Comparison of volumetric urine collection versus single-sample urine collection in horses consuming diets varying in cation-anion balance

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Objective—To determine daily variation in urinary clearance and fractional excretion (FE) of electrolytes and minerals within and between horses and to compare volumetric and single-sample urine collection for determining FE values of diets with a range of dietary cation-anion balance (DCAB).

Animals—5 Thoroughbred and 6 mixed-breed mares.

Procedure—3 isocaloric diets with low, medium, and high DCAB values (85, 190, and 380 mEq/kg of dry matter, respectively) were each fed for 14 days. Daily blood samples, single urine samples collected by using a urinary catheter (5 mares), and volumetric urine collections (6 mares) were obtained during the last 72 hours of each diet.

Results—Urine and plasma pH values, plasma concentrations, and FE values of sodium, chloride, potassium, magnesium, phosphorus, and calcium were altered by varying the DCAB. Noticeable variation in clearance and FE values was detected within horses from day-to-day on the same diet as well as between horses. Fractional excretion values were not significantly different between single-sample and volumetric methods, except for magnesium in the high DCAB diet. Volumetric and single-sample collections revealed similar patterns of change in urinary FE values with varying DCAB, except for calcium and magnesium.

Conclusions and Clinical Relevance—Substantial variation in clearance and FE of electrolytes and minerals are evident within horses between 24-hour periods as well as between horses fed a specific diet. Three daily urine samples provide similar information regarding dietary-induced changes in clearance and FE values (excluding calcium and magnesium) as that obtained by volumetric urine collection. (*Am J Vet Res* 2003;64:284–291)

Electrolyte and mineral balance in horses is controlled primarily via hormonal influences on the renal and gastrointestinal systems.[1,2] Aldosterone constitutes the major method of control over plasma concentrations and urinary excretion of sodium and potassium. Aldosterone is released primarily in response to even minor increases in serum potassium concentration, resulting in a direct stimulatory effect on the adrenal cortex.[3] Aldosterone is also secreted in response to factors that elicit secretion of ACTH or stimulate the renin-angiotensin system, including stress, decreases in vascular volume, and low dietary sodium intake. Aldosterone release results in substantial antinatriuresis and kaliuresis.[3,4] Other factors that affect the plasma concentration and urinary excretion of sodium and potassium include renal tubular integrity, glomerular filtration rate, diuresis, and acid-base status.[3]

Parathyroid hormone (PTH) controls plasma concentrations as well as urinary excretion of calcium and phosphorus and, to a lesser extent, magnesium. Parathyroid hormone is released primarily in response to minor decreases in serum ionized calcium concentration, and it promotes mobilization of calcium from the soluble bone pool, resorption of calcium and magnesium in the renal tubules, and increased urinary phosphorus excretion.[4] The release of PTH also stimulates renal production of active 1,25 dihydroxyvitamin D, which stimulates active absorption of calcium in the gastrointestinal tract.[4]

Dietary factors, particularly the **dietary cation-anion balance (DCAB)**, can have profound effects on electrolyte and mineral balance in many species including horses.[5-7] A low DCAB diet (high anion content) induces metabolic acidosis, which promotes increased sensitivity of tissue receptors to the effects of PTH, resulting in enhanced release of calcium from bone and enhanced absorption of calcium from the GI tract.[8] Acidosis overrides the effects of PTH on renal tubules, resulting in increased urinary excretion of calcium as a result of PTH.[9] A high DCAB diet induces nutritional metabolic alkalosis and increases urinary excretion of potassium and sodium.[5,6,10]

Fractional excretion (FE) of electrolytes and min-

Received April 10, 2002.
Accepted October 25, 2002.
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Supported by the Southern California Equine Foundation.
The authors thank Dr. Michael Murphy and Tom Arendt for technical assistance.
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erals in single urine samples obtained by use of a catheter has been used to evaluate electrolyte and mineral balance in healthy horses as well as horses with disorders such asexertional rabdomyolysis.[11,12] Dietary intake is 1 of the principal determinants of FE of electrolytes and minerals in most circumstances. Additional factors that affect FE of electrolytes and minerals include submaximal exercise, which increases urine flow and the FE of sodium and potassium and decreases the FE of chloride, and meal consumption, which can substantially decrease the FE of sodium and increase the FE of potassium.[13-15] Several authors have questioned the accuracy of a single urine sample obtained by use of a catheter because of these effects, as well as the substantial diurnal variation in the FE of chloride, sodium, calcium, and potassium documented in studies[16,17] that involved the use of volumetric urine collection during a 24-hour period. Volumetric urine collection, however, is laborious to perform, frequently requires confinement of animals, and often necessitates a period during which the animals adapt to the urine collection devices. Variability in urinary clearance and FE of electrolytes and minerals in the same individual during consecutive days does not appear to have been investigated.[17,18]

The purpose of the study reported here was to determine the degree of variability in clearance and FE of electrolytes and minerals within horses between consecutive 24-hour periods, as well as the variation among horses for a single urine sample obtained by use of a catheter and urine collected by use of a volumetric urine collection method. The degree of accuracy with which 3 daily single urine samples obtained by use of a catheter were able to reflect the pattern of change in electrolyte and mineral excretion for a range of DCAB, compared with results for 3 consecutive 24-hour volumetric urine collections, was also evaluated. This study was an extension of a set of experiments evaluating differences in electrolyte and mineral metabolism between horses with **recurrent exertional rhabdomyolysis (RER)** and control horses. As reported elsewhere,[10] we did not detect significant differences in electrolyte or mineral metabolism between RER and control horses, allowing inclusion of RER horses in the volumetric and single-sample urine collection groups.

