Effect of Furosemide on Urine Specific Gravity and Osmolality in Thoroughbred Racehorses*

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ABSTRACT

Postrace urine samples from thoroughbred horses were examined to compare osmolality and specific gravity between horses treated with furosemide and those not treated. Samples were assigned to groups in relation to reported medication (furosemide) status, race finish position, and distance of race. Urine osmolality was significantly ($P < .05$) lower in samples from horses treated with furosemide when compared with untreated horses. Specific gravity determinations are less precise at measuring urine osmolality at lower levels (1.01 g/ml or less). The measurement of osmolality is a superior method for determining the urine solute concentration and facilitating the regulation of furosemide.

INTRODUCTION

Furosemide is a potent diuretic that inhibits sodium reabsorption at the thick ascending loop of Henle in the kidney; furosemide is also often given to racehorses with a history of bleeding in the respiratory tract (exercise-induced pulmonary hemorrhaging).[1,2] In Kentucky, during the period encompassed by this study, the permitted dose of furosemide (Lasix [now Salix], Aventis) for thoroughbred racehorses was 250 mg administered by IV injection at least 4 hours before the start of the race. There are concerns that the administration of a medication such as furosemide can affect the urinary and plasma concentrations of other coadministered agents.[3] Of particular concern is that these effects cannot be predicted for drugs and medications that have not yet been tested. In addition to the concern regarding the diluting ability of furosemide is the question regarding the potential effects of this medication on performance.[4,5] A survey of results yielded a conclusion that the administration of furosemide to standardbred horses significantly decreased their racing time compared with horses that were not treated with furosemide.[5] Another study concluded that the administration of furosemide to thoroughbred horses improved their performance by three to five-and-a-half lengths in a 6-furlong race.[4]

Following maximal doses of furosemide, the kidney is less able to concentrate or dilute the final urine (the urine approaches or will become isosmotic to plasma).[1] As a consequence of the diuretic action of furosemide, the osmolality (and the specific gravity) will be lowered in proportion to the dose and duration following administration of the diuretic. These furosemide-related actions form the basis for the potential for misuse of the diuretic and provide the motivation for screening the urine from racehorses that finish in the top three positions. Typically, if the specific gravity of the urine sample is less than 1.010 g/ml, the con-

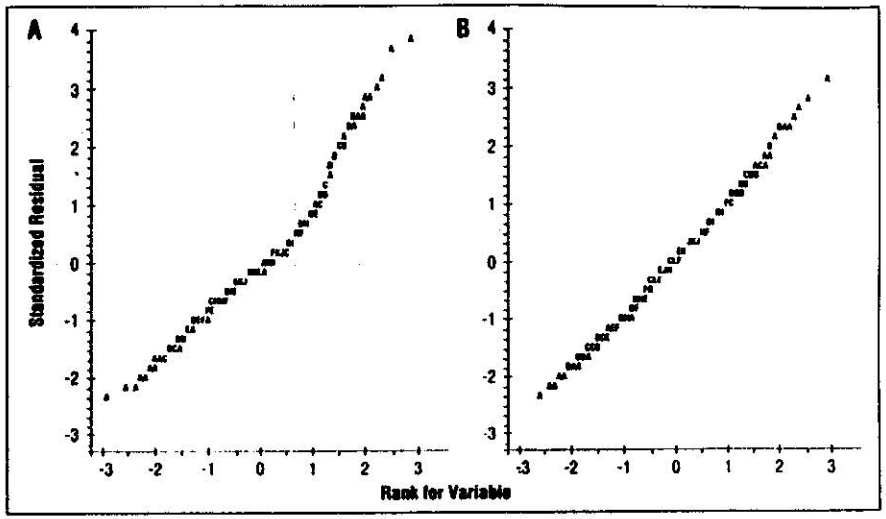

Figure 1. *Normality plot of osmolality* **(A)** *and specific gravity* **(B)** *results. The standardized residuals were plotted against their expected rank if they had come from a standard normal distribution. The symbols displayed are indicative of the number of data points they represent (e.g., A = 1, B = 2).*

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TABLE 1. Osmolality and Specific Gravity Data (Mean and SD) for Groups of Horses of Different Racing Status with and without Furosemide Treatment Before Racing

Group	Number of Horses	Racing Status*	Osmolality (mOsm/kg water)	SD	Specific Gravity (g/ml)	SD
Furosemide						
1	38	Sprint, Winner	797	279	1.0262	0.0095
2	71	Sprint, Nonwinner	762	262	1.0261	0.0094
3	31	Route, Winner	747	226	1.0255	0.0100
4	54	Route, Nonwinner	719	224	1.0241	0.0079
No furosemide						
5	23	Sprint, Winner	1124	387	1.0360	0.0102
6	39	Sprint, Nonwinner	1164	452	1.0345	0.0114
7	18	Route, Winner	1094	466	1.0346	0.0100
8	34	Route, Nonwinner	989	364	1.0308	0.0095

