Measurement of urine pH and net acid excretion and their association with urine calcium excretion in periparturient dairy cows

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ABSTRACT

Urine pH (U_{pH}) and net acid excretion (NAE) are used to monitor the degree of systemic acidification and predict the magnitude of resultant hypercalciuria when feeding an acidogenic ration to control periparturient hypocalcemia in dairy cattle. The objectives of this study were to evaluate the diagnostic performance of urine dipstick and pH paper for measuring U_{pH} , and to characterize the U_{pH} –NAE relationship and the association of urine Ca concentration ([Ca]) with U_{pH} and NAE. Urine samples ($n = 1,116$) were collected daily from 106 periparturient Holstein-Friesian cows fed an acidogenic ration during late gestation. Net acid excretion was measured by titration, and U_{pH} was measured by a glass-electrode pH meter (reference method), Multistix-SG urine dipsticks (Siemens Medical Solutions Inc., Ann Arbor, MI), and Hydriion pH paper (Micro Essential Laboratory Inc., Brooklyn, NY). Diagnostic performance was evaluated using Spearman correlation coefficient (r_s), Bland–Altman plots, and logistic regression. Urine pH measured by urine dipstick ($r_s = 0.94$) and pH paper ($r_s = 0.96$) were strongly associated with U_{pH} . Method-comparison studies indicated that the urine dipstick measured an average of 0.28 pH units higher, and pH paper 0.10 pH units lower, than U_{pH} . Urine [Ca] was more strongly associated with U_{pH} ($r_s = -0.65$) than NAE ($r_s = 0.52$). Goals for controlling periparturient hypocalcemia under the study conditions were $U_{pH} < 6.22$ and < 6.11 , based on achieving urine [Ca] ≥ 5 mmol/L and estimated urinary Ca excretion ≥ 4 g/d, respectively. Urine pH was as accurate at predicting urine [Ca] as NAE when $U_{pH} > 6.11$. We conclude that pH paper is an accurate, practical, and low-cost cow-side test for measuring U_{pH} and provides a clinically useful estimate of urine [Ca].

Key words: urine dipstick, pH paper, net acid excretion, acidogenic diet, hypercalciuria

INTRODUCTION

Feeding a low-DCAD ration during late gestation is an effective way to decrease the incidence of periparturient hypocalcemia (Charbonneau et al., 2006; Constable et al., 2017; Lean et al., 2019). Induction of a strong ion (metabolic) acidosis, increased Ca absorption from the gastrointestinal tract, and the resultant aciduria and hypercalciuria are considered prerequisites for successfully controlling periparturient hypocalcemia when low-DCAD (acidogenic) rations are fed (Grünberg et al., 2011; Megahed et al., 2018a). Ingestion of an acidogenic ration induces aciduria due to the dietary-induced decrease in urinary strong ion difference (Constable et al., 2009). Aciduria induces hypercalciuria by decreasing Ca reabsorption in the distal convoluted tubules and connecting tubules of the urinary tract (Grünberg et al., 2011; Martín-Tereso and Verstegen, 2011; Megahed et al., 2018a). The mean urine Ca excretion in late-gestation dairy cows fed a non-acidogenic ration is typically < 1 g/d but increases when an acidogenic ration is fed (Roche et al., 2003; Grünberg et al., 2011; Megahed et al., 2018a); the availability of additional Ca for reabsorption by the renal tubules appears to play an important role in decreasing the incidence and severity of periparturient hypocalcemia in dairy cows fed acidogenic diets in late gestation (Grünberg et al., 2011; Martín-Tereso and Verstegen, 2011; Megahed et al., 2018a).

Measuring urinary Ca concentration ([Ca]) appears to provide the best method for evaluating the effectiveness of feeding an acidogenic ration, based on our current understanding of Ca homeostasis in periparturient dairy cows (Grünberg et al., 2011; Martín-Tereso and Verstegen, 2011; Megahed et al., 2018a). Although a suitable on-farm method for measuring urine [Ca] is not currently available, measurement of urine pH (U_{pH}) provides a practical and inexpensive cow-side

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method for monitoring the degree of hypercalciuria and systemic acidification when feeding acidogenic rations (Constable et al., 2009). A range of values for mean herd U_{pH} (5.5–7.0) have been proposed for optimal control of periparturient hypocalcemia in Holstein-Friesian cows (Constable et al., 2009). Conversely, $U_{pH} > 8.25$ at 48 h before parturition indicated an increased risk of recumbency in periparturient dairy cows (Seifi et al., 2004). It is therefore important to determine whether U_{pH} can be used to predict urine [Ca], because U_{pH} can be measured rapidly and at low cost on the farm. Accordingly, the first objective of this study was to characterize the association between urine [Ca] and U_{pH} in periparturient dairy cows.

The reference method for measuring U_{pH} is the glass-electrode pH meter, and relatively inexpensive pH meters are available for on-farm use (Kandeel et al., 2019). However, the requirement for regular calibration and user training decreases the practicality of using glass-electrode pH meters for cow-side measurement of U_{pH} . Multistix-SG urine reagent dipsticks (Siemens Medical Solutions Inc., Ann Arbor, MI) are widely available in North America and provide an inexpensive (\$0.30 per test) cow-side method for measuring U_{pH} in increments of 0.5 or 1.0 pH units between pH of 5.0 and 8.5. Hydrion pH paper (Micro Essential Laboratory Inc., Brooklyn, NY) is an inexpensive (\$0.04 per test) colorimetric test paper designed to measure urine and saliva pH between 5.5 and 8.0 in increments of 0.2, 0.3, or 0.4 pH units. The pH paper contains proprietary indicator dyes that change from yellow-green to green and finally dark blue when in contact with fluid of increasing pH. We are not aware of any published method-comparison studies that have formally evaluated the accuracy of urine dipsticks and pH paper in measuring U_{pH} in cattle. We hypothesized that Multistix-SG urine reagent strips and Hydrion pH paper would provide accurate, low-cost, practical cow-side methods for measuring U_{pH} in periparturient dairy cattle. The second objective of this study was therefore to determine the clinical accuracy of using Multistix-SG strips and Hydrion pH paper to measure U_{pH} in periparturient dairy cows.