Materials and Methods

Animals—Eleven mares (5 Thoroughbred mares with RER that ranged from 2 to 15 years of age [mean, 8 years] and 6 clinically normal mixed-breed [control] mares that ranged from 4 to 10 years of age [mean, 6 years]) were used in the study. Control and RER mares were selected on the basis of criteria established elsewhere,[10,19] including serum concentrations of creatine kinase and results of contracture testing of intercostal muscles.[10,19] Horses were allowed to drink from an automated watering system during the study, and daily consumption was not determined.

Dietary formulation—Three diets with various DCAB values were created. Each diet consisted of a foundation pellet[a] that was fed twice daily in combination with grass hay (ratio of concentrate to roughage, 45:55). Dietary cation-anion balance was calculated by measuring concentrations of various electrolytes (ie, **sodium** [NA^+], **potassium** [K^+], **chloride** [CL^-], and **sulfur** [S^{2-}]) and use of the following equation:

$$DCAB = (NA^+ + K^+) - (CL^- + S^{2-})$$

Diets were designed to meet minimum requirements for vitamins, electrolytes, and trace minerals established by the National Research Council,[20] and each ration was fed at approximately 2.2% of body weight to provide 28.8 Mcal of digestible energy/d. Dietary energy content surpassed the daily requirements of resting horses by 75%, because results of another study[21] documented that a high-carbohydrate diet with an energy content that surpassed the daily energy requirements of sedentary horses by 70% was necessary to evoke significant increases in serum creatine kinase activity in horses with RER performing exercise on a treadmill.

Formulation and electrolyte and mineral composition of the diets used in the study has been reported elsewhere.[10] The foundation pellet of the low DCAB diet was supplemented with Cl^- and S^{2-} in the form of an organic soy product[b] to reduce the DCAB value of the total diet to 85 mEq/kg of dry matter. Sodium bicarbonate (4.2% dry matter) was added to the foundation pellet of the high DCAB diet to increase the DCAB of that diet to 380 mEq/kg of dry matter. The foundation pellet of the medium DCAB diet was not altered, resulting in a DCAB value of 190 mEq/kg of dry matter.

After formulation, analysis of each diet by a commercial laboratory[c] revealed the concentration of Na^+ in the medium diet was lower than requested, yielding a final dietary dry-matter Na^+ concentration of 0.17% for the low DCAB diet, 0.09% for the medium DCAB diet, and 0.56% for the high DCAB diet.[10] Therefore, the Na^+ concentration of the medium DCAB diet was slightly less than the recommended minimum of 0.1% dry matter for sedentary horses.[22] **Crude protein (CP)** content of the 3 diets also differed, with the low diet containing 17.8% CP (dry-matter basis), the medium diet containing 15.4% CP, and the high diet containing 14.4% CP.

Feeding protocol—To introduce the pelleted diet, horses were fed gradually increasing amounts of the foundation pellet for the medium diet during a 5-day period. Then each horse was fed the same diet concurrently for a 14-day period, commencing with the high DCAB diet, which was followed by feeding of the medium DCAB diet and then the low DCAB diet to prevent sudden extreme changes in DCAB.

Collection and analysis of samples—On the last 3 mornings that each diet was fed, jugular venous blood samples were collected from all horses into tubes containing sodium heparin. Samples were obtained prior to feeding on each of those mornings, and plasma was harvested and used to determine plasma concentrations of electrolytes, minerals, and **creatinine (Cr)**. Plasma concentrations of Cr, Na^+, Cl^-, K^+, **calcium (Ca^{2+})**, **phosphorus (P)**, and **magnesium (Mg^{2+})** were determined by use of an automated chemistry analyzer.[d]

Urine samples were collected and used to determine urine FE values. Urine samples were obtained from 5 mares once daily prior to the morning feeding simultaneously with venous blood collection; these samples were obtained by use of a catheter inserted into the bladder of each horse. The remaining 6 mares (3 control mares and 3 mares with RER) were restrained in tie stalls with continuous observation to allow 72 hours of volumetric urine collection by use of a rubber self-retaining urine collection harness.[e] Mares were under surveillance throughout collection periods to prevent urine spillage. Urine spillage rarely occurred; however, when it did occur, the amount of spilled urine was estimated and recorded. Volume and pH of urine samples were measured every 6 hours, and an aliquot of urine was retained after vigorous stirring. An aliquot was collected from each 6-hour sample and pooled to create a sample for each 24-hour period of collection, and each 24-hour pooled sample was submitted for analysis of mineral and electrolyte content. Fractional excretion was calculated by use of the following equation:

$$FE = \frac{[Cr]_{plasma}}{[Cr]_{urine}} \times \frac{[X]_{urine}}{[X]_{plasma}} \times 100$$

where $[Cr]_{plasma}$ and $[Cr]_{urine}$ are the Cr concentrations in plasma or urine, respectively, and $[X]_{plasma}$ and $[X]_{urine}$ are the concentrations of a specific mineral or electrolyte in plasma or urine, respectively.

