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Short communication

Urine pH as predictor of blood acid-base status in dairy cattle fed acidogenic diets

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ABSTRACT

We determined the association between urine pH and blood acid-base indicators and assessed a urine pH cut-off value to predict severe metabolic acidosis under field conditions in cows fed acidogenic diets. Eighty-six cows were sampled for urine and blood. Urine pH was evaluated immediately after collection, and blood acid-base status was evaluated within 2 hours of collection using a portable blood analyzer. Twenty-five cows were classified as having severe metabolic acidosis (blood pH $\leq$ 7.4; bicarbonate $<$ 24 mmol/L, base excess ≤ -0.5; PCO_2 low to normal concentrations and urine pH between 4.88 and 5.71. There was a positive linear association between urine pH and blood pH (r = 0.46), and between urine pH and base excess (r = 0.74). The area under the ROC curve was 0.91 (CI 95 %= 0.84–0.96; good-excellent test). The optimal cut-off value for urine pH to categorize a cow with severe metabolic acidosis was 5.5 (94 % specificity and 72 % sensitivity). For each 0.1 unit of decrease in urine pH below 5.5, cows were 1.6 times (95 % CI= 1.3–2.1) more likely to exhibit a severe metabolic acidosis. We conclude that a urine pH of 5.5 or less is indicative of more life-threatening metabolic acidosis in dairy cows.

Periparturient dairy cows are predisposed to hypocalcemia which is defined as total plasma Ca (tCa) and ionized plasma Ca (iCa) concentrations of $<$ 2.15 and $<$ 1.0 mmol/L, respectively (Goff, 2018; Couto Serrenho et al., 2021c). A recommended preventive strategy for clinical hypocalcaemia in dairy cows is feeding anionic compounds during the prepartum to induce mild metabolic acidosis (Melendez and Chelikani, 2022). Ruminants consuming forages high in K^+ and Ca^{2+}, and low in Cl^-, SO_4^{-2}, and PO_4^{-3}, have a slightly higher blood pH than non-ruminants, which makes ruminant blood more alkalotic than other species. The metabolic alkalosis reduces the ability of the cow to maintain normocalcaemia around parturition (Goff, 2014). Therefore, supplementation of anions during the prepartum has been shown to shift the alkalotic state to a mild acidotic state (Goff, 2018), potentially increase the sensitivity of bone and renal cell receptors to parathyroid hormone (Goff, 2014), enhance calcium mobilization from bone, decreasing calcium reabsorption from the kidney and increasing calcium absorption from the digestive tract (Goff, 2018; Wilkens et al., 2020). In contrast, other evidence indicates that when cows are fed anionic diets before calving, the excretion of calcium in urine is reduced to maintain plasma calcium homeostasis. For example, in primiparous cows fed acidogenic diets, mean urine Ca excretion decreased to 4.7 g/d at ~48 h before parturition and continued to decrease gradually up to 0.1 g/d at ~30 h postpartum, whereas, in multiparous cows, urine Ca excretion decreased from 5.5 g/d at ~24 h before parturition to 1.7 g/d at parturition (Megahed et al., 2018). Similarly, in cows fed acidogenic diets, urine Ca excretion decreased from 6.9 g/d at ~12 h before parturition to 3.4 g/d at ~12 h after parturition, and the reabsorption of Ca from the urine will increase the amount of Ca available in the exchangeable Ca pool to facilitate calcium homeostasis (Megahed et al., 2018). In addition to urine calcium changes, urine pH is also acidified by feeding acidogenic diets (Melendez et al., 2019).