Urinary net acid excretion (NAE) provides a more accurate method of predicting urinary [Ca] in healthy cattle than ration DCAD (Gelfert et al., 2004), and provides a more sensitive and accurate method of assessing systemic acid-base balance in healthy cattle than U_{pH} (Vagnoni and Oetzel, 1998; Constable et al., 2009; Grünberg et al., 2011). Urine NAE is commonly measured in late-gestation dairy cattle in northern Europe, and it is recommended that NAE should be > -50 mmol/L and urine [Ca] should be increased to ≥ 5 mmol/L for optimal control of periparturient hypocalcemia (Bender et al., 2003; Gelfert et al., 2004). We

were therefore interested in comparing the accuracy of U_{pH} and NAE in predicting urine [Ca]. Accordingly, the third objective of this study was to characterize the association between urine [Ca] and U_{pH} , to compare the association with that between urine [Ca] and NAE, and to characterize the U_{pH} –NAE relationship. An overarching goal of this study was to identify a practical method for monitoring the efficacy of feeding acidogenic diets to late-gestation dairy cows as part of an effective control program for periparturient hypocalcemia.

MATERIALS AND METHODS

All methods were evaluated and approved by the Purdue Animal Care and Use Committee. The prospective study reported here using a convenience sample was part of a larger series of studies investigating energy and mineral homeostasis during the periparturient period, and the prediction of parturition and dystocia in Holstein-Friesian cattle. Additional results have been published elsewhere (Megahed et al., 2015, 2016, 2017, 2018a,b; Hiew et al., 2016).

Animals and Sample Collection

Urine samples ($n = 1,124$) were obtained by perineal stimulation and free catch of voided urine from 106 healthy periparturient Holstein-Friesian cows (34 primiparous, 72 multiparous) fed an acidogenic TMR during late gestation (DCAD = -17.5 mEq/100 g of DM) and a standard lactating cow TMR (DCAD = $+20.0$ mEq/100 g of DM) where DCAD = $([Na^+] + [K^+]) - ([Cl^-] + [S^{2-}])$, as described elsewhere (Megahed et al., 2018a). Up to a total of 18 urine samples were collected daily from each cow starting 1 to 12 d before parturition, on d 0 (day of calving), d 1, 2, 3, and then at approximately weekly intervals from d 4 to d 35 lactation. Urine was collected into 15-mL vials that were completely filled with urine, immediately closed to minimize exposure to air (which can lead to loss of CO_2 and increased urine pH; Bender and Staufenbiel, 2003), and stored at $37^\circ C$ in a water bath. Body weight was calculated from the thoracic circumference (heart girth) as described by Megahed et al. (2018a).

Measurement of Urine pH

We measured U_{pH} at $37^\circ C$ within 15 min of collection using a rapid-response glass-electrode pH meter (Orion 520A; Thermo Electron Corporation, Beverly MA). The meter measured solution pH in increments of 0.001 with a reported accuracy of ± 0.002 and was used as the reference method. The meter was calibrated daily using 2 buffer solutions of pH 4.01 and 7.00 at room

temperature (Oakton Instruments, Vernon Hills, IL). The glass electrode was rinsed using deionized water, dried with a small disposable towel (Kimwipes, Kimtech; Kimberly-Clark, Roswell, GA), and dipped into a well-mixed urine sample. The U_{pH} reading was recorded after it stabilized within a few seconds.

Multistix-10-SG urine reagent dipsticks (Siemens Medical Solutions Inc.) were used to measure urine pH ($U_{\text{pH-MS}}$). The strips were originally designed to detect urinary tract infection and kidney disorders in humans and use a double-indicator colorimetric system (methyl red, bromothymol blue) to produce a pH-dependent change in color from orange (pH = 5.0) passing through yellow and green to greenish blue (pH = 8.5; Kandeel et al., 2019). The test strip was dipped into the urine sample and then blotted by touching the edge of the strip to the paper towel to remove excess urine. The strip was then left to stand on a flat clean surface with the indicator pad facing up. After 60 s of reaction time, the color change of the test strip was recorded and compared with the color chart provided by the manufacturer.

Finally, we used Hydrion pH paper for urine and saliva (Micro Essential Laboratory Inc.) to measure urine pH ($U_{\text{pH-HP}}$). Approximately 5 cm of pH paper was torn from the roll and dipped into the urine sample. The pH paper was then removed and its color immediately compared with the color chart provided by the manufacturer.

Measurement of NAE and Urine Ca Concentration

Urine samples were stored at -20°C for up to 2 mo after pH measurement, thawed at room temperature, and the sediment homogeneously mixed by vortexing for 10 s immediately before measurement of NAE and urine [Ca]. Urine NAE was determined by titration as described elsewhere (Constable et al., 2009; Grünberg et al., 2011). In brief, 2 mL of urine was acidified using 1 *N* HCl to pH <4 and heated to a slow boil for at least 2 min to expel CO_2 . Acidification and heating removed bicarbonate from the urine sample, dissolved phosphate crystals, and produced a solution containing strong cations and anions, as well as non-bicarbonate buffers such as phosphate and creatinine (Constable et al., 2009). The urine was allowed to cool to room temperature and then back-titrated with 0.1 *N* NaOH to a solution pH of 7.40. Formaldehyde was then added, which lowered the pH, and the sample back-titrated with 0.1 *N* NaOH for a second time to measure urine ammonium concentration ($[\text{NH}_4^+]$). Use of a 0.1 *N* NaOH solution further diluted the urine sample and prevented precipitation of phosphates by Ca and magnesium during alkalization. The volumes of 1 *N* HCl, 0.1 *N* NaOH, and

pH at each step were recorded, and NAE and $[\text{NH}_4^+]$ were calculated from the recorded values as described (Constable et al., 2009).

Urine [Ca] and creatinine (**Crea**) concentration ($[\text{Crea}]$) were measured spectrophotometrically (AU680; Beckman Coulter Inc., Brea, CA) using the cresolphthalein and picric acid methods, respectively, at the Veterinary Diagnostic Laboratory at the University of Illinois at Urbana-Champaign. The analyzer method acidified urine samples to a pH <2 with 6 *N* HCl to dissolve all Ca salts and dilute the sample. Calcium ions were then reacted with o-cresolphthalein complexone in an alkaline solution to form an intense violet-colored complex, with the reaction occurring in the presence of 8-hydroxyquinoline to remove interference by magnesium and iron. The absorbance of the Ca-o-cresolphthalein complexone was measured spectrophotometrically, with the increase in sample absorbance being directly proportional to the sample [Ca]. The manufacturer reported within-run CV <1.3% and between-run CV <2.5%. We calculated urine Ca to Crea concentration ($[\text{Ca}]/[\text{Crea}]$) to account for the effect of variations in urine concentration (free water content) on urine [Ca]. Urine Ca excretion (g/d) for each 24-h period was calculated from urine [Ca], urine [Crea], and the calculated BW (kg), assuming a constant urine Crea excretion of 0.029 g/kg of BW (Valadares et al., 1999; Martín-Tereso and Verstegen, 2011), such that urine Ca excretion = $([\text{Ca}]/[\text{Crea}]) \times 0.029 \times \text{BW}$.