Renal clearance of Cr, Na^+, Cl^-, K^+, Ca^{2+}, P, and Mg^{2+} was calculated from volumetric urine collections by use of the following equation:

$$Clearance_X = ([X]_{urine} / [X]_{plasma}) \times (V_u/\text{time/body weight})$$

where V_u is the 24-hour urine volume, time is the number of minutes per urine collection period, and body weight is the body weight of the horse (in kilograms).[17]

Urine pH was determined by use of pH indicator strips.[f] Aliquots of 24-hour urine collections were refrigerated at 4°C and analyzed within 36 hours after collection. Aliquots of urine were mixed with concentrated nitric acid at a ratio of 1 part urine:2 parts nitric acid to dissolve urine crystals prior to analysis. Urine concentrations of Na^+, K^+, Ca^{2+}, P, and Mg^{2+} were determined by use of emission spectrometry. Urine concentrations of Cl^- and Cr were measured by use of an automated chemistry analyzer.[d] Urine specific gravity was not measured.

Statistical analysis—Data from 3 horses in which urine was collected by use of volumetric urine collection during the period when the horses were consuming the low DCAB diet were excluded from analysis on the basis of inadequate consumption of the diet. The remaining data, incorporating results of plasma biochemical analysis, urinary clearance, and FE values, were analyzed by use of a 1-way ANOVA and simple linear regression, which controlled for repeated measures in a mixed-model approach by use of a software program.[23] Results were expressed as mean ± SEM values. Significance was set at $P < 0.05$. Pearson correlations and **coefficients of variation (CVs)** were calculated by using the software program after stratifying data on the basis of diet and day, when appropriate. The CVs were calculated within a univariate procedure.[23]

Results

Plasma concentrations of electrolytes and minerals—Plasma pH was significantly decreased when horses consumed the low DCAB diet, compared with values for horses when they consumed the other 2 diets (**Table 1**). Plasma Cr and total Ca^{2+} concentrations did not differ among horses when consuming each of the various diets. Consumption of the low DCAB diet resulted in a small but significant decrease in plasma Na^+ concentration and a markedly higher plasma Cl^- concentration, compared with values when horses were consuming the medium and high diets. Plasma K^+ and plasma Mg^{2+} concentrations were significantly lower in horses when consuming the low DCAB diet, compared with values when horses were consuming the medium DCAB diet. Plasma concentrations of Na^+, Cl^-, and Mg^{2+} did not differ between horses when consuming the medium and high diets. Consumption of the high DCAB diet resulted in higher plasma P and lower plasma K^+ concentrations, compared with values during consumption of the medium diet.

Table 1—Mean ± SEM values for plasma pH and plasma concentrations of creatinine, electrolytes, and minerals in horses consuming 3 diets that had low, medium, and high dietary cation-anion balance (DCAB), respectively

Variables	Low DCAB (8 horses, 19 plasma samples)	Medium DCAB (11 horses, 32 plasma samples)	High DCAB (11 horses, 28 plasma samples)
pH	7.33 ± 0.01[a]	7.38 ± 0.01[b]	7.38 ± 0.01[b]
Creatinine (mg/dL)	1.63 ± 0.04	1.48 ± 0.06	1.50 ± 0.05
Sodium (mEq/dL)	136.6 ± 0.38[a]	137.9 ± 0.29[b]	137.9 ± 0.35[b]
Chloride (mEq/dL)	109.4 ± 0.61[a]	103.8 ± 0.29[b]	104 ± 0.36[b]
Potassium (mEq/dL)	4.11 ± 0.11[a]	4.59 ± 0.15[b]	3.89 ± 0.15[a]
Magnesium (mg/dL)	1.85 ± 0.04[a]	2.01 ± 0.03[b]	1.94 ± 0.02[a,b]
Phosphorus (mg/dL)	3.54 ± 0.15[a,b]	3.36 ± 0.15[a]	3.72 ± 0.17[b]
Calcium (mg/dL)	11.9 ± 0.09	12.1 ± 0.04	12.0 ± 0.09

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

Urine concentrations of electrolytes and minerals—Urine pH was significantly lower (more acidic) when horses consumed the low diet (mean ± SEM pH, 5.3 ± 0.05), compared with values when horses consumed the medium (pH, 7.9 ± 0.07) and high (pH, 8.6 ± 0.07) diets. In horses in which a single urine sample was collected by use of a catheter, urine Cr and P concentrations did not differ significantly among samples obtained when horses consumed each of the diets (**Table 2**). Urine concentration of K^+ was significantly lower and urine concentration of Cl^- significantly higher when horses consumed the low DCAB diet, compared with electrolyte concentrations when horses consumed the other diets. Urine concentration of Na^+ was higher and urine concentration of Ca^{2+} lower when horses consumed the high DCAB diet, compared with values when horses consumed the medium diet. Urine concentration of Mg^{2+} was significantly higher in horses consuming the medium diet.

