being severely affected by strong single gene effects, as the only very high Pu value detected could be traced back to a case of ketosis. Thereby, our study does not indicate a genetic defect leading to the urinary excretion of large amounts of P in dairy cows.

The current study was based on data from a single experimental herd with repeated cross-sectional spot sampling from a small cohort of high-yielding cows of 2 breeds. To obtain reliable genetic parameters, data from larger cohorts are needed. The relative ease with which urine samples were collected using manual stimulation seemed effective for such a purpose and involved only very mild interactions with the cows; as such it is widely applicable. A further application might be in monitoring mineral supply and anion-cation balance in diets. Given the low repeatability, experimental power in any study is greatly enhanced by repeated sampling of each cow.

In conclusion, individual differences between cows were detected in their urine P concentrations, and individual differences are presumably anchored in genetic differences. We saw no indications that individual dairy cows consistently excrete very large amounts of phosphorus. Creatinine-to-Pu ratio was not useful for studying Pu excretion due to stage of lactation effects on creatinine concentrations. The Pu concentrations are seen as indicators of P recycling and absorption efficiency. Thereby, a subject for further research would be to investigate if genetic selection for cows demanding less P in diets is possible and feasible.

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