Urine pH-NAE Relationship

Acid is excreted in urine via 3 main pathways: (1) proton excretion via $\text{H}^+\text{-K}^+\text{-ATPase}$ and $\text{H}^+\text{-ATPase}$, with protons being buffered by the 2 main buffers in urine, phosphate, and Crea; (2) excretion of NH_4^+ from renal tubular metabolism of glutamine with simultaneous generation of HCO_3^- that is available systemically; and (3) increased excretion of phosphate that can buffer urine acidity and is measured as titratable acidity (**TA**; Lemann et al., 2003). Net acid excretion is quantified using the following equation: $\text{NAE} = \text{TA} + [\text{NH}_4^+] - [\text{HCO}_3^-]$, with $[\text{HCO}_3^-]$ being dependent on the urine carbon dioxide tension (**Pco₂**) and strong ion difference (Constable et al., 2009). Experimental studies have demonstrated that phosphate is quantitatively unimportant as a urine buffer in ruminants with metabolic acidosis and acidemia (Scott, 1969; Scott et al., 1971; van Mosel et al., 1993), and bicarbonate is quantitatively unimportant whenever $U_{\text{pH}} < 6.0$ to 6.4, because urine $[\text{HCO}_3^-]$ is quantitatively low in acidic urine (Lemann et al., 2003; Constable et al., 2009). Consequently, increased excretion of the ammonium ion, resulting in increased urine $[\text{NH}_4^+]$ and NAE, is

the predominant mechanism for ensuring acid–base homeostasis in the presence of a nutritionally induced systemic acidosis in ruminants and all animals studied to date (Weiner and Verlander, 2017).

Although increased urine $[\text{NH}_4^+]$ directly increases NAE and therefore indicates the presence and magnitude of systemic acidosis, an increase in urine $[\text{NH}_4^+]$ does not cause a corresponding decrease in U_{pH} . This is because an increase in urine $[\text{NH}_4^+]$ increases U_{pH} unless the magnitude of the increase in $[\text{NH}_4^+]$ approximates the magnitude of the increase in NAE, in which case U_{pH} is unchanged. This outcome has been previously reported in bovine urine (Scott et al., 1971) and is best understood by examining the physicochemical equation for ruminant urine relating U_{pH} to NAE, such that: $U_{\text{pH}} = c + \log_{10}([\text{NH}_4^+] + d - \text{NAE})$, where $c = \text{pK}_1' - \log_{10}(S \times \text{Pco}_2)$ from the Henderson–Hasselbalch equation and d is the actual strong ion difference when urine pH is 7.40 (Constable et al., 2009). Constable's physicochemical equation for urine (Constable et al., 2009) indicates that a marked increase in urine $[\text{NH}_4^+]$, in response to increased systemic acidity, does not result in further decreases in U_{pH} in ruminants because an increase in urine $[\text{NH}_4^+]$ directly increases NAE. The clinically relevant outcome of these physicochemical relationships is increased variability in the U_{pH} –NAE relationship in ruminants with marked aciduria (Constable et al., 2009). The range of values where the U_{pH} –NAE relationship exhibits increased variability can be identified in dairy cows fed an acidogenic diet by first characterizing the urine $[\text{NH}_4^+]$ –NAE relationship to identify the NAE value where urine $[\text{NH}_4^+]$ starts to rapidly increase, and to then apply this NAE value to the U_{pH} –NAE relationship and calculate the corresponding U_{pH} value where urine $[\text{NH}_4^+]$ starts to rapidly increase.

Statistical Analysis

Statistical analyses were performed using SAS 9.4 (PROC CORR, PROC NLMIXED; SAS Inc., Cary, NC) and MedCalc Statistical Software version 18.6 (MedCalc Software bvba, Ostend, Belgium); $P < 0.05$ was considered significant. Spearman correlation coefficients (r_s) were calculated to characterize the curvilinear relationships between U_{pH} , $U_{\text{pH-MS}}$, and $U_{\text{pH-HP}}$, urine $[\text{Ca}]$, urine Ca to Crea ratio, NAE, and urine $[\text{NH}_4^+]$. We did not include daily calculated urine Ca excretion in this calculation because it was calculated from urine $[\text{Ca}]$. One of the assumptions of correlation analysis (independence of samples) was met for this analysis by randomly selecting 1 urine sample from each cow from urine samples obtained over a 7-d period (d 3 antepartum to d 3 postpartum) using a random

number generator (Microsoft Excel 2010; Microsoft Corp., Redmond, WA). We selected the 7-d time span for randomization because it provided a large range of U_{pH} values in cows consuming an acidogenic diet in late gestation and a lactating cow diet immediately after parturition (Megahed et al., 2018a).

Method comparisons evaluated the linear relationship between $U_{\text{pH-MS}}$ or $U_{\text{pH-HP}}$ and the reference method (U_{pH}) using scatterplots and Bland–Altman difference plots. The upper and lower limits of agreement were calculated from the bias $\pm 1.96 \times \text{SD}$; the bias estimate from Bland–Altman plots reflected the mean bias over the range of measured values (Bland and Altman, 2007; Giavarina, 2015). The acceptable limits for $U_{\text{pH-MS}}$ and $U_{\text{pH-HP}}$ were considered to be ≤ 0.5 pH units compared with the reference method (U_{pH}). Acceptable limits were based on the results of other studies (Heuter et al., 1998; Kwong et al., 2013; Abbott et al., 2017), the resolution of $U_{\text{pH-MS}}$ measurement, and personal observations that variations in U_{pH} of this magnitude commonly occur over a 24-h period in cattle fed a TMR.

Accurate minimum target values for U_{pH} and NAE that indicate effective control of periparturient hypocalcemia when an acidogenic ration is fed to late-gestation multiparous dairy cows have yet to be determined. Nevertheless, for effective control of periparturient hypocalcemia a research group in Germany recommends that urine $[\text{Ca}]$ should be ≥ 5 mmol/L (Bender et al., 2003; Gelfert et al., 2004), whereas a research group in the United States suggests that daily urine Ca excretion should be ≥ 4 g (Grünberg et al., 2011; Megahed et al., 2018a). Therefore, mixed-models analysis was applied to data from multiparous cows to characterize the relationship between urine $[\text{Ca}]$, urine Ca to Crea ratio, and daily urinary Ca excretion (dependent variables) and NAE or U_{pH} measured by the glass-electrode pH meter. The 3 dependent urine Ca indices were regressed against NAE, or logarithmically transformed to base 10 and regressed against U_{pH} , to produce linear relationships for analysis based on known physicochemical relationships and derived mathematical equations (Lemann, 1999; Lemann et al., 2003; Constable et al., 2009), as well as examination of residual plots. The mixed-models procedure used an unstructured covariance matrix and random intercept. This method accounted for repeated measures on each cow and produced a common slope estimate and a mean estimate for the intercept that was adjusted for each cow. Values for pH and NAE when urine $[\text{Ca}] \geq 5$ mmol/L and estimated daily urine Ca excretion ≥ 4 g were calculated from the developed equations. These cutpoints were selected because they currently provide the best operational definitions for effective control of periparturient hypocalcemia.