In the volumetric collection group, urine Cr and K^+ concentrations were significantly lower and urine P concentration significantly higher when horses consumed the low DCAB diet, compared with values for horses consuming the other diets. Urine Mg^{2+} and Ca^{2+} concentrations differed significantly among horses consuming each of the various diets and were lowest when horses consumed the low DCAB diet. Urine concentration of Na^+ was significantly greater for horses consuming the high DCAB diet. Urine concentration of Cl^- differed significantly between horses when consuming the medium and high diets.

When horses consumed the low DCAB diet, urine concentrations of Na^+, K^+, Cl^-, and Mg^{2+} were significantly greater in horses from which a single urine sample was collected by use of a catheter, compared with values for horses from which urine was collected by use of the volumetric collection method. When horses consumed the high DCAB diet, urine concentrations of Cl^- were significantly greater in horses from which a single urine sample was collected by use of a catheter, compared with values for horses from which urine was collected by use of the volumetric collection method.

Subjectively, urine was clear and almost entirely

Table 2—Mean ± SEM (range) values for urine volume and concentrations of creatinine, electrolytes, and minerals in urine samples obtained by use of a single-sample catheter collection or volumetric collection method during 3 consecutive days from horses consuming 3 diets that varied in DCAB

	Low DCAB		Medium DCAB		High DCAB	
Variable	**Volumetric (3 horses)***	**Catheter (5 horses)**	**Volumetric (6 horses)**	**Catheter (5 horses)**	**Volumetric (6 horses)**	**Catheter (5 horses)**
Creatinine (mg/dL)	124.2 ± 8.9^{A} (87.8–178.9)	180.9 ± 18.8 (101.2–251.7)	224.9 ± 19.3^{B} (93.2–397.1)	237.2 ± 34.6 (57.7–458.0)	200.9 ± 18.9^{B} (68.7–382.4)	246.7 ± 37.7 (102.8–491.0)
Sodium (mEq/L)	$25.1 \pm 3.7^{\dagger A}$ (10.3–42.3)	$50.4 \pm 7.9^{a,b}$ (11.8–90.4)	9.4 ± 2.7^{A} (1.3–36.6)	13.4 ± 6.2^{b} (1.0–82.5)	84.4 ± 13.4^{B} (13.3–176.9)	79.1 ± 24.4^{a} (9.8–285.0)
Potassium (mEq/L)	$58.4 \pm 11.3^{\dagger A}$ (13.9–99.9)	161.1 ± 15.8^{a} (93.6–235.5)	199.7 ± 14.9^{B} (96.0–349.3)	241.5 ± 28.2^{b} (35.6–371.0)	206.3 ± 20.0^{B} (11.7–382.0)	246.5 ± 29.2^{b} (88.3–374.8)
Chloride (mEq/L)	$75.0 \pm 11.8^{\dagger A,B}$ (20.0–109.0)	184.0 ± 19.8^{a} (79.0–281.0)	103.8 ± 7.9^{A} (36.0–142.0)	105.6 ± 18.2^{b} (15.0–265.0)	$69.3 \pm 6.1^{\dagger B}$ (29.0–118.0)	126.8 ± 30.8^{b} (10.0–276.0)
Magnesium (mg/dL)	$16.4 \pm 2.2^{\dagger A}$ (6.76–24.2)	36.0 ± 3.7^{a} (22.4–62.7)	64.3 ± 5.5^{B} (29.2–103.3)	63.6 ± 9.5^{b} (8.4–132.8)	49.4 ± 4.3^{C} (16.8–83.1)	37.3 ± 7.1^{a} (6.6–81.6)
Phosphorus (mg/dL)	14.7 ± 3.4^{A} (5.11–32.3)	27.4 ± 11.4 (0.2–118.0)	4.7 ± 1.84^{B} (0.1–24.2)	16.0 ± 9.2 (0.1–111.5)	6.6 ± 2.1^{B} (0.4–36.7)	20.3 ± 6.6 (0.4–50.6)
Calcium (mg/dL)	95.4 ± 16.8^{A} (26.3–154.5)	$156 \pm 33.4^{a,b}$ (18.1–293.9)	235.4 ± 22.9^{B} (98.6–436.3)	187.4 ± 39.8^{a} (12.4–461.7)	168.6 ± 18.6^{C} (58.6–332.1)	89.8 ± 30.9^{b} (21.9–350.2)
Urine volume (L/d)	17.60 ± 1.68^{A} (12.0–26.1)	NA	8.11 ± 0.91^{B} (2.0–14.87)	NA	10.21 ± 1.39^{B} (2.3–26.2)	NA

*Only 3 horses consumed the diet and were used for analysis. †Within a diet, values differ significantly ($P < 0.05$) for urine samples collected by use of the volumetric collection method or by use of a catheter. [a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$). [A,B,C]Within a row, values with differing superscript letters different significantly ($P < 0.05$). NA = Not applicable.

free of sediment when horses consumed the low DCAB diet, whereas urine appeared thick and cloudy when horses consumed the medium and high diets. Urine volume was significantly greater in horses when consuming the low diet, compared with the volume in horses when consuming the medium and high diets (Table 2).

