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Research article

Comparative evaluation of urinary dipstick and pH-meter for cattle urine pH measurement

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ABSTRACT

Urinary dipsticks are often used for the diagnosis of kidney, liver, metabolic, and urogenital diseases in cattle clinical practice for the simplicity of use and ease of access. The aims of the present study were to evaluate the agreement between urinary dipsticks and pH-meter for the urine pH in dairy cattle, and also to compare the urine pH before and after centrifugation from each method. The agreement between urine dipstick and pH-meter methods before and after centrifuge were calculated by Passing-Bablok regression analysis demonstrated constant differences (intercept) at 0.60 and -1.01 and positive proportional differences (slope) at 0.94 and 1.13; respectively. Total bias estimated by Bland-Altman plot analysis before (0.20) and after (0.14) centrifuge were lower than the acceptable bias in urine samples. The regression analysis of this study emphasized that the urinary dipstick can be used to determine the cattle urine.

1. Introduction

An integral part of the complete urinalysis is urine pH, affects the results of urine chemistry and sediment tests and is affected by plasma pH alterations and urinary tract infections (Reine and Langston, 2005; Defontis et al., 2013). The high content of dietary K, Na, Cl, and S in dairy cows increases and decreases urinary pH, respectively (Kume et al., 2011). The changing of urine pH can also induce the urolithiasis formation of calcium oxalate, calcium carbonate, and magnesium ammonium phosphate (triple phosphate) crystals (Videla and van Amstel, 2016). Measuring urine pH with the benchtop pH-meter is a standard method, but urinary dipstick often uses for easy to use in veterinary practice (Kwong et al., 2013). They have color indicators that are primarily designed for human urine, and therefore should be validated in veterinary species. The advantage of urinary dipsticks in addition to easy to handle is that they require only a small urine sample to measure the pH and well-suited for farm monitoring of urine pH by the owners (Reine and Langston, 2005; Kwong et al., 2013). Despite these features, the accuracy of urinary dipsticks is often questionable when compared with pH-meter (Seifi et al., 2004). Therefore, before using urinary dipstick in cattle, the accuracy of the pH dipstick should be carefully validated. There are few references of evidence of the performance of urinary dipstick on herbivores (Nappert and Naylor, 2001; Athanasiou et al., 2018). Furthermore, most of these studies have not provided a clear correlation between urinary dipstick and benchtop pH-meter to determine the urine pH of healthy cows, before and after centrifugation.

Considering the lack of sufficient knowledge in this regard the first aim of the study was to compare the urinary dipstick and benchtop pH-meter on urine pH in healthy cows. There is limited data reported on the possible effect of centrifugation on urinary pH in animals (Athanasiou et al., 2018) and it is noteworthy that this data is controversial. Therefore, the second aim of this study was to determine the correlation between the two methods before and after centrifugation. The urinary dipstick and benchtop pH-meter tested in this study are commonly used by most veterinary clinicians in clinical practice in Iran.

2. Materials and methods

This study was conducted between Octobers to December 2018 on consecutive urine samples obtained from dairy farm of the Faculty of Veterinary Medicine, Semnan University, Semnan, Iran. All stages of this study was approved by the Animal Care Committee of Veterinary Medicine, Semnan University (permit number SEC-97-10). The minimum sample size required to conduct this study was 12 animals, which was calculated by the MedCalc Statistical Software 17.2 (MedCalc Software, Mariakerke, Belgium). The Kolmogorov–Smirnov test was used to

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determine the normality of the data. If the distribution of data was normal, then t-test and otherwise used Kruskal-Wallis test to determine the statistical difference between urine pH values measured by pH-meter and dipstick before and after centrifuge. Passing-Bablok regression analysis was calculated to determine the systematic and proportional differences between urine dipstick and pH-meter methods before and after centrifuge (Passing and Bablok, 1984). Bland-Altman difference plot analyses was used to evaluate each of the 2 methods (Bland and Altman, 1986). The minimum sample size calculated by the univariate analysis approach to repeated measures using GLIMMPSE software (http://glimmpse.samplesizeshop.org/) for the present study was 20 samples. Finally, 20 fresh urine specimens were obtained from lactating cows (15–50 days postpartum, $\geq$ 3 parity) with a minimum volume sample of 5 ml were inclusion criteria for our study.