We used mixed-models segmented linear regression (Gonçalves et al., 2016; Trefz et al., 2018) to characterize the urine $[\text{NH}_4^+]$ -NAE relationship for all urine samples, adjusting for the effect of cow, with μ_0 representing the random background coefficient assuming the distribution of random effects to be normal with mean (μ_0) = 0 and variance = s^2e . A mixed models approach was used because up to 10 data points were obtained from each cow. The model equations assumed a positive linear relationship between $[\text{NH}_4^+]$ and NAE when $\text{NAE} \leq X_c$ (X_c = the cutpoint identified by segmented regression) and a second positive linear relationship with higher slope between $[\text{NH}_4^+]$ and NAE when $\text{NAE} > X_c$, such that: $\text{NAE} = \mu_0 + b_0 + b_1 \times \text{NAE}$ when $\text{NAE} \leq X_c$, and $[\mu_0 + b_0 + b_1 \times X_c + b_2 \times (x - X_c)]$ when $x > X_c$. The segmented regression approach permits objective identification of the NAE value that indicates a rapid increase in urine $[\text{NH}_4^+]$. Accurate identification of this cutpoint is clinically important because urine pH becomes less accurate than NAE in predicting the presence and severity of metabolic acidosis when quantitatively important concentrations of NH_4^+ are present in urine.

Mixed-models nonlinear regression was then used to characterize the curvilinear U_{pH} -NAE relationship, such that $U_{\text{pH}} = a + \log_{10}(b - \text{NAE})$. This equation was derived from the physicochemical equation for bovine urine, with the value for a approximating the estimated value in bovine urine for $[\text{pK}_1' - \log_{10}(S \times \text{Pco}_2)]$ from the Henderson-Hasselbalch equation and the value for b is a constant (Constable et al., 2009). The equation was expressed as the equivalent algebraic equation to assist in producing an iterative solution when $\text{NAE} < b$, such that $\text{NAE} = b - 10^{(U_{\text{pH}} - a)}$. The nonlinear mixed model was fit by applying the adaptive Gaussian quadrature approximation for the marginal likelihood function and dual quasi-Newton optimization. Model fit was evaluated using plots of actual and predicted values and residual plots. The U_{pH} value identified as corresponding to a daily urine Ca excretion ≥ 4 g ($U_{\text{pH-DailyCa} \geq 4\text{g}}$) was then applied to the fitted equation to identify the corresponding NAE cutpoint value.

Binary logistic regression was used to characterize the relationship between U_{pH} and $U_{\text{pH-MS}}$ or $U_{\text{pH-HP}}$ ($1 = U_{\text{pH}} < U_{\text{pH-DailyCa} \geq 4\text{g}}$; $0 = U_{\text{pH}} \geq U_{\text{pH-DailyCa} \geq 4\text{g}}$). We evaluated the adequacy of the logistic regression model fit using plots of deviance influence statistics against the predicted values. We constructed receiver operating characteristic (ROC) curves for each logistic regression model and calculated the area under the curve (AUC) as a global index of test performance (Swets, 1988). Sensitivity (Se) and specificity (Sp) were calculated at the optimal cutpoint of the ROC determined by the

Youden index (the cutpoint where the following expression had its maximum value: $\text{Se} + \text{Sp} - 1$), which equally weighted Se and Sp. The positive likelihood ratio (+LR) was calculated as $+LR = \text{Se}/(1 - \text{Sp})$ (Grimes and Schulz, 2005). The kappa coefficient (κ) was calculated at the optimal cutpoint identified from the ROC to characterize the level of agreement between the tests (Landis and Koch, 1977).

RESULTS

We identified 8 data points as outliers during statistical analysis and eliminated them from further analysis; inclusion of all 8 data points yielded similar variable estimates during mixed-models segmented linear and mixed-models nonlinear regression but larger 95% CI for the estimates. Descriptive statistics summarizing U_{pH} values measured by the pH meter in 1,116 urine samples for the different pH categories measured by the Multistix-SG urine dipstick and Hydrion pH paper are presented in Table 1. Urine pH measured by the reference method ranged from 5.00 to 8.58.

Urine Ca-pH Relationship

Urine [Ca], [Ca] to [Crea] ratio, and calculated daily urine Ca excretion were moderately and negatively associated with U_{pH} in primiparous and multiparous cows (r_s -0.65 to -0.69; Table 2). Mixed-models nonlinear analysis indicated that urine [Ca] (in mmol/L) had a negative exponential association with U_{pH} (urine [Ca] = $10^{(3.53 - 0.455 \times U_{\text{pH}})}$) based on analysis of 356 samples from 47 multiparous cows, with up to 10 samples per cow (Figure 1A). The mixed-models equation predicted that urine [Ca] ≥ 5.0 mmol/L when $U_{\text{pH}} < 6.22$. Mixed-models analysis indicated that the urine [Ca] to [Crea] ratio had a negative exponential association with U_{pH} [urine [Ca]:[Crea] = $10^{(0.85 - 0.208 \times U_{\text{pH}})}$] based on analysis of 350 samples from 47 multiparous cows, with up to 10 samples per cow (data not shown). Finally, mixed-models analysis indicated that estimated daily urine Ca excretion had a negative exponential association with U_{pH} [daily urine Ca excretion = $10^{(3.21 - 0.427 \times U_{\text{pH}})}$] based on analysis of 350 samples from 47 multiparous cows, with up to 10 samples per cow (Figure 1B). The mixed-models equation predicted that calculated daily urine Ca excretion was ≥ 4 g/d in multiparous cows when $U_{\text{pH}} < 6.11$.