Renal clearance and FE of electrolytes and minerals—For horses consuming all diets, data on renal clearance obtained from 24-hour volumetric urine collection (**Table 3**) revealed identical patterns of change to FE values (**Table 4**). Mean 24-hour clearance of creatinine calculated from three 24-hour periods of volumetric urine collection did not differ significantly among horses when consuming each of the 3 diets. Mean 24-hour clearance of Na^+ differed significantly among horses consuming each of the various diets, and it was lowest in horses when consuming the medium diet and highest in horses when consuming the high diet. Mean FE of Na^+ was also lowest for horses when consuming the medium diet, compared with values for horses when consuming the other 2 diets. Mean clearance and FE of K^+ increased with increasing DCAB in the diet. Mean clearance and FE of Cl^- and P were significantly greater when horses were consuming the low diet, compared with values when horses were consuming the other 2 diets. Mean clearance of Ca^{2+} was not different among horses when consuming each of the diets, whereas FE of Ca^{2+} was higher when horses consumed the medium diet, compared with values for horses when consuming the low and high diets. Mean clearance and FE of Mg^{2+} were significantly lower when horses consumed the low DCAB diet.

Daily variation in renal clearance and FE values—A significant degree of variation between consecutive 24-hour clearance and FE values was detected within each horse as well as among horses. Mean CV

Table 3—Least-square mean ± SEM (95% confidence interval) values for renal clearance of creatinine, electrolytes, and minerals in horses consuming 3 diets that varied in DCAB

Clearance (mL/min/kg)	**Low DCAB (3 horses)***	**Medium DCAB (6 horses)**	**High DCAB (6 horses)**
Creatinine	1.5935 ± 0.140^{a} (1.316, 1.871)	1.4021 ± 0.10^{a} (1.198, 1.606)	1.5043 ± 0.10^{a} (1.300, 1.709)
Sodium	0.0041 ± 0.001^{a} (0.002, 0.007)	0.0007 ± 0.0002^{b} (–0.001, 0.002)	0.0079 ± 0.001^{c} (0.006, 0.010)
Chloride	0.0143 ± 0.001^{a} (0.011, 0.017)	0.0095 ± 0.001^{b} (0.007, 0.012)	0.0078 ± 0.0010^{b} (0.006, 0.010)
Potassium	0.3229 ± 0.070^{a} (0.180, 0.466)	0.4453 ± 0.05^{a} (0.347, 0.544)	0.5879 ± 0.05^{b} (0.490, 0.686)
Calcium	0.1614 ± 0.04^{a} (0.089, 0.233)	0.1952 ± 0.03^{a} (0.143, 0.247)	0.1673 ± 0.03^{a} (0.115, 0.219)
Phosphorus	0.0821 ± 0.010^{a} (0.066, 0.098)	0.0110 ± 0.004^{b} (0.008, 0.030)	0.0170 ± 0.006^{b} (0.006, 0.028)
Magnesium	0.2101 ± 0.03^{a} (0.157, 0.263)	0.2980 ± 0.02^{b} (0.263, 0.333)	0.2866 ± 0.02^{b} (0.252, 0.322)

Values represent mean of data for samples collected for 3 consecutive days of volumetric urine collection from each horse on each diet.
*Only 3 horses consumed the diet and were used for analysis. [a,b,c]Within a row, values with differing superscript letters differ significantly ($P < 0.05$).

values for within- and between-horse variation in 24-hour clearance values were calculated (**Table 5**). Mean CVs for within-horse variation were greatest for the clearances of Na^+, P, and Ca^{2+}. The greatest degree of variation within specific horses between consecutive 24-hour clearance values occurred when horses were consuming the medium diet. Mean CVs for between-horse variation in clearance values within a 24-hour period were also greatest for Na^+, P, and Ca^{2+} when horses consumed the medium and high diets, and mean CVs were greatest for K^+, Na^+, and Ca^{2+} when horses consumed the low diet. Large CVs for the FE of electrolytes and minerals within horses between consecutive 24-hour periods were found for the single-sample and volumetric urine collection groups (**Table 6**). Large CVs for the FE of electrolytes and minerals between horses within 24-hour periods were also

Table 4—Least-square mean ± SEM (95% confidence interval) values for fractional excretion (FE) of electrolytes and minerals in urine samples obtained by use of a single-sample catheter collection or volumetric collection method during 3 consecutive days from horses consuming 3 diets that varied in DCAB