Assessing urine pH in cows fed anionic compounds is a quick and cost-effective tool to monitor the level of metabolic acidification (Goff, 2018; Wilkens et al., 2020). A target urine pH between 6.0 and 6.8 has been recommended for Holstein cows; however, in several countries, certain nutritional strategies are still focused on benchmarking the urine pH close to 5.5 or lower (Melendez and Chelikani, 2022). Over-acidification may have no advantages and, on the contrary, may have detrimental effects on both the dam and her offspring because of a potential uncompensated metabolic acidosis (Klein, 2020; Melendez

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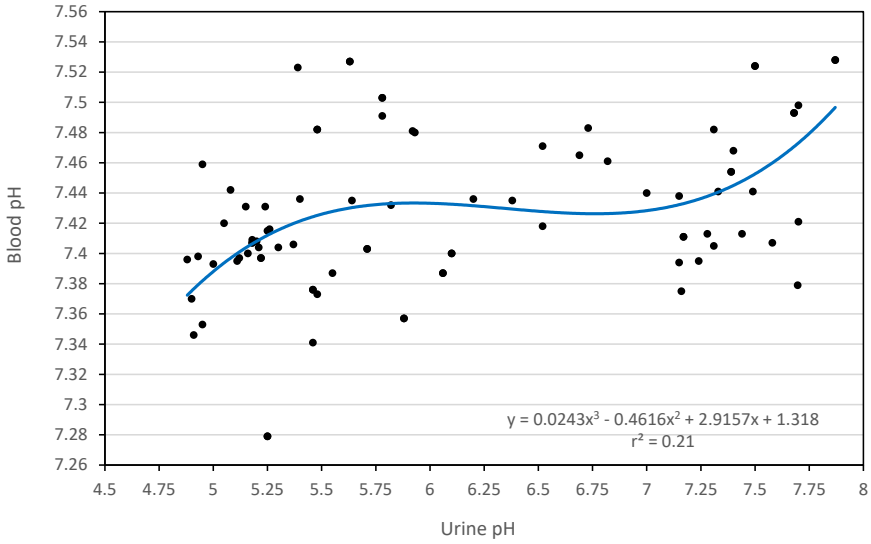

Fig. 1. Regression curve between urine pH and blood pH in cows (n=86) fed anionic compounds.

et al., 2022b). The association between urine pH and blood acid-base status in cows with urine pH $\leq$ 5.5 is largely unknown. We hypothesized that cows with a urine pH lower than 6.0 will have an increased risk of uncompensated metabolic acidosis. Therefore, the objectives of this study were to determine the association between urine pH and blood acid-base indicators including blood pH, base excess, bicarbonate, and CO_2 concentration, and to set a urine pH cut-off value to predict a potential uncompensated metabolic acidosis under field conditions in cows fed anionic compounds.

The study was approved by the Institutional Animal Care and Use Committee of the Texas Tech University (IACUC protocol ID: 21045-05). Based on our prior experience and animal availability, 70 prepartum multiparous cows (> 3 parity number) from one commercial Holstein dairy farm in Texas, USA, were used once for urine and blood sampling. Additional 16 non-lactating, non-pregnant Jersey cows from the Texas Tech Teaching University School of Veterinary Medicine, with urine pH between 5.0 and 8.0, were also sampled for urine and blood. Jersey cows were used for their major variability in urine pH.

Urine samples were collected after cleaning the area around the vulva with absorbent paper, stimulating the escutcheon area by massaging with the fingers. After ensuring that the urine stream was intense and clean, the sample was collected in a plastic container and measured immediately with a portable calibrated pH meter (Hanna Ins., Smithfield, RI 02917, USA). Next, an anaerobic blood sample was obtained from the coccygeal blood vessels in heparinized vaccutainer tubes to measure acid-base blood parameters [blood pH, PCO_2 (mmHg), PO_2 (mmHg), HCO_3^- (mmol/L), lactate (mmol/L), and calculated HCO_3, base excess (BE, mmol/L), sO_2 (%), and TCO_2 (mmol/L)] using a portable blood analyser at 22°C (I-stat, Abbott, Orlando, Florida 32810, USA). Samples were assessed within 30 minutes of collection.

