

Urine Concentration in Healthy and Diseased Dairy Cows during the First Month after Calving: Comparison of the Refractometry and Reagent Strip Methods

Gregorio José Alcántara-Isidro², M Belén García-Rodríguez^{1*}, Inmaculada Diez-Prieto M¹, Angeles Ríos-Granja¹, Maria Cano-Rábano¹, Carlos César Pérez-García¹

¹Department of Veterinary Medicine, Surgery and Anatomy, University of León, León, Spain.

² Federación Frisona de Castilla y León (FEFRICALE), Valladolid, Spain.

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*For Correspondence

M Belén García-Rodríguez, Department of Veterinary Medicine, Surgery and Anatomy, University of León, León, Spain, Tel: +34 987 29 1884.

E-mail: mbgarr@unileon.es

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ABSTRACT

Osmolality is a standard parameter to measure urine concentration, however clinicians often use refractometry or reagent strip to measure urine specific gravity (SG).

Objective: To evaluate refractometry and reagent strip for urine SG determination compared with osmolality, and to determine the range of urine concentration in healthy and diseased cows during the first month after calving.

Methods: Urine samples were obtained from 197 Holstein cows during the first month after calving and analyzed by reagent strip and refractometry immediately, and then frozen until osmolality determination.. The animals were assigned to two groups: healthy and diseased animals.

Results: Refractometry SG showed a good correlation with osmolality, but there was no correlation between them and SG by reagent strip. The mean urine osmolality was 781 mOsm/kg in healthy and 677 mOsm/kg in diseased cows.

Conclusions: Refractometry is a sensible device to use at a clinical level in cows to determine urine concentration, but not reagent strip. Urine concentration in dairy cows during the first month after calving was lower than previously reported as reference range in bovines, being even lower in diseased cows.

INTRODUCTION

The major pathologies of lactating dairy cows are known to occur during the peripartum period. The physiological changes that characterize this period make these animals prone to suffer health modifications that alter their productivity^[1]. Therefore, this study was performed during this period, in which the postpartum revision is standardized in order to achieve the restoration of health status and milk production.

The urine concentration determination is an important data in the urinalysis because it provides information related to the renal ability to appropriately respond to the hydric homeostasis variations, and moreover, it allows to properly interpreting the other parameters analyzed in the same sample^[2]. Furthermore, the urine concentration can be indicative of the hydration status when there is no renal, adrenal, or pituitary disease^[3].

Osmolality is a standard parameter used to determine urine concentration. However, clinicians often estimate urinary concentration by quantifications of urinary gravity, using direct (gravimetry) or indirect methods (refractometry and dipstick)^[4]. The urinary specific gravity (SG) value obtained by these methods can be affected by normal urine components^[5,6].

The urine test strip provides fast results without the need for external laboratory analysis ^[7] and is a practical method to obtain information during the postpartum examination protocol of lactating dairy cows, including urine SG, nevertheless there has been reported a lack of correlation between SG results by reagent strips and refractometry or osmolality in other animal species ^[8,9] and in the man ^[10,11].

To our knowledge the only comparison of methods for measuring urine concentration in bovine was reported by Thornton and English ^[12] that studied the correlation between SG, measured by urinometer, and osmolality in calves with diarrhea, showing good correlation between these two methods.

The aims of the present work were to compare the refractometry and reagent strip methods for determination of urinary concentration (urinary SG) compared to osmolality in order to set the utility of these methods at the clinical level, and to determine the range of urine concentrations in healthy and diseased cows (affected by several diseases) during the first month after calving.

MATERIALS AND METHODS

Animals

Urine samples obtained from 197 Holstein cows on 17 different dairy farms located in northwest Spain were analyzed in this study. The average milk yield was 32 kg/day. Only animals up to the fifth lactation stage were included.

The herds were in a reproductive control program that involved weekly, two weeks, or monthly visits, depending on the herd size. The program visit included the postpartum checks of all the animals calved since the previous visit, thus the maximum period between calving and the visit was 30 days, and urine samples were collected at this time. The study was carried out over 24 months.

