Evaluation of creatinine as a urine marker and factors affecting urinary excretion of magnesium by dairy cows

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ABSTRACT

Nutrient balance studies require measuring urine volume, and urinary excretion can be used to assess Mg bioavailability. A less laborious method than total collection of urine could make balance studies more feasible and expand the utility of using urinary Mg as an index of bioavailability, but the method needs to be accurate and sensitive. Sampling interval can affect accuracy because excretion must be at steady state. Two experiments were conducted to (1) determine whether urinary creatinine could be used to accurately estimate urinary output of nutrients markedly excreted via urine (N, K, Na, S, and Mg; experiment 1) and (2) determine the appropriate sampling schedule to evaluate Mg excretion after abrupt diet changes (experiment 2). Experiment 1 was originally designed to evaluate the interaction of monensin [0 vs. 14 mg of monensin/kg of dry matter (DM)] and Mg source (MgO vs. MgSO₄; total diet Mg: 0.36% of DM) under antagonism from increased dietary K (2.11% of DM) on urinary Mg excretion. Experiment 2 evaluated the interaction of Mg concentration (basal vs. supplemental MgO; total diet Mg: 0.20 vs. 0.42% of DM) and K (basal vs. supplemental K₂CO₃; total diet K: 1.60 vs. 2.57% of DM) on urinary Mg excretion over time. Using 4-d composite samples from total collection of urine (n = 34 cow-periods), the average daily excretion of creatinine was similar to previous estimates (29.0 ± 1.16 mg of creatinine/kg of body weight) but was variable among cows (root mean squared error = 2,980 mg/d; 14% of mean). Treatment-average estimated excretion of urine and urinary N, K, Na, S, and Mg were similar to actual values; however, differences between actual and estimated values could be substantial for individual cows. Using the mean creatinine excretion per kilogram of body weight for all cows to estimate urine eliminates the lack of fit variance resulting in artificially low within-treatment variation for estimated urine

volume. The standard error of the mean for estimated urine volume was 23% less (1.93 vs. 2.51) than that for actual urine production. This inflated the type I error rate, and, consequently, statistical inferences on N and K excretion differed when urine output was estimated rather than measured. The standard error of the mean for excretion of Mg calculated with actual or estimated urine production were almost identical (0.92 vs. 0.97); however, similar standard error of the mean was likely caused by differences in the covariance of urinary Mg concentration with estimated or actual urine output. Based on spot sampling (experiment 2), urinary Mg reached steady state by 2 d following an increase in dietary K regardless of Mg level, whereas excretion of urinary Mg following an increase in dietary Mg continued to increase through 7 d. Estimating nutrient excretion with urinary creatinine and body weight on average is accurate, but variance is likely underestimated. Knowing the time course of urinary Mg excretion will improve the value of using urinary Mg concentration to assess diet adequacy or Mg bioavailability.

Key words: magnesium, urinary marker, creatinine, nutrient excretion

INTRODUCTION

Assessing mineral utilization (Faulkner and Weiss, 2017), environmental impact (de Boer et al., 2002), or dietary requirements (NRC, 2001) often requires measuring nutrient balance in vivo. This involves quantifying urine volume; however, measuring urine volume via total collection is expensive and not feasible under many conditions. Urinary creatinine has been used previously to estimate urine volume and excretion of purine derivatives (Valadares et al., 1999; Chizzotti et al., 2008), but method validity has not been evaluated for elements markedly excreted via urine. For example, 20 to 90% of the intakes of N, Na, K, Mg, and S can be excreted in the urine (Tebbe et al., 2018).

Urinary excretion measurements also can be used to estimate relative bioavailability of Mg (Jesse et al., 1981; Van Ravenswaay et al., 1989, 1992). Under many practical conditions, diets may not provide adequate

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Mg, and deficiencies can develop quickly because lactating dairy cows have only a small reservoir of labile Mg (Blaxter and McGill, 1956). Several dietary factors alter Mg absorption, including chemical and physical form of supplemental Mg (Jesse et al., 1981; Van Ravenswaay et al., 1989, 1992), differences within a Mg source (i.e., MgO; Jesse et al., 1981), inclusion of monensin (Greene et al., 1986; Tebbe et al., 2018), and dietary K (Weiss, 2004; Schonewille et al., 2008). A method for quickly evaluating Mg bioavailability in cows would be useful to ensure a sufficient absorbable supply and quantify factors affecting absorption.

Urinary excretion of Mg fluctuates over the course of several days after abrupt diet changes (Schonewille et al., 2000), but urinary Mg concentrations must be at steady state to make accurate comparisons of bioavailability (Van Ravenswaay et al., 1992). Therefore, the main objectives of this study were to (1) evaluate using creatinine for estimating urine output and excretion of major nutrients (N, K, Na, S, and Mg) in lactating dairy cattle and (2) investigate the timeline of urinary Mg excretion following abrupt dietary changes.