A multivariable regression analysis between urine pH and blood parameters was carried out. Covariables were the breed, lactation number, and body condition score (BCS) of the cows. A receiving operating characteristic (ROC) analysis to set a cut-off value of urine pH to predict a potentially life-threatening metabolic acidosis was conducted. Severe metabolic acidosis was considered as defined by Constable et al. (2009) and Klein (2020) with all 4 of the following features present: blood pH $\leq$ 7.4, blood bicarbonate concentration $\leq$ 24 mmol/L, base excess $\leq$ −0.5 mmol/L, and low to normal PCO_2 (45 mm Hg). An analysis of variance for blood parameters between cows

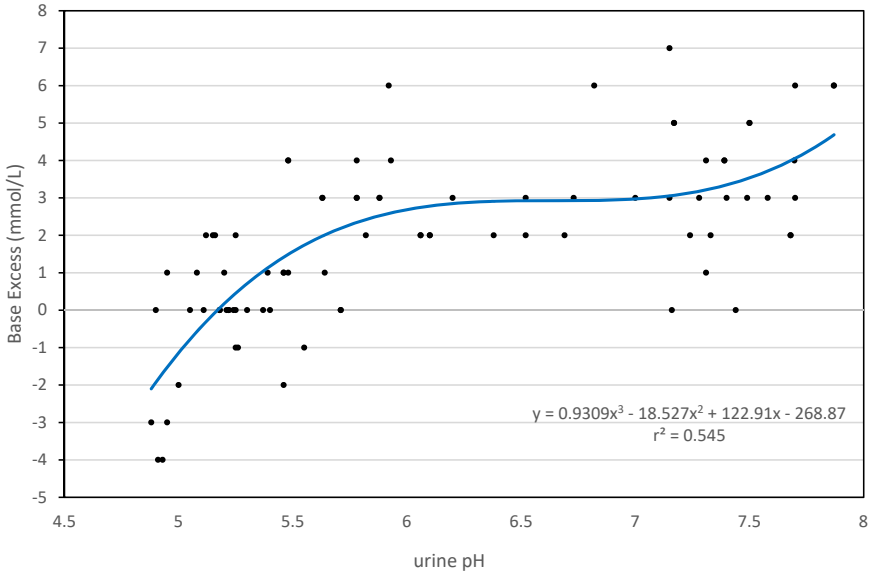

Fig. 2. Regression line between urine pH and blood base excess (mmol/L) in cows (n=86) fed anionic compounds.

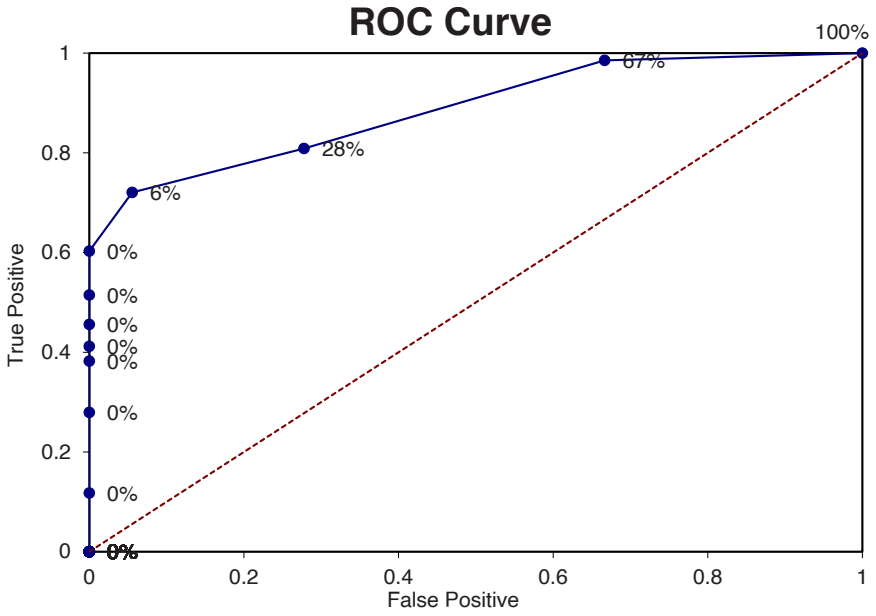

Fig. 3. ROC curve for urine pH as predictor of severe metabolic acidosis in cows fed anionic compounds.

Table 1
Blood acid-base parameters in cows fed anionic compounds with urine pH ≤ 5.5 (n=36) and urine pH > 5.5 (n=50).