*Route = races of 1 mile or more; sprint = races of 6.5 furlongs or less.

centration of furosemide would then be determined in the blood sample collected concurrently with the urine sample. Chu and others concluded that horses would be less likely to be misclassified as being in violation of regulatory concentrations of furosemide if plasma furosemide were determined in addition to urine specific gravity.[6] On the other hand, Uboh and coworkers concluded that urine specific gravity values were not reliable for predicting the dose or time of administration of furosemide to racehorses.[7] Consequently, it was speculated that measurement of the urine osmolar concentration as a screening method may be a more accurate indication of the administration of furosemide because the type of solute contained in a given sample does not affect the osmolality of the solution. In contrast, specific gravity is only an approximation of the total solute concentration and can be affected by a number of physiologic variables, including the amount of protein in the sample.[8] Therefore, in the current study, specific gravity and osmolality of urine samples from thoroughbred horses (n = 308) were compared following races.

MATERIALS AND METHODS

Urine Samples

A total of 308 postrace urine samples were obtained from thoroughbred racehorses at Turfway Park, Florence, Kentucky during the racing meet from September through December 1995; samples were stored at –70°C until the time of analysis. Horses selected for sampling included the first three finishers in each race plus other randomly selected horses. Samples were sequentially numbered. The furosemide status of each horse was declared before the race and was independently verified during the postrace regulatory analysis. Upon completion of all analyses, these "blind" samples were then identified for classification. Fewer than 10 horses were sampled more than once during the study period. These duplicate samples were identified and excluded from analysis. The remaining identified samples were then assigned to one of eight corresponding groups:

Group 1: Furosemide, sprint (6.5 furlongs or less) winners

Group 2: Furosemide, sprint, nonwinners

TABLE 2. Statistical Comparison of Urine Osmolality Data Among Groups of Racehorses with Different Racing Status with and without Furosemide Treatment Before Racing

	Furosemide				No Furosemide		
	Sprint, Winner	Sprint, Nonwinner	Route, Winner	Route, Nonwinner	Sprint, Winner	Sprint, Nonwinner	Route, Winner
No furosemide							
Route, nonwinner	Y	Y	Y	Y	N	N	N
Route, winner	Y	Y	Y	Y	N	N	—
Sprint, nonwinner	Y	Y	Y	Y	N	—	—
Sprint, winner	Y	Y	Y	Y	—	—	—
Furosemide							
Route, nonwinner	N	N	N	—	—	—	—
Route, winner	N	N	—	—	—	—	—
Sprint, nonwinner	N	—	—	—	—	—	—

Data were analyzed by one-way analysis of variance and the Student-Newman-Keuls post hoc test. Y indicates values were significantly different ($P < .05$). N indicates no significant [illegible]. Route = races of 1 mile or more; sprint = races of 6.5 furlongs [illegible].

Group 3: Furosemide, route (1 mile or more), winners

Group 4: Furosemide, route, nonwinners

Group 5: No furosemide, sprint, winners

Group 6: No furosemide, sprint, nonwinners

Group 7: No furosemide, route, winners

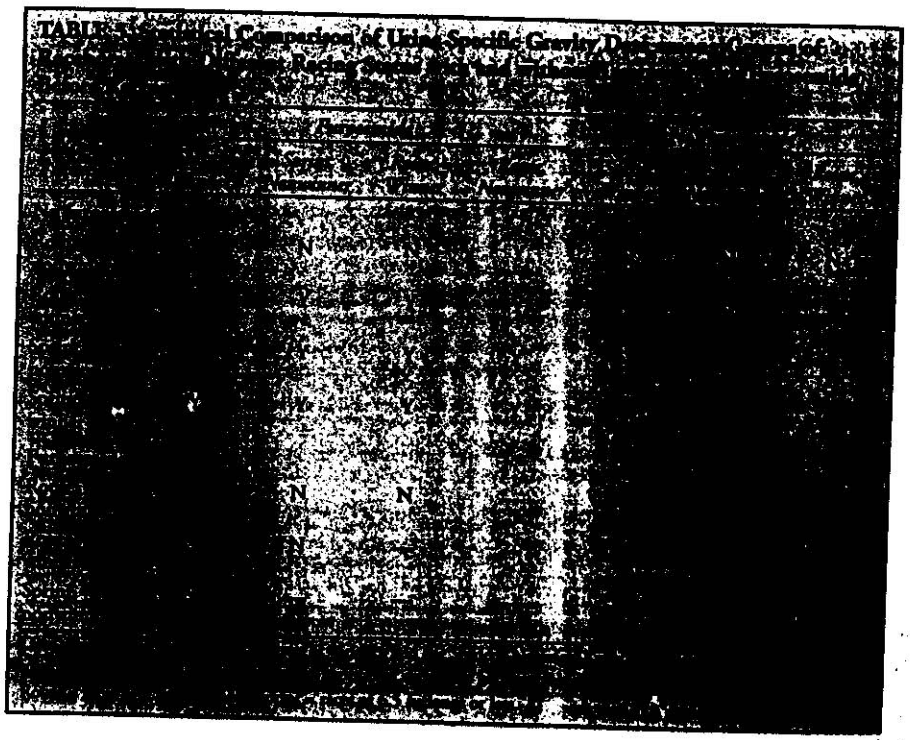
Group 8: No furosemide, route, nonwinners.