The animals were assigned to one of two groups that differed in dry matter intake: healthy animals (n=123), with daily food intake that was considered normal by the farmer; and diseased animals (n=74), with daily food intake that was reduced or abolished. The animals identified as sick by the farmer were examined by the veterinary clinician in order to diagnose the disease involved.

Husbandry and feeding

The production system of the dairy farms involved was intensive and very similar. The animals were located in a free stall barn with a feed alley, which allowed tying of the animals. The animals received a ration formulated to provide a consumable quantity of feedstuffs, which would supply all required nutrients ^[13]. The feeding was performed once or twice daily. The morning feeding coincided with the program examination visit. Drinking water was provided ad libitum by different storage systems.

Temperature and humidity were very variable along the study period that, as was previously stated, was carried out for two years. The range of variation throughout the year in the region has been from a monthly mean temperature of -1.5 °C to 29.6°C, and from a monthly mean of relative humidity of 43% to 87%.

Sampling procedure

Urine samples was obtained by catheterization and stored in plastic tubes.

The urine was immediately analyzed using test strips and the urinary specific gravity was measured by refractometry. Samples were then refrigerated for 1-12 hr, until freezing at - 18°C. The frozen urine samples were stored until further analysis.

Urine analysis

Reagent strip: The urine samples obtained were immediately analyzed by Multistix 10 visual (Bayer Corporation, USA) and the results were recorded.

Refractometry: The urine SG was measured by refractometry using a portable refractometer (Zuzi, Japan), with a range of 1.000-1.040 and without temperature compensation. The coefficient of variation of repeated measures was 1%.

Urinary osmolality: The urine osmolality was determined by the freezing-point depression method with a FISKE One-Ten Osmometer (Needham Heights, USA). Prior to analysis, the samples were centrifuged in order to eliminate every particle that could experience crystallization before reaching the appropriate degree of freezing ^[14]. The coefficient of variation of repeated measures was 2.3%.

Statistical analysis

Data were analyzed using commercial software package (Statistica 7.0, Statsoft Inc. and MedCalc® version 11.0.1.0, MedCalc software). Correlation coefficient between the three analytical methods was calculated. Linear regression analysis was used to assess the relationship between urine concentrations measured by refractometry, and urine osmolality determinations. Comparison between methods was made using Deming regression and Bland- Altman difference plots.

One-way analysis of variance (ANOVA) was used in healthy cows to determine the effect of lactation number on urine concentration. The Newman-Keuls test was applied to establish the significant differences between lactations. ANOVA was used to determine the effects of health status (healthy compared to diseased cows) and the Newman-Keuls test showed the significant

differences between healthy animals and the subgroup of animals affected by the same disease. Statistical significance level of every test was $P < 0.05$.

RESULTS

Reagent strip results

In healthy and diseased cows were obtained negative results to leukocyte, nitrite, bilirubin and glucose and normal results to urobilinogen in all cases. The results obtained to hemoglobin, pH, ketone bodies, urinary protein, and specific gravity are presented as percentage in healthy and diseased cows (**Table 1**).

Table 1. Reagent strip result in healthy and diseased cows.

Hemoglobine	NEG	TR	40	80	200	ENT
Healthy (%)	74.1	0.6	0	0.6	7.4	17.3
Diseased (%)	64.6	11.1	1	3	15.2	5.1
pH	6	6.5	7	7.5	8	8.5
Healthy (%)	1.9	0	0.6	1.2	6.2	90.1
Diseased (%)	14.2	1	12.1	17.2	55.5	0
Ketone bodies	NEG	5	15	40	80	160
Healthy (%)	77.8	9.3	6.2	4.3	1.2	1.2
Diseased (%)	38.4	24.2	12.1	8.1	5.1	12.1
Protein	NEG	TR	30	100	300	2000
Healthy (%)	21.6	19.8	30.2	14.2	14.2	0
Diseased (%)	31.3	17.2	43.4	5.1	2	1
Specific gravity	1.000	1.005	1.010	1.015	1.020	1.030
Healthy (%)	3.7	34	49.4	9.9	2.5	0.6
Diseased (%)	1	13.4	44.3	17.5	15.5	8.2

Results of the urine analysis by reagent strip in healthy and diseased cows expressed as percentage.