	Blood pH	PCO_2 mmHg	PO_2 mmHg	HCO_3 mmol/L	BE mmol/L	sO_2%	TCO_2 mmol/L	Lactate mmol/L	Urine pH
Urine pH									
≤ 5.5 (n=36)	7.40 ±0.04	39.6±4.6	49.1±18.7	24.6±1.58	-0.06±1.83	77.9±14.1	25.8±1.58	0.78±0.52	5.18±0.18
>5.5 (n=50)	7.44±0.04	39.9±4.7	48.8±22.6	27.1±1.60	2.95±1.83	76.3±16.5	28.2±1.69	0.86±0.52	6.78±0.30
P-value	< 0.001	0.76	0.96	< 0.001	< 0.001	0.70	< 0.001	0.53	< 0.001

with above and below the cut-off urine pH value obtained by the ROC analysis was also built. All the analyses were run using the computer software SAS 9.4.

The association between urine pH and blood pH (Fig. 1) fitted a polynomial third-order curve, where blood pH = $0.0243x^3$ - $0.4616x^2$ + 2.9157x + 1.318 (r^2 = 0.21); as well as for base excess (mmol/L) = $-268.9 + 0.931x^3 - 18.53x^2 + 122.9x$ (r^2 = 0.55) (Fig. 2).

The area under the ROC curve was 0.91 ± 0.03 (CI 95 %= 0.85–0.97) (good to excellent test). The best cut-off value for urine pH to categorize a cow with severe metabolic acidosis was 5.5, with 94 % specificity and 72 % sensitivity (Fig. 3). The interpretation of the Odds Ratio estimates was that for each 0.1 unit of decrease in urine pH below 5.5, cows were 1.6 times (95 % CI= 1.3–2.1) more likely to exhibit severe metabolic acidosis. In Table 1, acid-base parameters in cows with urine pH ≤ 5.5 and > 5.5 is shown.

One of the biggest challenges of feeding acidogenic diets in prepartum dairy cows is achieving a urine pH with low variability (Goff, 2014; 2018). Reaching a moderately acidic pH that prevents clinical hypocalcaemia is the main goal of acidogenic diets without putting the cows at risk for uncompensated metabolic acidosis. In fact, cows with urine pH between 6.0 and 7.0 are consistently less likely to develop clinical hypocalcaemia (Charbonneau et al., 2006; Melendez and Poock, 2017; Melendez et al. 2019; 2021a; 2021b); however, there are still recommendations on achieving urine pH ≤ 5.5. At this level, the variability of urine pH is very low, therefore the producer and the nutritional consultant feel satisfied. Low variability is because the cow is unlikely to keep compensating for the severe metabolic acidosis, where she may reach a point of no return towards a terminal state of death (Constable et al., 2009; Klein, 2020; Melendez and Chelikani, 2022). Many studies with very acidic treatments have shown to improve calcium homeostasis compared to control groups, but treated cows excrete 10 times more calcium in the urine than control groups, and dramatically they reduce feed intake (Charbonneau et al., 2006; Glosson et al., 2023). More moderate acidogenic diets also improve calcium status without producing marked calcium losses in the urine and/or reducing feed intake. In fact, a recent study reported that cows with urine pH slightly higher than 6 had a better feed intake, a lower urine Ca excretion, and a plasma calcium concentration similar to more acidogenic diets, showing that moderate acidogenic diets are adequate enough to control milk fever in dairy cattle (Thompson and Ferreira, 2023).

In summary, this study offers a practical tool to use urine pH as a predictor of severe metabolic acidosis in prepartum cows fed anionic compounds to prevent milk fever. A urine pH ≤ 5.5 indicates a severe metabolic acidosis that may become uncompensated. Though we used portable pH meters for urine pH measurements, perhaps a faster and cheaper alternative using pH paper could be considered and validated in future studies for monitoring severe acid-base disturbances in cows fed acidogenic diets.

CRediT authorship contribution statement

Daniela Redrovan: Writing – review & editing, Validation, Supervision, Project administration, Investigation, Conceptualization. **Pedro Melendez:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Prasanth K. Chelikani:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Conflicts of interest

The authors of this manuscript declare no conflict of interest.

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