There were no races between the distances of 6.5 furlongs and 1 mile (8 furlongs).

Urine Analysis

Frozen urine samples were thawed at room temperature before analysis. Specific gravity was determined with a refractometer (National Instrument) set to 1.000 g/ml with deionized water and a hydrometer (Squibb Urometer). The refractometer displayed specific gravity values up to 1.045 g/ml, and the hydrometer's range of measurement was from 1.000 to 1.060 g/ml, with a stated accuracy of ± 0.002 g/ml. Using linear regression analysis, there was a strong correlation between the specific gravity values obtained between the two methods ($r^2 = 0.86$). The values for specific gravity of samples that were greater than 1.045 g/ml by refractometry were taken from the corresponding hydrometer measurement.

Osmolality was determined using a freezing-point depression osmometer (Precision Systems). The resolution of the osmometer was 1 mOsm/kg. The repeatability and linearity of the osmometer were ± 2 mOsm/kg between 0 and 400 mOsm/kg and ± 0.5% between 400



and 2,000 mOsm/kg. Calibrator solutions of 100 and 500 mOsm/kg were tested, and the osmometer was adjusted to these solutions before samples were assayed.

Statistical Evaluation

Normality of the residuals by one-way analysis of variance (ANOVA) was determined using the rank and plot procedure of SAS version 8 (SAS Institute). In the normality plot, the standardized residuals were plotted against their expected rank if they were from a standard normal distribution (Figure 1). The residuals were plotted against the predicted values from the model. Differences between groups were evaluated using a one-way analysis of variance and the Student-Newman-Keuls post hoc test. Correlation between specific gravity and osmolality was evaluated using linear regression analysis, a sigmoidal (Boltzman), or both fit to the data. Data are presented as mean ± SD, and differences were considered to be statistically significant when $P < .05$.

Both normal plots from the ANOVA for the specific gravity and osmolality values (Figure 1) could be fitted by a straight line. Both residual plots exhibited heteroskedasticity. Based on these findings, it was concluded that a nonparametric test of medians would add no extra statistical power.

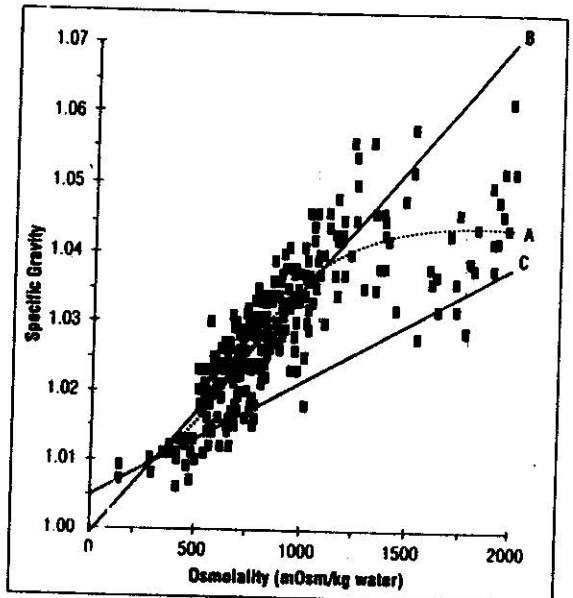


Figure 2. *Urine specific gravity plotted as a function of urine osmolality for all samples (n = 308). Line A* (dashed) *represents a sigmoidal (Boltzman) fit to the data (Chi square = 0.00003). Line B reflects a linear regression fit to the urine osmolality data between 500 and 1,000 mOsm/kg; correlation coefficient (r) = 0.71862, slope (m) = 0.00004, n = 206. Line C reflects a linear regression fit to the urine osmolality data less than 500 mOsm/kg; r = 0.51961, m = 0.00002, n = 24.*

RESULTS

The urine specific gravity for all the furosemide-treated horses (Groups 1 through 4, n = 194) was 1.0255 ± 0.009 g/ml. Urine specific gravity (1.0337 ± 0.010 g/ml) was significantly ($P < .05$) higher in horses in groups not treated with furosemide (Groups 5 through 8, n = 114) than in groups that did receive furosemide. The urine osmolality of horses that were not treated with furosemide (1,093 ± 418 mOsm/kg) was also significantly ($P < .05$) higher than that of furosemide-treated horses (755 ± 249 mOsm/kg).