Urine Ca-NAE Relationship

Urine [Ca], Ca to Crea ratio, and daily Ca excretion were positively associated with NAE in primiparous

Table 1. Urine pH values measured by glass-electrode pH meter for the different pH categories measured by the Multistix-SG urine dipstick and Hydrion pH paper in 1,116 free-catch urine samples collected by perineal stimulation from 106 periparturient Holstein-Friesian cows

pH category	Frequency	Urine pH	
		Mean $\pm$ SD	Range
Multistix-SG urine dipstick ¹			
5.0	12	5.21 $\pm$ 0.14	5.00–5.39
6.0	204	5.67 $\pm$ 0.32	5.06–6.80
6.5	73	6.30 $\pm$ 0.22	5.78–6.80
7.0	35	6.80 $\pm$ 0.11	6.60–7.07
7.5	64	7.25 $\pm$ 0.17	6.84–7.61
8.0	109	7.70 $\pm$ 0.24	6.75–8.31
8.5	619	8.20 $\pm$ 0.17	7.22–8.58
Hydrion pH paper ¹			
5.5	81	5.39 $\pm$ 0.19	5.00–5.69
5.8	44	5.72 $\pm$ 0.14	5.28–5.92
6.0	52	5.83 $\pm$ 0.30	5.10–6.60
6.2	56	5.92 $\pm$ 0.31	5.06–6.61
6.4	36	6.28 $\pm$ 0.21	5.73–6.68
6.6	22	6.61 $\pm$ 0.15	6.26–6.86
6.8	37	6.84 $\pm$ 0.21	6.49–7.35
7.0	25	7.11 $\pm$ 0.19	6.69–7.54
7.2	33	7.30 $\pm$ 0.17	6.94–7.81
7.4	46	7.52 $\pm$ 0.12	7.27–7.90
7.6	70	7.78 $\pm$ 0.14	7.49–8.07
8.0	614	8.21 $\pm$ 0.15	7.60–8.58

¹Multistix-SG urine dipstick: Siemens Medical Solutions Inc. (Ann Arbor, MI); Hydrion pH paper: Micro Essential Laboratory Inc. (Brooklyn, NY).

and multiparous cows (r_s 0.52 to 0.57; Table 2). Mixed-models analysis indicated that urine [Ca] (in mmol/L) was linearly associated with NAE (urine [Ca] = $7.01 + 0.039 \times \text{NAE}$) based on analysis of 236 samples from 32 multiparous cows, with up to 10 samples per cow; Figure 1C). The mixed-models equation estimated that urine [Ca] ≥ 5.0 mmol/L when NAE > -52 mmol/L. Mixed-models analysis indicated that the urine [Ca] to

[Crea] ratio was linearly associated with NAE (urine [Ca]:[Crea] = $0.27 + 0.0013 \times \text{NAE}$) based on analysis of 231 samples from 32 multiparous cows, with up to 10 samples per cow (data not shown). Finally, mixed-models analysis indicated that estimated daily urine Ca excretion was linearly related to NAE (daily urine Ca excretion = $4.43 + 0.021 \times \text{NAE}$) based on analysis of 231 samples from 32 multiparous cows, with up to 10

Table 2. Spearman correlation coefficients for the relationships between urine Ca concentration ([Ca]; mg/dL), urine Ca to creatinine concentration ([Ca]/[Crea]), net acid excretion (NAE), ammonium concentration ($[\text{NH}_4^+]$), glass-electrode pH meter (U_{pH} , reference method), Multistix-SG urine dipstick pH ($U_{\text{pH-MS}}$), and Hydrion pH paper ($U_{\text{pH-HP}}$) in 1 randomly identified urine sample from d 3 antepartum to d 3 postpartum in 106 periparturient Holstein-Friesian cows¹

Variable	[Ca]	[Ca]/[Crea]	NAE	$[\text{NH}_4^+]$	U_{pH}	$U_{\text{pH-MS}}$	$U_{\text{pH-HP}}$
[Ca] (mmol/L)	1.00	0.92*** (n = 77)	0.52*** (n = 39)	0.41** (n = 39)	−0.65*** (n = 78)	−0.62*** (n = 78)	−0.63*** (n = 78)
[Ca]/[Crea]		1.00	0.57*** (n = 39)	0.36* (n = 39)	−0.69*** (n = 78)	−0.64*** (n = 78)	−0.66*** (n = 78)
NAE (mmol/L)			1.00	0.87*** (n = 46)	−0.97*** (n = 46)	−0.93*** (n = 46)	−0.96*** (n = 46)
$[\text{NH}_4^+]$ (mmol/L)				1.00	−0.82*** (n = 46)	−0.81*** (n = 46)	−0.81*** (n = 46)
U_{pH}					1.00	0.94*** (n = 106)	0.96*** (n = 106)
$U_{\text{pH-MS}}$						1.00	0.96*** (n = 106)
$U_{\text{pH-HP}}$							1.00

¹Multistix-SG urine dipstick: Siemens Medical Solutions Inc. (Ann Arbor, MI); Hydrion pH paper: Micro Essential Laboratory Inc. (Brooklyn, NY).

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

Figure 1. (A) Scatterplot of the exponential relationship between urine Ca concentration ($[Ca]$) and urine pH (U_{pH}) for 356 urine samples from 47 periparturient multiparous Holstein-Friesian cows. The solid line is the regression line $[Ca] = 10^{(3.53 - 0.455 \times U_{pH})}$. The horizontal dashed line is the recommended minimum urine $[Ca]$ (≥ 5 mmol/L) used in this study as an operational construct for effective control of periparturient hypocalcemia. The vertical dashed line indicates that $U_{pH} < 6.22$ when urine $[Ca] \geq 5$ mmol/L. (B) Scatterplot of the exponential relationship between daily Ca excretion and U_{pH} for 350 urine samples from 47 periparturient multiparous Holstein-Friesian cows. The solid line is the regression line $y = 10^{(3.21 - 0.427 \times U_{pH})}$. The horizontal dashed line is the recommended minimum daily Ca excretion (≥ 4 g/d) used in this study as an operational construct for effective control of periparturient hypocalcemia. The vertical dashed line indicates that $U_{pH} < 6.11$ when daily urine Ca excretion ≥ 4 g/d. (C) Scatterplot of the linear relationship between urine $[Ca]$ and net acid excretion (NAE) for 236 urine samples from 32 periparturient multiparous Holstein-Friesian cows. The solid line is the linear regression line ($y = 7.01 + 0.039 \times NAE$). The horizontal dashed line is the recommended minimum urine $[Ca]$ (≥ 5 mmol/L) used in this study as an operational construct for effective control of periparturient hypocalcemia. The vertical dashed line indicates that $NAE > -52$ mmol/L when urine $[Ca] \geq 5$ mmol/L. (D) Scatterplot of the linear relationship between daily Ca excretion and NAE for 231 urine samples from 32 periparturient multiparous Holstein-Friesian cows. The solid line is the linear regression line ($y = 4.43 + 0.021 \times NAE$). The horizontal dashed line is the recommended minimum daily Ca excretion (≥ 4 g/d) used in this study as an operational construct for effective control of periparturient hypocalcemia. The vertical dashed line indicates that $NAE > -20$ mmol/L when daily urine Ca excretion ≥ 4 g/d.

samples per cow (Figure 1D). The mixed-models equation predicted that calculated daily urine Ca excretion was ≥ 4 g/d when $NAE > -20$ mmol/L.