MATERIALS AND METHODS

Cows and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee. Experiment 1 evaluated the use of creatinine as a marker to estimate urine output and urinary excretion of nutrients. The experiment was conducted ancillary to a larger balance study (Tebbe et al., 2018). Briefly, 18 multiparous Holstein cows (139 ± 35 DIM) were placed into 3 groups of 6 cows. Within each group, 3 cows were randomly assigned to receive no monensin and 3 were fed monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN) at 14 mg/kg of DM. Cows remained on their monensin treatment for the duration of the experiment (i.e., whole-plot treatment). Within each whole-plot treatment (i.e., split-plot treatment), cows were assigned to 1 of 2 Mg source sequences: (1) MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD) and then MgSO₄ (Magriculture; Giles Chemical, Waynesville, NC) or (2) MgSO₄ and then MgO. Periods within a sequence were 21 d, with total collection of feces and urine on d 16 to 20 (Weiss et al., 2009). Based on the time course of Mg excretion in dry cows (Schonewille et al., 2000), a 16-d diet adaptation period should have been adequate to eliminate carryover effects. Diets were formulated to contain about 40% Mg from supplemental Mg sources and have similar concentrations of total dietary Mg (target: 0.35% of

DM; Table 1). The diets were balanced to have similar concentrations of major nutrients and to have elevated K concentrations (basal diet was 1.3% K plus 0.8% K from K₂CO₃) to create antagonism on Mg absorption.

To evaluate the timeline of urinary Mg excretion following abrupt diet changes, experiment 2 used 32 multiparous Holstein cows (215 ± 32 DIM) that were placed into 8 blocks of 4 cows based on milk yield (range in average block yields: 20.8–41.8 kg/d). The experiment was conducted in 2 groups of 4 blocks each. Before the experimental period, cows were moved to tiestalls and fed a common diet with typical dietary concentrations of Mg and K (0.24 and 1.6% of DM, respectively). The diet was the same ration the cows had been consuming for several weeks prior. After 7 d in tiestalls, cows were abruptly changed to 1 of 4 diets (Table 2) for 7 d. Diets were (1) basal (no supplemental Mg and K; total diet Mg and K: 0.2 and 1.6%, respectively); (2) basal Mg, high K [**HiK**; total diet K: 2.6% of DM, 40% from K₂CO₃ (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ)]; (3) basal K, high Mg [**HiMg**; total diet Mg: 0.4% of DM, 50% as MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD)]; and (4) high K, high Mg (**HiK+Mg**; total dietary K and Mg: 2.6 and 0.4% of DM, respectively; 40% from K₂CO₃ and 50% from MgO).

In both experiments, rations were fed once daily with a target refusal rate of 5%. Individual feed delivery and refusal amounts were weighed and recorded daily. Cows were milked twice daily at approximately 0200 and 1400 h, and weights were measured electronically (Afimilk; Kibbutz Afikim, Israel). Body weights were measured on 2 consecutive days at the beginning and end of each experimental period. All 4 BW were then averaged for all calculations.

Samples and Analyses

Silages and concentrates were sampled once at the end of each experimental period for each group and assayed for DM (100°C for 48 h). For other analyses, silages were dried (lyophilized in experiment 1 or 55°C for 48 h in experiment 2) and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Ground silages and concentrate samples were analyzed for DM (100°C for 24 h), NDF (Ankom Fiber Analyzer, Ankom Technology, Fairport, NY) with sodium sulfite and amylase, CP (Kjeldahl N $\times$ 6.25; AOAC International, 2000, method 984.13.4.09), and minerals (OARDC Star Laboratory, Wooster, OH) by inductively coupled plasma emission spectroscopy (**ICP**; Isaac and Johnson, 1985) after microwave digestion in nitric acid (Jones et al., 1991). The analyzed nutrient composition of silages and concentrates was

used to calculate TMR composition. One refusal sample per cow was collected during each experimental period, analyzed for DM (100°C for 48 h), and used to calculate daily DMI.