FE (%)	Low DCAB		Medium DCAB		High DCAB	
	Volumetric (3 horses)*	Catheter (5 horses)	Volumetric (6 horses)	Catheter (5 horses)	Volumetric (6 horses)	Catheter (5 horses)
Sodium	0.25[A]	0.33[a]	0.05[A]	0.05[b]	0.51[B]	0.39[a]
	(0.08, 0.43)	(0.19, 0.47)	(–0.07, 0.17)	(–0.06, 0.16)	(0.39, 0.63)	(0.25, 0.54)
Chloride	0.88[A]	1.48[a]	0.70[A]	0.88[b]	0.53[B]	0.70[b]
	(0.68, 1.08)	(1.08, 1.89)	(0.54, 0.86)	(0.52, 1.25)	(0.49, 0.56)	(0.29, 1.10)
Potassium	16.38[A]	32.55[a]	32.41[B]	37.58[a]	42.60[C]	46.85[a]
	(6.56, 26.19)	(20.83, 44.26)	(23.79, 41.03)	(26.67, 48.48)	(33.98, 51.21)	(34.56, 59.15)
Calcium	9.0[A]	10.74[a]	13.85[B]	9.82[a,b]	10.03[A]	4.94[b]
	(4.28, 13.71)	(6.09, 15.39)	(10.05, 17.65)	(5.39, 14.25)	(6.23, 13.83)	(0.11, 9.77)
Phosphorus	5.54[A]	6.98[a]	0.73[B]	2.50[a]	1.25[B]	2.51[a]
	(4.36, 6.73)	(2.47, 11.49)	(–0.10, 1.56)	(–1.79, 6.80)	(0.42, 2.08)	(–2.17, 7.19)
Magnesium	11.30[A]	15.91[a,b]	21.82[B]	17.85[a]	19.58†[B*]	11.80[b]
	(7.69, 14.92)	(12.18, 19.63)	(18.89, 24.76)	(14.45, 21.25)	(16.64, 22.51)	(7.88, 15.73)

See Table 2 for key.

Table 5—Within- and between-horse coefficient of variation (CV) for the clearance of creatinine, electrolytes, and minerals in urine samples obtained by use of a volumetric collection method during 3 consecutive days from horses consuming 3 diets that varied in DCAB

CV (%)	Low DCAB		Medium DCAB		High DCAB	
	Within-horse	Between-horses	Within-horse	Between-horses	Within-horse	Between-horses
Creatinine	17.31	16.04	32.84	30.33	26.05	27.56
Sodium	53.22	56.62	92.81	126.63	85.92	69.67
Chloride	29.01	50.06	35.74	30.69	34.72	30.93
Potassium	21.25	68.50	37.11	32.71	34.26	38.64
Calcium	40.17	52.48	49.11	49.37	37.52	51.25
Phosphorus	51.57	29.54	89.48	135.40	39.72	92.44
Magnesium	21.57	28.30	35.17	27.09	21.96	26.19

Values represent variation in renal clearance values between and within horses during 3 consecutive 24-hour periods of volumetric urine collection during consumption of each diet.

Table 6—Within-horse CV for FE of electrolytes and minerals in urine samples obtained by use of a single-sample catheter collection or volumetric collection method during 3 consecutive days from horses consuming 3 diets that varied in DCAB

CV (%)	Low DCAB		Medium DCAB		High DCAB	
	Volumetric	Catheter	Volumetric	Catheter	Volumetric	Catheter
Sodium	51.63	44.96	71.06	48.70	83.30	44.33
Chloride	22.90	17.60	24.83	72.30	24.82	97.93
Potassium	23.20	24.30	32.59	31.70	14.50	50.97
Calcium	38.50	52.97	24.00	73.50	36.02	62.68
Phosphorus	53.61	61.56	85.11	72.01	44.93	62.13
Magnesium	22.60	27.96	14.68	42.12	10.49	57.36

Values represent variation in FE values within specific horses between 3 consecutive 24-hour periods of volumetric urine collection or between 3 consecutive daily single urine samples obtained by use of a catheter during consumption of each diet.

observed in data from the catheter and volumetric urine collection groups (values not reported).

Comparison of results from volumetric and catheter urine collections—Samples obtained by use of a catheter or volumetric urine collection had similar alterations among horses consuming each of the 3 diets with regard to FE of Na^+, Cl^-, K^+, and P. We did not detect significant differences in FE of Na^+, Cl^-, K^+, Ca^{2+}, or P between samples obtained by each of the urine collection methods, which is in contrast to results in another report.[10] However, FE of Ca^{2+} and Mg^{2+} did not have similar patterns of change for horses consuming each of the diets for the 2 urine collection groups; for horses consuming the high diet, FE of Ca^{2+} and Mg^{2+} was much lower in single urine samples obtained by use of a catheter, compared with values for urine samples obtained by use of the volumetric collection method (Table 4).

Correlation of clearance and FE in volumetric collections—Simple linear regression identified strong associations between renal clearance and FE values for Na^+, Cl^-, K^+, Ca^{2+}, P, and Mg^{2+} when controlling for other factors, which included day and diet. The Pearson technique was used to further investigate correlations between clearance and FE values of each electrolyte and mineral in horses when consuming each of the diets. Strong positive correlations existed between clearance and FE of Na^+, Ca^{2+}, and P for all diets. Clearance of K^+ was significantly correlated to FE of K^+ when horses were consuming the low and medium diets, but not when horses were consuming the high DCAB diet. Significant correlations between clearance and FE of Cl^- were observed when horses consumed the low and high diets. Significant correlations between clearance and FE of Mg^{2+} were observed only when horses consumed the low DCAB diet.

Correlation among clearance of electrolytes and minerals in volumetric collections—In horses consuming the low DCAB diet, clearance of Cr was not significantly correlated with the clearance of any electrolyte or mineral, whereas clearance of Mg^{2+} was significantly correlated with the clearance of Cl, K^+, and Ca^{2+}, and the clearance of Cl^- was correlated with the clearance of K^+. When horses consumed the medium DCAB diet, clearance of Cr was correlated with clearance of K^+, Ca^{2+}, and Mg^{2+}, and the clearance of Mg^{2+} was correlated with the clearance of Cl^-, K^+, and Ca^{2+}. In addition, clearance of K^+ was correlated with clearance of Ca^{2+} and Cl^-. In horses consuming the high DCAB diet, clearance of Cr was correlated only with clearance of Mg^{2+}, clearance of Mg^{2+} was significantly correlated with clearance of Ca^{2+}, and clearance of Na^+ was significantly correlated with clearance of Cl^-.