Methods Comparison for Urine pH

Excellent correlation existed between U_{pH} , U_{pH-MS} , and U_{pH-HP} in primiparous and multiparous cows (r_s 0.94 to 0.96; Table 2). The horizontal box plot of Multistix-SG urine dipstick pH versus U_{pH} is presented in Figure 2A. The Bland-Altman plot indicated that the Multistix-SG urine dipstick read, on average, 0.28 pH units higher than the pH meter, with 95% limits of agreement between -0.15 and 0.72 (Figure 2B). The horizontal box plot of Hydrion pH paper versus U_{pH} is presented in Figure 3A. The Bland-Altman plot indicated that the pH paper read, on average, 0.10 pH units lower than the pH meter, with 95% limits of agreement between -0.57 and 0.36 (Figure 3B). The Bland-Altman plot indicated the presence of proportional bias, based on the negative slope of the difference versus mean relationship.

Urine Ammonium Concentration-NAE Relationship

An excellent correlation existed between urine $[NH_4^+]$ and NAE in primiparous and multiparous cows (r_s 0.87; Table 2). Segmented mixed-models regression fitted 2 lines to the urine $[NH_4^+]$ -NAE relationship for 291 samples from 44 multiparous cows with up to 10 samples/cow and identified a breakpoint at $NAE = +14.4$ mmol/L (95% CI 12.8–16.0; Figure 4). This breakpoint represented the value for NAE where the excretion rate of urine $[NH_4^+]$ increased markedly, and consequently identified the point where U_{pH} became less accurate as a predictor of the magnitude of systemic acidosis. The model equations were $[NH_4^+] = 9.36 + 0.0377 \times NAE$ when $NAE \leq 14.4$ mmol/L, and $[NH_4^+] = 9.90 + 1.044 \times (NAE - 14.4)$ when $NAE > 14.4$ mmol/L. The 95% CI for the second slope (0.977–1.11) included 1, indicating that all of the increase in NAE due to marked systemic acidosis was due to urine ammonium excretion.

Urine pH-NAE Relationship

A strong negative correlation existed between U_{pH} and NAE in primiparous and multiparous cows (r_s -0.97 ; Table 2). We determined NAE in 291 urine samples from 44 multiparous Holstein-Friesian cows, with up to 10 urine samples analyzed per cow. A negative exponential relationship existed between U_{pH} and NAE, such that $U_{pH} = 6.10 + \log_{10}(14.3 - NAE)$ (Figure 5). This equation only has a solution when

NAE ≤ 14.3 mmol/L; interestingly, this was the same cutpoint identified using segmented linear regression (<14.4 mmol/L) to identify at what value for NAE we observed a marked increase in the rate of ammonium

excretion. In other words, U_{pH} does not provide an accurate insight into the magnitude of systemic acidosis when $U_{pH} < 6.11$ and NAE >14.4 mmol/L.

We measured urine pH with the reference method in 765 multiparous cows. Logistic regression analysis

Figure 2. (A) Box and whisker plot of urine pH measured by Multistix-SG urine dipstick (Siemens Medical Solutions Inc., Ann Arbor, MI) and pH meter (reference method) for 1,116 urine samples obtained periodically from 106 Holstein-Friesian periparturient cows. The shaded boxes represent the first and third quartiles, the vertical lines in the shaded boxes represent the median value, the whiskers represent the 10th and 90th percentiles, and the filled circles represent data points outside the percentile range. The long dashed diagonal line is the line of identity. The horizontal dashed line is the optimal cutpoint (Multistix-SG urine dipstick pH ≤ 6.0) for identifying urine samples with pH < 6.11 (vertical dashed line) in periparturient multiparous cows. (B) Bland-Altman mean difference plot. The horizontal solid line at 0.28 is the mean bias, and the dashed lines reflect the 95% limits of agreement, which are equivalent to the range of differences that contains 95% of future measurements. The plot indicates that the Multistix-SG urine dipstick measures urine pH 0.28 units higher than the reference method.

Figure 3. (A) Box and whisker plot of urine pH measured by Hydrion pH paper (Micro Essential Laboratory Inc., Brooklyn, NY) and pH meter (reference method) for 1,116 urine samples obtained periodically from 106 Holstein-Friesian periparturient cows. The shaded boxes represent the first and third quartiles, the vertical lines in the shaded boxes represent the median value, the whiskers represent the 10th and 90th percentiles, and the filled circles represent data points outside the percentile range. The long dashed diagonal line is the line of identity. The horizontal dashed line is the optimal cutpoint (Hydrion pH paper ≤ 6.2) for identifying urine samples with pH < 6.11 (vertical dashed line). (B) Bland-Altman mean difference plot. The horizontal long-dashed line is the mean bias, and the horizontal short-dashed lines reflect the 95% limits of agreement, which are equivalent to the range of differences that contains 95% of future measurements. The plot indicates that the Hydrion pH paper measures urine pH 0.10 pH units lower than the reference method.

indicated that the Multistix-SG urine dipstick accurately predicted urine pH (AUC = 0.991; 95% CI: 0.987–0.996). A Multistix-SG urine dipstick pH reading ≤ 6.0 accurately identified $U_{\text{pH}} < 6.11 = U_{\text{pH-DailyCa} \geq 4\text{g}}$, as demonstrated by Se = 0.926 (95% CI: 0.872–0.963), Sp = 0.987 (95% CI: 0.975–0.994), +LR = 71.3 (95% CI: 35.8–142.2), and $\kappa = 0.92$ (95% CI: 0.88–0.96).

Logistic regression analysis indicated that the Hydrion pH paper accurately predicted urine pH (AUC = 0.995; 95% CI: 0.992–0.998), which was similar ($P = 0.10$) to that for the Multistix-SG dipstick. A Hydrion pH paper reading ≤ 6.2 accurately identified $U_{\text{pH}} < 6.11 = U_{\text{pH-DailyCa} \geq 4\text{g}}$, as demonstrated by Se = 0.960 (95% CI: 0.914–0.985), Sp = 0.968 (95% CI: 0.950–0.980), +LR = 29.6 (95% CI: 19.2–45.5), and $\kappa = 0.90$ (95% CI: 0.86–0.93).