In experiment 1, a 4-d composite sample from total collection of urine was used for analysis. To collect urine, external urine cups were attached to the cow's vulva (Weiss et al., 2009). After the urine left the cow, flow was split into 2 streams. One went into a container without acid and was sampled for minerals and creatinine, and the other went into a container with H₂SO₄ (pH of urine <5) and was sampled for N analysis. For experiment 2, urine was collected from cows via vulva stimulation on the day before treatment diets were fed (d 0) and on d 2, 4, and 7 approximately 5 h (at 0700 h) after feeding. All total collection samples and urine spot samples were assayed for creatinine (creatinine urinary assay no. 500701; Cayman Chemical Co., Ann Arbor, MI), N by the Kjeldahl method (AOAC International, 2000, method 984.13.4.09), and minerals (OARDC

Star Laboratory) by ICP (Isaac and Johnson, 1985) after microwave digestion in nitric acid (Jones et al., 1991). The creatinine intra- and interassay coefficients of variation were 2.4 and 11.1%, respectively. All urine samples from experiments 1 and 2 were also assayed for Mg using a colorimetric assay (magnesium liquicolor no. 0130; Stanbio Laboratory, Boerne, TX). The colorimetric assay was evaluated because of the lower cost and greater practicality compared with ICP. The Mg colorimetric assay intra- and interassay coefficients of variation were 7.8 and 8.8%, respectively.

During experiment 2, blood was collected from the tail vein into sodium heparin BD Vacutainers (Franklin Lakes, NJ) on d 0, 3, and 7 of treatment feeding. Packed cell volume (**PCV**) was assayed using capillary tubes that were centrifuged (C-MH30; Unico, Dayton, NJ) at room temperature and read with a microcapillary reader (cat. no. 2201; Damon/IEC Division, Needham Heights, MA). Plasma was separated by centrifugation (2,500 × *g* at 10°C), frozen, and later analyzed for Mg

Table 1. Ingredient and nutrient composition of diets¹ fed in experiment 1 (% of DM)

Item	Control		Monensin	
	MgO	MgSO ₄	MgO	MgSO ₄
Ingredient				
Corn silage	34.2	34.2	34.2	34.2
Alfalfa silage	22.0	22.0	22.0	22.0
Corn grain, ground	18.4	17.5	18.4	17.5
Soybean meal, 48% CP	12.9	13.0	12.9	13.0
Soy hulls	8.67	7.96	8.67	7.96
Animal-vegetable fat	0.49	0.48	0.49	0.48
Potassium carbonate ²	1.51	1.49	1.51	1.49
Dicalcium phosphate	0.35	0.35	0.35	0.35
Magnesium oxide ³	0.19	—	0.19	—
Magnesium sulfate ⁴	—	1.65	—	1.65
Trace-mineralized salt ⁵	0.70	0.70	0.70	0.70
Mineral-vitamin premix ⁶	0.62	0.62	0.62	0.62
Monensin ⁷	—	—	0.007	0.007
Nutrient composition				
DM, %	59.0	58.6	59.2	58.6
CP	17.0	16.7	16.6	16.8
NDF	31.6	30.8	31.8	30.9
Mg	0.33	0.38	0.34	0.37
K	2.12	2.09	2.12	2.11
Na	0.26	0.29	0.24	0.27
S	0.21	0.43	0.21	0.42

¹Control = 0 mg/kg of monensin and Mg from MgO or MgSO₄; monensin = 14 mg/kg of monensin and Mg from MgO or MgSO₄.

²Contained 56% K (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ).

³Contained 58% Mg (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD).

⁴Contained 9.8% Mg (Magriculture; Giles Chemical, Waynesville, NC).

⁵Contained 95% NaCl, 3,500 mg/kg of Zn, 2,800 mg/kg of Mn, 1,750 mg/kg of Fe, 400 mg/kg of Cu, 70 mg/kg of I, 70 mg/kg of Co (Morton Salt Inc., Chicago, IL).

⁶Contained 60 mg/kg of Se (sodium selenate), 111 mg/kg of biotin, 3.8 g/kg of Zn (Zinpro 120; Zinpro Corp., Eden Prairie, MN), 0.8 g/kg of Cu (copper sulfate), 500 kIU/kg of vitamin A, 200 kIU/kg of vitamin D, and 3,600 IU/kg of vitamin E.

⁷Contained 198 g/kg of monensin (Rumensin 90; Elanco Animal Health, Greenfield, IN).

(magnesium liquicolor no. 0130). Blood Mg from experiment 1 is reported in Tebbe et al. (2018) and was not affected by treatments.

Calculations and Statistical Analysis

Two cows during experiment 1 in the MgO with monensin treatment unknowingly received diets devoid of trace-mineralized salt (apparent by urine Na excretion <1 g/d and total dietary level <0.02% of DM) and subsequently had decreases in DMI and milk yield; all data from these 2 cow-periods were removed.