Osmolality and specific gravity data for each group are presented in Table 1. The means (and medians) of osmolality for each group differed by less than 10% and the means (and medians) of specific gravity for each group differed by less than 0.002% in all cases.

The statistical evaluation of osmolality data (ANOVA and Student-Newman-Keuls post hoc test) are presented in Table 2 (osmolality) and Table 3 (specific gravity). Urine osmolality values of each furosemide-treated group were significantly ($P < .05$) different from each group not treated with the diuretic. In contrast, a similar analysis of urine specific gravity measurements demonstrated that group urine specific gravity was different in only 13 of the 16 comparisons between the furosemide-treated and untreated groups (Table 3).

Further analysis of these data is illustrated in Figure 2, which presents a plot of the specific gravity measurement as a function of the corresponding osmolality determination in each of the 308 samples. This graphic includes a sigmoidal (Boltzman) fit to the data; Chi square value for this analysis was 0.00003. In addition, linear regression analysis of the urine osmolality values between 500 and 1,000 mOsm/kg and corresponding specific gravity measurements (n = 206) is illustrated; the correlation coefficient (r) was 0.71862 ($P < .001$) and the slope (m) was 0.00004. By contrast, a similar linear regression analysis of the data (n = 24) below 500 mOsm/kg yielded an r of 0.51961 ($P < .01$) with m equal to 0.00002. These values were significantly lower than the corresponding values for data points between

500 and 1,000 mOsm/kg. Finally, for values greater than 1,000 mOsm/kg (n = 78), the linear regression analysis yielded an r of 0.31359 and an m of 0.000007 (line not shown).

DISCUSSION

As expected, solute concentrations of the postrace urine samples were significantly affected by the medication status of the animal. Thus, all horses treated with furosemide had significantly lower urine osmolalities compared with horses not treated with furosemide. However, not all of the horses in groups treated with furosemide had urine specific gravities that were lower than the values for horses not treated with furosemide, supporting a conclusion that measurement of specific gravity is not as accurate as measurement of osmolality for estimating urine solute concentration.

This conclusion is further supported by correlation of osmolality values with urine specific gravity. Urine specific gravity correlates less well with osmolality below 500 mOsm/kg (as well as with osmolality above 1,000 mOsm/kg) compared with its correlation with osmolality values between 500 and 1,000 mOsm/kg. This is particularly important given the fact that the corresponding plasma or urine samples from horses with urine specimens with specific gravity values in the range of 1.010 are likely to be subjected to further regulatory evaluation for elevated plasma or serum concentrations of furosemide.

In summary, these data demonstrate that urine osmolality is more precise as an indicator of the diuretic state of the racehorse than is urine specific gravity.

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Efficacy of Extended Pirlimycin Therapy for Treatment of Experimentally Induced *Streptococcus uberis* Intramammary Infections in Lactating Dairy Cattle*

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ABSTRACT

Streptococcus uberis is an important cause of mastitis in dairy cows throughout the world, particularly during the dry period, around the time of calving, and during early lactation. Strategies for controlling *S. uberis* mastitis have not received adequate research attention and are therefore poorly defined and inadequate. Objectives of the present study were to evaluate the efficacy of extended therapy regimens with pirlimycin for treatment of experimentally induced *S. uberis* intramammary infections in lactating dairy cows during early lactation and to evaluate the usefulness of the *S. uberis* experimental infection model for evaluating antimicrobial efficacy in dairy cows. The efficacy of extended pirlimycin intramammary therapy regimens was investigated in 103 mammary glands of 68 dairy cows that became infected following experimental challenge with *S. uberis* during early lactation. Cows infected with *S. uberis* in one or both experimentally challenged mammary glands were randomly allocated to three groups, representing three different treatment regimens with pirlimycin, including 2-day (n = 21 cows, 31 mammary quarters), 5-day (n = 21 cows, 32 quarters), and 8-day (n = 26 cows, 40 quarters). For all groups, pirlimycin was administered at a rate of 50 mg of pirlimycin hydrochloride via intramammary infusion. A cure was defined as an experimentally infected mammary gland

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