DISCUSSION

The major findings of this study were 3-fold: (1) U_{pH} provides a clinically useful method for predicting NAE and urine [Ca] in periparturient dairy cattle whenever $U_{\text{pH}} > 6.11$; (2) $U_{\text{pH}} < 6.11$ predicted daily Ca excretion ≥ 4 g/d and $U_{\text{pH}} < 6.22$ predicted urine [Ca] ≥ 5 mmol/L in the current study; and (3) the Multistix-SG urine dipstick and Hydrion pH paper were clinically valuable tests for predicting U_{pH} . Specifically, the test performance of the dipstick and pH paper were excellent based on AUC > 0.90 (Swets, 1988), +LR > 10 (Grimes and Schulz, 2005), and $\kappa > 0.80$ (Landis and Koch, 1977). Our findings suggested that Hydrion pH paper is preferred to Multistix-SG urine dipsticks for monitoring U_{pH} in dairy cows fed an acidogenic ration.

Figure 4. Scatterplot of the relationship between urine ammonium concentration ($[\text{NH}_4^+]$) and net acid excretion (NAE) for urine samples from 44 periparturient multiparous Holstein-Friesian cows. Segmented mixed-models regression fitted 2 lines to the urine $[\text{NH}_4^+]$ -NAE relationship for 291 urine samples and identified a break point (NAE = +14.4 mmol/L) where the excretion rate of urine $[\text{NH}_4^+]$ increased markedly.

This is because the pH paper estimated U_{pH} more accurately and to a greater degree of resolution over the pH range of interest than the urine dipstick, and it was less expensive.

Net acid excretion and U_{pH} provide clinically useful insights into acid–base status, because both indices change in a predictable manner with alterations in systemic acid–base balance, although U_{pH} is much simpler to measure (Jørgensen, 1957; Kutas, 1965; Constable et al., 2009). The first clinical application of NAE in veterinary medicine was by Kutas in 1965, who adapted Jørgensen's technique for human urine to urine from dairy cattle. In a subsequent study, Kutas suggested the following guidelines for interpreting NAE

based on mean values from 4 to 8 healthy animals: < –100 mmol/L, typical lactating dairy cow ration; –100 to 0 mmol/L, danger of metabolic acidosis; and >0 mmol/L, presence of metabolic acidosis (Kutas, 1967). The strong ion difference theory was applied to urine in 2009 and a physicochemical equation developed that characterized, for the first time, the mathematical relationship between U_{pH} and NAE (Constable et al., 2009). In a validation study of the theoretically derived equation, it appeared that NAE could be accurately estimated from U_{pH} when $U_{pH} > 6.3$; however, this cut-point was determined subjectively and without applying objective statistical methods. We used segmented regression of the urine $[NH_4^+]$ –NAE relationship and

Figure 5. Scatterplot of the relationship between urine pH (U_{pH}) measured by a glass-electrode pH meter and urine net acid excretion (NAE) for 291 urine samples from 44 periparturient multiparous Holstein-Friesian cows. Some data points are superimposed. A negative exponential relationship existed between U_{pH} and NAE, such that $U_{pH} = 6.10 + \log_{10}(14.3 - \text{NAE})$. The dashed horizontal lines indicate target U_{pH} values used in this study as an operational construct for effective control of periparturient hypocalcemia: $U_{pH} < 6.22$, target urine $[Ca] \geq 5$ mmol/L; $U_{pH} < 6.11$, target daily urinary Ca excretion > 4 g/d.

nonlinear regression of the U_{pH} -NAE relationship in the study reported here and determined that U_{pH} provided a clinically acceptable method for predicting NAE in urine from healthy cows when $U_{pH} > 6.11$ and $NAE < 14.4$ mmol/L. It needs to be emphasized that the magnitude of a nutritionally induced systemic acidosis cannot be accurately predicted whenever $U_{pH} < 6.11$, and that in this circumstance measurement of NAE or urine $[NH_4^+]$ is required to accurately quantify the magnitude of the systemic acid-base derangement. This conclusion is likely to be valid only in healthy animals ingesting a ration that affects acid-base status, as in the current study. This is because U_{pH} in sick lactating dairy cows is primarily influenced by the urine concentration of strong electrolytes, which depend, in turn, on the plasma concentration of strong electrolytes (Buscher and Klee, 1993; Constable et al., 2009). For example, the incidence of aciduria is 30% during d 5 to 14 of lactation despite the fact that cows are fed an alkalinizing ration, and the incidence of aciduria is increased in lactating cows with diseases such as metritis, retained placenta, and ketosis (Markusfeld, 1987) that are often accompanied by abnormal strong ion concentrations in plasma.

Urine Ca indices, such as urine $[Ca]$ and daily Ca excretion, represent clinically useful goals when feeding an acidogenic ration to late-gestation dairy cattle as part of a control program for periparturient hypocalcemia. The linear relationships between the 2 urine Ca indices and NAE and the exponential relationships between the 2 urine Ca indices and U_{pH} in multiparous cows (Figure 1) were similar to the relationships observed in human urine (Lemann, 1999; Lemann et al., 2003) and consistent with the presence of fundamental physicochemical relationships between ingestion of an acidogenic diet and hypercalciuria. Under the conditions of the current study, daily urine Ca excretion = $10^{(3.21 - 0.427 \times U_{pH})}$, and this equation predicted that $U_{pH} < 6.11$ should provide sufficient hypercalciuria (≥ 4 g of Ca/d) to control periparturient hypocalcemia in multiparous cows (Grünberg et al., 2011; Megahed et al., 2018a). Similarly, under the conditions of the current study, urine $[Ca] = 10^{(3.53 - 0.455 \times U_{pH})}$, and this equation predicted the U_{pH} should be < 6.22 to provide sufficient hypercalciuria to control periparturient hypocalcemia in multiparous cows if urine $[Ca] \geq 5$ mmol/L is the preferred monitoring goal (Bender et al., 2003; Gelfert et al., 2004). Our finding that mixed-models analysis estimated that urine $[Ca] \geq 5.0$ mmol/L when $NAE > -52$ mmol/L was of interest; this NAE cutpoint approximated that recommended by Bender and colleagues ($NAE > -50$ mmol/L) based on data from 10 dairy herds in Germany (Bender et al., 2003). It needs

to be emphasized that the cutpoints for the 2 urine Ca indices provide the best operational definitions for effective control of periparturient hypocalcemia, based on our current understanding of the mechanism by which acidogenic diets mitigate periparturient hypocalcemia. It is likely that the U_{pH} cutpoints will change as more studies are conducted and the preferred operational construct (urine $[Ca]$ or daily urinary Ca excretion) for monitoring the systemic response to ingesting an acidogenic diet is identified.