Comparison of reagent strip, refractometry, and osmolality

The urinary SG measured via reagent strips did not exhibit correlation with urinary SG determined by refractometry or urinary osmolality, for either healthy or diseased cows (**Table 2**). Nevertheless, urinary SG determined by refractometry demonstrated a high correlation with urinary osmolality, when healthy and diseased cows were analyzed separately (**Table 2**) or when all obtained data were included ($r=0.92$; $p < 0.0001$). The following linear equation was obtained to calculate osmolality from SG measures by refractometry: $\text{Osm (mOsm/kg)} = -27567.2749 (\pm 890) + 27.649 (\pm 0.87) \times \text{refractometry SG}$ (**Figure 1**). When osmolality was calculated from refractometry SG according to the above equation 189 over 199 results (95%) were within ± 171 mOsm/kg of the osmolality measured value (**Figure 2**). The Deming regression analysis showed the existence of systematic constant differences (intercept: $-23,5952 \pm 22,5522$) but no systematic proportional differences (slope: $1,0318 \pm 0,03130$) between the measured osmolality and the calculated osmolality obtained applying the previous equation to refractometry SG results (**Figure 3**).

Table 2. Correlation coefficients between analysis methods.

	Reagent strip SG	Osmolality	Refractometry SG	Healthy cows
Reagent strip SG	1	-0.09	0.07	Reagent strip SG
Osmolality	-0.03	1	0.90	Osmolality
Refractometry SG	0.09	0.93	1	Refractometry SG
Diseased cows	Reagent strip SG	Osmolality	Refractometry SG	

Correlation coefficient (r) between urinary gravity determined by refractometry and reagent strip, and urinary osmolality, which were obtained separately in healthy and diseased cows.

Results obtained by reagent strip underestimated SG by refractometry by a mean value of 0.014 (**Figure 4**). Moreover, reagent strip results lower or equal to 1.010 account for 87.1% of the results in healthy animals and 58.7% in diseased animals while these results only account for 4% of the samples measured by refractometry (**Table 1**).

Healthy cows

The osmolality (**Figure 5**) and SG by refractometry (**Figure 6**) results obtained in healthy cows follows a normal distribution pattern (D'Agostini-Pearson Test: Osmolality, $p=0.0504$; Refractometry SG, $p=0.1725$).

The mean, standard deviations, minimum and maximum values of urinary SG determined by refractometry and osmolality in the group of healthy cows are shown taking the lactation number into consideration (**Table 3**). The ANOVA determined that the lactation number had a significant effect on urine osmolality ($P < 0.01$) and SG by refractometry ($P < 0.05$), and the Newman-Keuls

test demonstrated significant differences between the samples obtained from fifth lactation cows and samples obtained from first to fourth lactations cows, both in urinary osmolality ($P < 0.005$) and SG by refractometry ($P < 0.05$; except for the third lactation cows with $P < 0.01$).

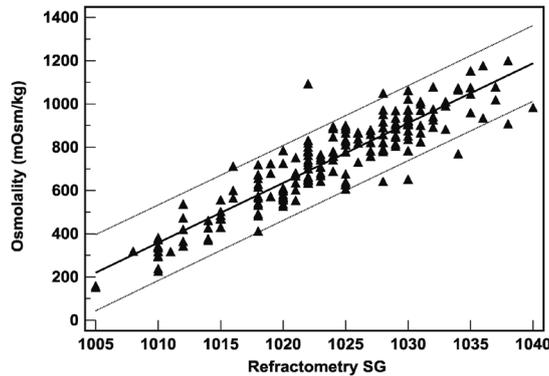


Figure 1. Linear regression scatter diagram of osmolality (mOsm/kg) versus SG by refractometry.

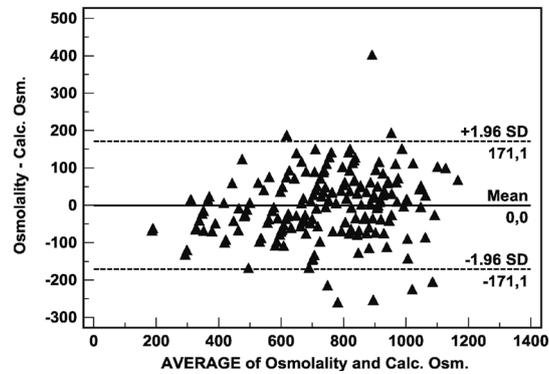


Figure 2. Bland-Altman difference plot of measured osmolality (Osmolality) versus calculated osmolality (Calc. Osm.) by the linear regression equation: $\text{Osmolality} = -27567.2749 + 27.649 \times \text{refractometry SG}$.