Discussion

The study reported here was initially designed to compare electrolyte and mineral metabolism between clinically normal horses and horses with RER when consuming diets with a range of DCAB. However, significant differences were not found between RER and control horses in electrolyte and mineral balance.[10] The original design of the study did not permit comparison of urine collection methods within the same horse; however, it did provide a unique opportunity to compare clearance and FE of electrolytes and minerals for horses consuming a range of DCAB between volumetric and single-sample urine collections in horses kept in a standardized environment. The major finding of the study was that despite significant within- and between-horse variation in electrolyte and mineral excretion from day to day, 3 daily urine samples obtained by use of a catheter provided accurate information regarding urinary excretion of Na^+, Cl^-, K^+, and P for horses consuming a range of DCAB. Similar to results of another study,[24] changes in the FE of electrolytes and minerals for horses consuming a range of DCAB in our study accurately reflected renal clearance, except for renal clearance of Ca^{2+}. The advantage of the use of urine samples collected daily by use of a catheter when investigating the effects of various diets or diseases, including RER, nutritional hyperparathyroidism, and acute renal failure, on urinary excretion of electrolyte and minerals is the ease of collection.[2,11,25]

Urinary excretion of electrolytes and minerals varies widely within a 24-hour period as a result of diurnal variation, exercise, feed or water intake, feed or water deprivation, and other factors.[13,15,16,26] Thus, it has been suggested that total volumetric urine collection for 24 hours is the shortest period that will accurately reflect these homeostatic processes.[16,17]

Analysis of results of the study reported here suggests that variation in FE and clearance of electrolytes and minerals are not only diurnal but also evident between consecutive 24-hour periods within horses even when diet, time of urine collection relative to feeding, daily routines, and exercise are standardized. In particular, excretion of Na^+, P, and Ca^{2+} within specific horses had high CVs for urine samples obtained by catheter and volumetric collection methods. For example, FE of Na^+ varied 11-fold between consecutive 24-hour periods in 1 horse, and FE of P varied by 20-fold, whereas FE of K^+, Mg^{2+}, and Cl^- varied 2- to 3-fold within that same horse. Urinary excretion of Na^+, P, and Ca^{2+} would be expected to be the most prone to fluctuation, because minor perturbations in plasma concentrations of these substances result in altered renal excretion under the influence of aldosterone (Na^+) and PTH (Ca^{2+} and P).[4] In the study reported here, hormonal release could also have been influenced by daily variation in voluntary water consumption and rate of feed ingestion.[15,26] High between- and within-horse CVs for excretion of Na^+, P, and Ca^{2+} were similar among consecutive 24-hour periods, and FE of these minerals and electrolytes did not differ substantially between samples collected by use of a catheter or volumetric collection methods. Thus, it appears that the high CVs for FE of Na^+, Cl^-, K^+, and P are a reflection of physiologic variation rather than a result of the method of urine collection. However, it is important to mention that the concentration of Na^+ in equine urine is normally so minimal that a minor change in renal reabsorption may result in a several-fold alteration in FE.

Measurement of the excretion of Ca^{2+} and Mg^{2+} in urine was affected by the method of urine collection with greater CVs observed when urine was alkaline (medium and high diets) and collected by use of a catheter. Equine urine commonly contains a large amount of calcium carbonate crystals, calcium oxalate crystals, and, occasionally, struvite cystals.[18,27,28] Subjectively, crystalluria was greatest when urine pH was most alkaline and almost undetectable when urine pH was lowest and urine volume was greatest. A reason that excretion of Ca^{2+}, P, and Mg^{2+} was highly variable in urine samples obtained by use of a catheter may have been the inability to retrieve a representative amount of mineral-containing crystals from the bladder, especially when horses were consuming alkalinizing diets.[27,29] For example, FE values of Ca^{2+} and Mg^{2+} were lower and had the highest variation in samples obtained by use of a catheter from horses consuming the medium and high diets, compared with values for samples obtained by use of the volumetric collection system from horses consuming those same diets. In contrast, when horses consumed the low DCAB diet, FE values of Ca^{2+} and Mg^{2+} were similar in samples obtained by use of a catheter or the volumetric collection method. Therefore, accurate measurement of Ca^{2+} and Mg^{2+} may require analysis of urine obtained via volumetric collection or analysis of an aliquot obtained after thorough mixing of an entire voided sample, particularly when crystalluria is evident. Voided samples commonly contain few crystals at the start of urination and a large amount of sediment at the end of urination. Therefore, it is important to completely empty the bladder to obtain a representative sample for mineral analysis.