This appears to be the first study to demonstrate that U_{pH} was similarly or more closely associated with urine Ca indices compared with NAE. A low luminal pH in the second half of the distal convoluted tubule and connecting tubule decreases the number and activity of epithelial Ca channels termed transient receptor potential vanilloid member 5 (**TRPV5**). The pH-induced change in TRPV5 receptor number and activity causes hypercalciuria, because TRPV5 channels are the primary gatekeeper of active Ca reabsorption in the distal region of the mammalian urinary tract (Constable et al., 2010; Martín-Tereso and Verstegen, 2011; Bonny and Edwards, 2013). Therefore, there is a strong physiologic basis for preferring to measure U_{pH} instead of a marker of systemic acidosis (NAE) when measuring proxies for urine Ca indices. Our findings suggest a preference for U_{pH} instead of NAE to monitor the systemic acid-base response in cattle fed an acidogenic diet whenever $U_{pH} > 6.11$ because of the simplicity of measuring U_{pH} relative to NAE, and because U_{pH} accurately predicts NAE when $U_{pH} > 6.11$. An important remaining issue is identifying the optimal mean herd U_{pH} that balances efficacy in controlling periparturient hypocalcemia with the potentially deleterious effects of excessive systemic acidification. A marked reduction in U_{pH} should be avoided when feeding acidogenic rations, because $U_{pH} < 5.5$ indicates excessive acidification, acidemia, and the presence of decreased DMI (Charbonneau et al., 2006).

Two studies have previously evaluated the agreement between urine dipstick pH and pH determined by the glass-electrode pH meter for measuring U_{pH} in cattle (Nappert and Naylor, 2001; Defontis et al., 2013). Nappert and Naylor (2001) used simple linear regression to characterize the relationship between the 2 tests and identified a strong correlation ($r = 0.89$, $n = 57$). In 2013, Defontis and colleagues (2013) also identified a strong correlation between the 2 tests ($r_s = 0.91$, $n = 100$). Neither of these studies used currently recommended methods for evaluating the degree of agreement between the 2 tests through Bland-Altman plots (Bland and Altman, 2007; Giavarina, 2015). The results of the current study identified a strong association (r_s

= 0.94, $n = 106$) between $U_{\text{pH-MS}}$ and U_{pH} . The predictive performance of the Multistix-SG urine dipstick in bovine urine was therefore similar to that reported in urine from humans ($r = 0.89$; $n = 390$; Kwong et al., 2013), dogs ($r = 0.92$, $n = 201$, Johnson et al., 2007; $r_s = 0.87$, $n = 101$, Defontis et al., 2013), and cats ($r_s = 0.80$, $n = 50$, Defontis et al., 2013). More importantly, the Bland–Altman plot indicated that the dipstick measured urine pH 0.28 units higher than the reference method, which was within the limits of acceptability of ≤ 0.50 pH units. The small positive bias was similar to that reported in dog urine (+0.22; Johnson et al., 2007) but different from findings in other species, where a bias of -0.22 was identified in human urine (Abbott et al., 2017), -0.02 in sheep urine (Athanasίου et al., 2018), and -0.12 in cat urine (Raskin et al., 2002). The difference in analytical performance of the dipstick may be due to species differences in urine color.

To the best of our knowledge, this is the first study to evaluate the diagnostic performance of urine pH paper with a narrow measurement increment (primarily 0.2 pH units) for monitoring urine pH in dairy cattle. Three studies have evaluated the diagnostic reliability of urine pH paper with a wider measurement increment (0.5 to 1.0 pH units). Nappert and Naylor (2001) reported a weaker association between pH paper with a 1.0 pH unit increment and U_{pH} ($r = 0.86$, $n = 45$), compared with the association between $U_{\text{pH-MS}}$ and U_{pH} in cattle. In cats, pH paper with a measurement increment of 1.0 pH units was less accurate than a urine dipstick with a 0.5 pH unit measurement increment (Raskin et al., 2002). In dogs, pH paper with a measurement increment of 0.5 pH units performed similarly to a urine dipstick with bias of +0.26 pH units (Johnson et al., 2007). Taken together, the findings of these studies suggest that the measurement increment contributes to the diagnostic accuracy of pH paper and dipstick when measuring urine pH. Our finding of lower bias between urine pH paper with narrow colorimetric scale (0.2 to 0.4 pH unit increment) and the reference method compared with the urine dipstick (0.5 pH unit increment) supports this conclusion.

The major limitation of the current study involving 106 Holstein-Friesian cows was that it was conducted on one farm that fed typical Midwest dairy rations for late-gestation and lactating Holstein-Friesian cows. However, the observed U_{pH} –NAE relationship was very similar to that observed in 11 nonpregnant, nonlactating Holstein-Friesian cows in Germany that received intraruminal infusions of different formulations of acidifying salts (Gelfert et al., 2007), and similar to that observed in 8 nonlactating Holstein cows in Wisconsin fed different anionic salts (Vagnoni and Oetzel, 1998). Moreover, the urine $[\text{Ca}]$ – U_{pH} relationship in late-ges-

tation dairy cows is influenced primarily by the ration DCAD (Vagnoni and Oetzel, 1998; Roche et al., 2003; Martín-Tereso and Verstegen, 2011) and minimally influenced by the Ca content of the ration (Ramberg et al., 1984; van de Braak et al., 1986; Kronqvist et al., 2011). This is because the dairy cow is able to control the rate of Ca uptake from the gastrointestinal tract and Ca mobilization from bone in response to changes in ration Ca content (Ramberg et al., 1976, 1984). It is therefore likely that operational definitions for controlling periparturient hypocalcemia based on urine $[\text{Ca}]$ or daily urinary Ca excretion as used in the current study will be preferred because they are mechanistic. A second study limitation was the use of 1 spot urine sample to calculate daily Ca excretion assuming a constant daily Crea excretion. Although 24 h urine collection would have been ideal, our intent was to characterize the performance of a practical test for routine herd monitoring. The assumption of constant Crea excretion appears reasonable, because mean urinary Crea excretion in dairy cows remained constant during the week before or after parturition (van de Braak et al., 1986).

CONCLUSIONS

Both U_{pH} and NAE provided a clinically useful insight into the magnitude of hypercalciuria and systemic acid–base status in dairy cows fed an acidogenic diet during late gestation. In the current study, clinically relevant U_{pH} goals for an optimal control program for periparturient hypocalcemia, based on operational definitions of urine $[\text{Ca}] \geq 5$ mmol/L and daily urinary Ca excretion ≥ 4 g, were $U_{\text{pH}} < 6.22$ and < 6.11 , respectively. Hydrion pH paper and Multistix-SG urine reagent dipsticks are accurate, practical, and low-cost (\$0.30/dipstick; \$0.04/paper test) on-farm methods for monitoring urine pH in dairy cows fed an acidogenic diet. It would be helpful to confirm our findings in dairy herds fed different rations in a variety of management systems.

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