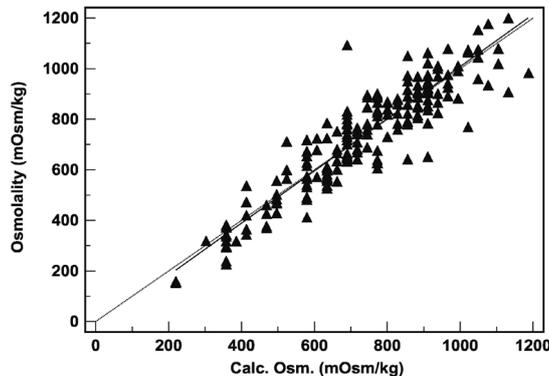


Figure 3. Deming regression scatter diagram of measured (Osmolality) versus calculated osmolality (Calc. Osm.).

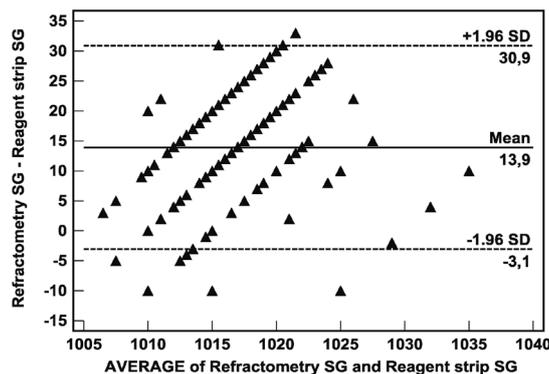


Figure 4. Bland-Altman difference plot of SG by refractometry and reagent strip.

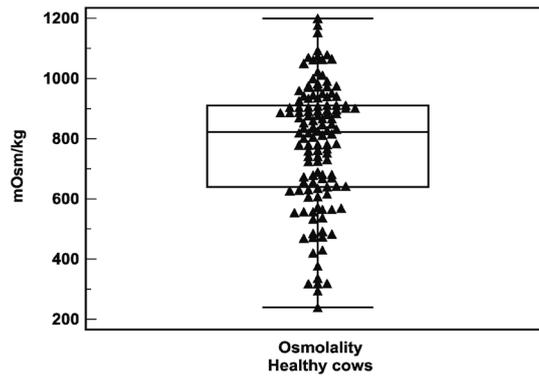


Figure 5. Box-and-whisker plot of osmolality (mOsm/kg) distribution in healthy animals.

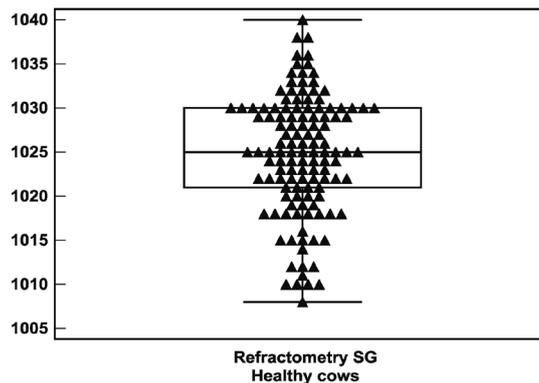


Figure 6. Box-and-whisker plot of SG by refractometry distribution in healthy animals.

Table 3. Urine osmolality and SG by refractometry in healthy cows.