The urinary dipstick and pH-meter were two methods adopted for the urinary pH study. Both techniques were applied concurrently for the examination of specimens in the laboratory. Urine samples were collected from the mid-stream by using the perineal skin stimulation and kept in a cooler container for transferring the samples from the farm to the laboratory. A study by Athanasiou et al. (2018) showed that the maximum urine sample storage time was 2 h before the pH analysis. Estimation of pH was executed on the complete urine and urine supernatants after centrifugation at 300 x g for 10 min. Urinary dipsticks utilized to analyze the pH of all animals involved in this study were the Multistix 10 SG (Siemens), urine reagent strips. The methyl red/-bromothymol blue color system has been designed as indicators of pH test reaction for urinary dipstick which produces pH readings from 5.0 to 8.5 with a range interval of 0.5. This pH range changes the color of the test pad from orange to green to blue. The visual reading of test pads was made by the corresponding individual to avoid interobserver variability. In Brief, one drop of urine sample was placed on the bottom of each test pad using a plastic pipette, careful to avoid urine combining among test pads and oversaturation. Reading the color change of the test pad happen within 60 s and recorded the color change. Urine pH of all samples was also measured using a digital benchtop pH-meter (Piccolo; Hanna Instruments Japan, Tokyo, Japan). The calibration of the digital pH-meter was performed every 24 h with buffer solutions of pH 4.00, 7.00, and 10.00, according to the manufacturer's instructions, and after each use, the electrode sensor was washed with deionized distilled water. In this study, the pH analysis of each sample was repeated three times for both methods.

Table 1. Mean, standard deviation (SD), and range values for both methods before and after centrifugation.

Method	cattle		
	Mean	SD	Range
pH-meter before centrifugation	7.81	0.34	(7.19–8.44)
pH-meter after centrifugation	7.83	0.40	(7.10–8.59)
Dipstick before centrifugation	7.67	0.42	(7.00–8.00)
Dipstick after centrifugation	7.62	0.37	(7.00–8.00)

3. Results

The imprecision of the 2 urine pH methods was evaluated by repeated measurements of each urine sample 6 times at three different pH values. The coefficient of variation (CV) for the dipstick method in cows was 0 for all pH values. The CV for the pH-meter in cow samples were 0.047% at 7.19, 0.043% at 7.82, and 0.040% at 8.44.

In Table 1 are given all values including the mean, standard deviation, and range of the pH measured by both methods before and after centrifugation. No difference ($P > 0.05$) was observed between the value of pH measured by dipstick (median 7.75, 95% CI of 7.57–7.93) and pH-meter (median 7.82, 95% CI of 7.67–7.97). The equation established by Passing-Boblok scatter plots to evaluate the agreement between the pH-meter and the dipstick in the cow showed a constant bias at 0.60 and a positive proportional bias at 0.90 (Figure 1A). In the cow, a total bias calculated by Bland-Altman plot analysis was 0.20 (Figure 1B), which this pH was within the acceptable pH range (0.25).

The equation created by Passing-Boblok scatter plots to evaluate the effect of centrifugation on the pH values measured by the urinary pH-meter before (x) and after centrifugation (y) in the cow (Figure 2A) was as follows:

$$y = -0.5490(95\%CI: -1.55–0.72) + 1.0680(95\%CI: 0.90 – 1.20)x$$

4. Discussion

In this study, we were looking to determine if a pH-meter can be replaced with a dipstick for measuring the urine pH in cow, given that the use of pH-meter in the farm is time-consuming and requires regular calibration with standard buffer. Another subject that was evaluated in present study, was the effect of centrifugation on pH values in both methods.

A bias determined by the Bland-Altman plot analysis was -0.01 (Figure 2B), and based on the results of the Passing-Bablok regression analysis, the pH values measured by the pH-meter are affected by centrifugation. Consequently, due to the presence of a constant and relative bias in the regression equation, urine specimens of the cow before and after centrifugation are not interchangeable. For this reason, the total bias created by the Bland-Altman plot analysis of urine pH measured by two methods after centrifugation was 0.14, which is clinically acceptable (Figure 3). Since the urine pH value measured by dipstick were close to each other before and after centrifugation, further