The greater urine volume that was observed when horses consumed the low DCAB diet is likely attributable to the higher CP content of this diet, compared with the CP content of the other 2 diets. This resulted in increased water intake and urinary excretion of urea, although this was not confirmed by measurement of water consumption.[30] The low urine volume (< 3 L in 24 hours) that was observed 1 time in a mare while consuming the medium DCAB diet and 1 time in another mare while consuming the high DCAB diet possibly could have reflected urine spillage, although this was not documented during observation. Effects of the low DCAB diet on urine pH and results from nutritional metabolic acidosis evoked by consumption of an anionic diet have been reported elsewhere.[10]

Accurate determination of urinary concentrations of Ca^{2+}, P, and Mg^{2+} also requires appropriate processing of the urine sample prior to analysis. To prevent loss of crystals in the study reported here urine samples were not centrifuged prior to analysis, and urine was acidified with concentrated nitric acid to dissolve crystals. Most other studies[17,18,27] of urinary excretion of Ca^{2+} and P in horses on a basal DCAB diet (medium diet) used centrifuged, nonacidified urine for analysis, potentially resulting in the loss of substantial amounts of Ca^{2+}, P, and Mg^{2+} prior to analysis. Mean clearance and FE values of Na^+, K^+, and Cl^- from other studies[2,17,18,27] are similar to those reported here for horses consuming the basal DCAB diet; however, the ranges for FE of P and Ca^{2+} in those other studies are much lower than in the study reported here, in which we used acidified urine. For example, 95% confidence intervals for FE of P and Ca^{2+} in our study ranged from 0 to 6.8% and from 5.4 to 17.6%, respectively, when horses were consuming the medium diet, compared with values of 0 to 0.5% for FE of P and –0.158 to 6.723% for FE of Ca^{2+} in urine samples collected by use of a catheter and volumetric collection methods in other studies.[2,17,27,31] Dietary variation and differences in number of animals between studies cannot be ruled out as an additional contributing cause of these differences. However, acidification of urine samples prior to analysis, 24-hour volumetric collection, and avoidance of centrifugation of samples seem most appropriate when analyzing content of Ca^{2+}, P, and Mg^{2+} in alkaline horse urine. The FE values for Ca^{2+} and Mg^{2+} are likely to be at the lower end of these ranges in single urine samples obtained by use of a catheter and at the higher end in samples obtained during natural voiding or by use of volumetric collection methods.

A strong correlation between clearance and FE was found for most electrolytes and minerals; however, a weak or nonexistent relationship was observed between clearance and FE of K^+ when horses consumed the high DCAB diet, clearance and FE of Cl^- when horses consumed the medium diet, and clearance and FE of Mg^{2+} when horses consumed the medium and high diets. In a study[32] of cattle, there was poor correlation between clearance and FE of K^+ and Cl^-, which was attributed to dietary fluctuations. However, because diet was standardized in our study, it appears that other unidentified factors play a role in influencing the correlation between these variables. Correlation of clearance of Cr to clearance of K^+, as well as to clearance of Cl^-, has been attributed to a close link of these electrolytes with glomerular filtration and less intense hormonal regulation, compared with that for Ca^{2+} and Na^+.[17] Correlation between clearance of Na^+ and clearance of Cl^- could be a result of a response of Cl^- excretion to changes in Na^+ excretion.[17,27,33] In the study reported here, correlation between clearance of Na^+ and clearance of Cl^- was only detected when horses were consuming a high Na^+ diet (ie, high DCAB diet). Although high Na^+ and K^+ excretion has been linked with high Cl^- excretion,[18,27] in our study of horses, the high DCAB diet resulted in low FE of Cl^-, compared with values for the diets that contained lower amounts of Na^+. Thus, in the studyreported here, Cl^- excretion appeared to be affected by dietary Cl^- intake and changes in acid-base homeostasis rather than simply a response to altered Na^+ excretion.[10] Clearance of Ca^{2+} and Mg^{2+} had the most consistent correlation with each other for horses consuming all of the various diets. Changes in Mg^{2+} excretion in horses reportedly reflect those of Ca^{2+} excretion as a result of PTH, which stimulates resorption of Ca^{2+} and Mg^{2+}.[8,34,35] The low DCAB diet, an anionic diet, should induce increased tissue sensitivity to PTH.[36] In our study, FE and clearance of Mg^{2+} were significantly lower in urine samples obtained by use of volumetric collection methods in horses consuming this diet.

Considerable physiologic variation in urinary electrolyte and mineral excretion between consecutive 24-hour periods may be evident even when the major factors influencing urinary electrolyte and mineral excretion, including diet and exertion, are held constant. Repeated collection of urine samples obtained by use of a catheter on consecutive days at the same time of day and the same stage of the daily routine appears to offer information comparable to that obtained from volumetric urine collection with regard to excretion of electrolytes and minerals, except for Ca^{2+} and Mg^{2+}, when comparing samples obtained from horses consuming diets with a wide range of DCAB.

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[a]DCAB pellets, Hubbard Feeds, Mankato, Minn.
[b]Soychlor, West Central Cooperative, Ralston, Iowa.
[c]Basic Plus minerals feed analysis, Dairy One, Ithaca, NY.
[d]Beckman CX4 analyzer, Beckman ICS, Brea, Calif.
[e]Self-retaining urine collection harnesses, Natural Biologics, Albert Lea, Minn.
[f]ColorpHast indicator strips, EM Science, Gibbstown, NJ.

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