Lactation	N	Urine osmolality (mOsm/kg)		Refractometry SG	
		mean ± SD	min.- max.	mean ± SD	min.-max.
first	40	813 ± 197 ^a	319 - 1200	1.026 ± 0.007 ^a	1.008 - 1.038
second	34	803 ± 183 ^a	377 - 1153	1.025 ± 0.006 ^a	1.014 - 1.040
third	27	765 ± 214 ^a	336 - 1177	1.024 ± 0.007 ^a	1.010 - 1.036
fourth	17	812 ± 146 ^a	483 - 1050	1.026 ± 0.005 ^a	1.018 - 1.036
fifth	9	541 ± 275 ^b	239 - 960	1.018 ± 0.0010 ^b	1.010 - 1.035
Total	127	781 ± 206	239 - 1200	1.025 ± 0.007	1.008 - 1.040

The mean ± standard deviation (SD), minimum and maximum values of the urinary osmolality (mOsm/kg) and urinary SG by refractometry, obtained in healthy cows at the different lactation numbers as well as the total values. The same superscript letter indicates no significant difference, while different superscript letters indicate significant differences between samples from different lactation cows by the Newman-Keuls test ($P < 0.05$).

Diseased cows

The diseases diagnosed were: ketosis (62%), displaced abomasum (26%), retained placenta/metritis (46%), mastitis (7%), puerperal paresis (5%), and lameness (4%). Some of the animals showed more than one disease.

The urine osmolality obtained from diseased cows group exhibited a mean value of 677 mOsm/kg, with a minimum value of 153 mOsm/kg and a maximum value of 1079 mOsm/kg. Urinary SG by refractometry obtained a mean value of 1.022, with a minimum value of 1.005 and maximum value of 1.037. The ANOVA and Newman-Keuls test demonstrated a significant effect of the health status on urine osmolality ($P < 0.005$) and SG by refractometry ($P < 0.05$). The urine osmolality and SG were lower in the animals affected by the different diseases diagnosed compared with healthy animals, and the difference was statistically significant with the cows affected of ketosis, retained placenta/metritis and mastitis (**Table 4**).

DISCUSSION

The comparison of different methods to determine the urine concentration demonstrated a significant positive correlation between urinary osmolality and SG from refractometry, as was previously reported by Thornton and English^[12] for calves with diarrhea. In contrast, the results presented herein demonstrated a lack of correlation between the SG determined by reagent strip and the two other methods studied, as has been also reported in other animal species^[8,9] and in man^[10,11]. Dorizzi and

Caputo [10] had excluded that urine pH and content of protein and glucose had an important effect in this discrepancy; thought the effect of urine protein can be variable depending on the pH of the solution [4]. On the other hand, the ionic components of urine have shown an important effect on the specific gravity measured by reagent strip, and particularly the ketone bodies have been associated to an underestimation of urine SG [15]. Nevertheless, our results showed the higher levels of urine specific gravity by reagent strip method in the diseased group of animals which ketone bodies excretion was also higher; moreover, the lowest measures of SG were obtained in healthy group cows which urine pH was more alkaline than the diseased group cows. It seems that the alkaline urine, mainly in the healthy cows group, may have some effect in the lack of correlation between the reagent strip and the refractometry or osmometry methods and in the extraordinarily low values obtained measuring the SG by reagent strip [16].

Table 4. Urine osmolality and SG by refractometry in diseased cows.

Disease	N	Urine osmolality (mOsm/kg)		Refractometry SG	
		mean \pm SD	min.- max.	mean \pm SD	min.-max.
Ketosis	44	675 \pm 210 ^{**}	298- 1076	1.023 \pm 0.008	1.010 - 1.037
Retained placenta/metritis	32	628 \pm 201 ^{**}	159 - 1079	1.021 \pm 0.007 [*]	1.005 - 1.037
Displaced abomasum	18	690 \pm 200	344 - 1045	1.022 \pm 0.007	1.010 - 1.035
Mastitis	5	616 \pm 198 ^{**}	365 - 836	1.020 \pm 0.007	1.012 - 1.030
Puerperal paresis	5	492 \pm 261	153 - 792	1.019 \pm 0.011	1.005 - 1.030
Lameness	4	667 \pm 367	153 - 938	1.022 \pm 0.012	1.005 - 1.030

The mean \pm standard deviation (SD), minimum and maximum values of the urinary osmolality (mOsm/kg) and urinary SG by refractometry, obtained in disease cows affected by different diseases. Significant differences with healthy animals by the Newman-Keuls test are expressed as: *P<0.01, ** P<0.005.