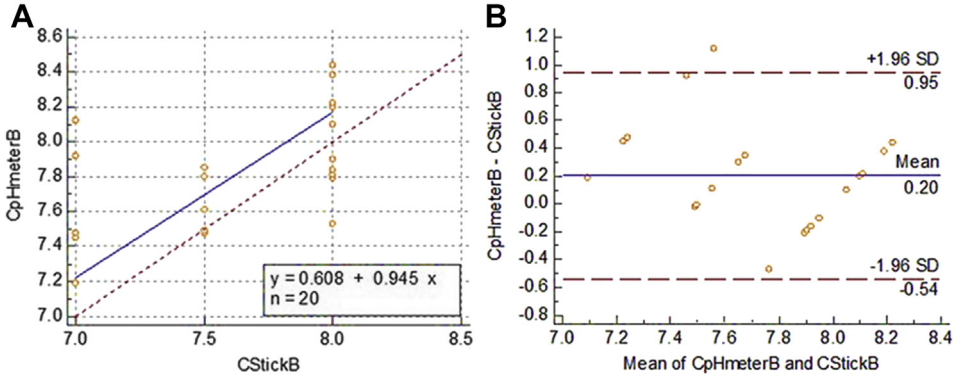

Figure 1. A. Passing and Bablok regression plots between the pH measurement by dipstick (Cattle Dipstick Before centrifugation—CStickB) vs the pH-meter (Cattle pH-meter Before centrifugation—CpH-meterB) in cattle urine. This graph shows the observations with the regression line (solid line), the confidence interval for the regression line (dashed lines). B. Bland–Altman plots showing the difference between the pH measured by the pH-meter (Cattle pH-meter Before centrifugation —CpHmeterB) vs dipstick (Cattle Dipstick Before centrifugation—CStickB), against the mean of the 2 methods in cattle urine.

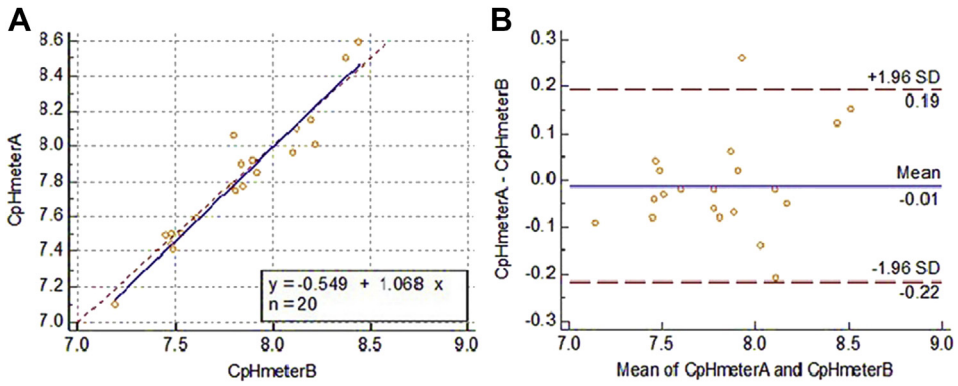

Figure 2. A. Passing and Bablok regression plot between the values measured by the pH-meter Before (CpHmeterB) and After centrifugation (CpHmeterA) in cattle. B. Bland–Altman plot showing the difference between pH measurements by the pH-meter Before and After centrifugation (CpHmeterB–CpHmeterA), against the mean of the 2 measurements (mean of CpHmeterA and CpHmeterB) in cattle urine.

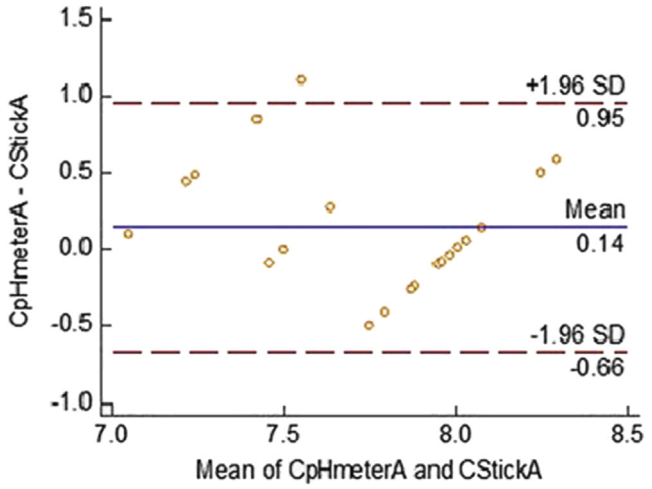

Figure 3. Bland–Altman plots showing the difference between the pH measured by the pH-meter (Cattle pH-meter After centrifugation —CpHmeterA) vs dipstick (Cattle Dipstick After centrifugation—CStickA), against the mean of the 2 methods in cattle urine.

validation was not required. This result was completely consistent with the results of other researchers (Seifi et al., 2004; Defontis et al., 2013). The findings of this study for the first time indicated that a good correlation was established by the Passing-Bablok regression analysis and Blond-Altman plot analysis between the urinary dipstick and the pH-meter to measure the urine pH in the cow.

Declarations

Author contribution statement

Ali Afsahi: Conceived and designed the experiments; Wrote the paper.
Mahmood Ahmadi-hamedani: Performed the experiments.
Mohammad Khodadi: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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