The differences in osmolality and osmolarity of organic fluids, such as urine, are negligible [47]. Therefore, the urine concentration data obtained in this study can be compared with the little available information on bovines that is expressed as osmolality or osmolarity. The mean urine osmolality in healthy cows during the first month after calving was lower than the urine osmolarity obtained by Osbaldiston and Moore [18] in lactating cows, which was 1080 mOsm/L. Similarly, the mean value obtained was lower than 1000 mOsm/kg considered normal for bovines by Gründer [19]. The lower mean urine concentration observed in the present study could be related to the lactation period when samples were collected. Osbaldiston and Moore [18] obtained samples at different moments of lactation period, while samples were only obtained during the first 30 days of lactation in the present study.

The dry matter intake ability is known to be inconsistent throughout the lactation period. During the first few weeks, there is an 18% reduction in the intake ability, which increases as the lactation progresses [13,20]. The reduction in dry matter intake may be concern in the lower urine concentration, considering the hormonal changes that occur after feeding of ruminants. The ruminal content is hypertonic with respect to the plasma after a meal due to the increase in salivary secretion produced at the start of the meal, which promotes an osmotic displacement of fluid from the blood to the rumen [24]. The displacement of fluid causes a transitory extracellular dehydration that stimulates the secretion of antidiuretic hormone and the renin-angiotensin system [22,23], contributing to a higher urine concentration. Therefore, the reduced dry matter intake during the first weeks after calving could partially explain the lower urinary concentration observed during this period. Another factor that could result in urine concentration variations compared to other studies could involve easy access to good quality water, as was in the animals included in this study, which could be related to a decreased requirement for conservation of free water. But it would be interesting to study urine concentration over the whole lactation period in animals under the same conditions of this study.

The mean urinary SG by refractometry in healthy cows was within the reference range determined by Gründer [19], and within the lower limits of the reference range from Kaneko et al. [24].

There was an unexpected and significant reduction in both the urine osmolality and the SG by refractometry for animals experiencing fifth lactation. This effect of lactation number could be due to the reduction in the urinary concentration capability with increasing age, as shown in other species [25,26]. In this respect, the fifth lactation cows would be within the limit of productive longevity, as indicating in a study by Rohrer and et al. [27] with average values of 2,496 days in Holstein cows.

The range of variation of urine concentration in dairy cows during the first month of the postpartum period was lower than that determined in other non-ruminant species, such as the dog, with a range of osmolality from 161 to 2830 and urine SG from 1.006 to 1.050 [25]. Moreover, the upper limit of the variation in dogs is clearly higher for both parameters than in dairy cows. The ruminal intervention on hydric homeostasis acting as a reservoir, compensating for the temporal effects of a deficiency or an excess of water [28], may be account for this differences.

The diseased cows demonstrated a statistically significant reduction in the average urine osmolality and SG in relation to healthy cows. The reduced urine concentration is also evident when are considered separately the subgroups of animals affected by different diseases, though not all with significant differences. The different pathologic mechanisms involved in each disease process make difficult to explain the similar changes in urine concentration, but considering that a reduced or abolished dry matter intake was a common characteristic of the diseased cows group, the reduction in salivary secretion and limited increase in ruminal osmolality [21] could be one factor that influenced the lower urine osmolality in this group of animals.

There are some limits in this study. The information over hydration status (hematocrit and plasma proteins) and renal function (urea and creatinine) would be interesting data to interpret the urine concentration, but it requires blood sampling and the farmers didn't accept the additional stress. Another limitation of the study is the lack of data of the urine concentration during the whole lactation and dry periods in similar conditions, and more data from five lactation cows.

In conclusion, urine SG by refractometry is a method that is comparable to urine osmolality. Furthermore, the small dimensions and easy use of the clinic refractometer make this a practical device at the clinical level. Nevertheless, the reagent strip is not a useful method to determine the urine SG in dairy cows.

The reference range of urine SG and osmolality in healthy dairy cows during the first month after calving was lower than previously reported average values and even lower in disease cows irrespective of the associated disease and the fifth lactation cows exhibited an unexpected reduction in the mean urine concentration, which must be investigated.

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