Daily urinary excretion of creatinine was estimated from total collection samples of experiment 1 ($n = 34$ cow-periods) and used to calculate creatinine (mg/d) excreted per kilogram of BW. Urinary creatinine excretion was regressed on average period BW using PROC MIXED (SAS 9.4; SAS Institute, 2015) and adjusted for the random effects of group, cow within a group $\times$ monensin, and period within a group. The fixed effects of treatments (monensin and Mg source) and their interactions with the independent variable (BW)

were tested but were not significant ($P < 0.96$). The intercept was not different from zero ($b = 5,741$ mg/d; $SE = 6,660$; $P < 0.40$) and was dropped from the model because it lacks biological significance. The slope of the final model (29.0 ± 1.16 mg of creatinine/kg of BW; $P < 0.01$; Figure 1) was the same as a previous estimate (29 mg of creatinine/kg of BW; Valadares et al., 1999) and was used to estimate urine volume and nutrient excretion from all urine spot samples in experiment 2.

Total collection urinary output and composite samples of experiment 1 were used to evaluate (1) accuracy of estimating urine output using creatinine and BW, (2) analytical method (ICP or colorimetric assay) for measuring urinary Mg concentration, (3) interaction between analytical method and method of obtaining urinary output on urinary Mg excretion, and (4) the accuracy of using estimated urine output to estimate excretion of nutrients (N, K, Na, S, Mg). The mixed model included the fixed effects of monensin (1 df), Mg source (1df), method (1 df for volume, Mg concentration, and nutrient excretion models; 3 df for measuring urinary Mg output with the 4 methods model), and

Table 2. Ingredient and nutrient composition of diets¹ fed in experiment 2 (% of DM)

Item	Basal	HiK	HiMg	HiK+Mg
Ingredient				
Corn silage	40.0	40.0	40.0	40.0
Alfalfa silage	16.0	16.0	16.0	16.0
Ground corn	16.2	15.8	16.2	15.9
Soybean meal, 48% CP	11.5	12.2	11.4	12.2
Corn gluten feed, dried	4.54	3.77	4.45	3.65
Soybean hulls	4.50	3.70	4.45	3.65
Wheat middlings	4.54	3.77	4.41	3.56
Limestone	1.00	1.00	1.00	1.00
Animal-vegetable fat	0.39	0.39	0.39	0.39
Potassium carbonate ²	—	2.00	—	2.00
Magnesium oxide ³	—	—	0.36	0.36
Trace-mineralized salt ⁴	0.70	0.70	0.70	0.70
Mineral-vitamin premix ⁵	0.62	0.62	0.62	0.62
Nutrient composition				
DM, %	57.4	57.1	57.5	56.8
CP	14.9	15.3	15.0	15.2
NDF	35.6	34.5	35.4	34.3
Mg	0.20	0.18	0.42	0.43
K	1.60	2.51	1.56	2.62
Na	0.32	0.36	0.37	0.30
S	0.20	0.19	0.19	0.20

¹Basal = no supplemental Mg and K (total diet Mg and K: 0.2 and 1.6%, respectively); HiK = supplemental K_2CO_3 [total diet K: 2.6% of DM, 40% from K_2CO_3 (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ)]; HiMg = supplemental MgO [total diet Mg: 0.4% of DM, 50% as MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD)], HiK+Mg = supplemental K_2CO_3 and MgO (total dietary K and Mg: 2.6 and 0.4% of DM, respectively; 40% from K_2CO_3 and 50% from MgO).

²Contained 56% K (DCAD Plus; Church & Dwight Co. Inc.).

³Contained 58% Mg (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC).

⁴Contained 95% NaCl, 3,500 mg/kg of Zn, 2,800 mg/kg of Mn, 1,750 mg/kg of Fe, 400 mg/kg of Cu, 70 mg/kg of I, and 70 mg/kg of Co (Morton Salt Inc., Chicago, IL).

⁵Contained 54 mg/kg of Se (sodium selenate), 116 mg/kg of biotin, 4.0 g/kg of Zn (Zinpro 120; Zinpro Corp., Eden Prairie, MN), 1.0 g/kg of Cu (copper sulfate), 590 kIU/kg of vitamin A, 200 kIU/kg of vitamin D, and 3,500 IU/kg of vitamin E.

Figure 1. Relationship between BW and creatinine excretion (experiment 1). The Y-intercept was not different than zero ($P > 0.40$) and was removed from the model: $Y = 29.0 (\pm 1.16) X$; root mean squared error = 2,980. Each symbol represents a cow-period ($n = 34$ cow-periods) from 1 of the 4 dietary treatments and is adjusted for group, cow within a group $\times$ monensin, and period within a group. MgO = (gray) squares; MgSO₄ = (red) triangles; no monensin = open shape; monensin = solid shape. Treatment did not affect ($P < 0.96$) the slope. Color version available online.

all 2- and 3-way interactions among fixed effects and the random effects of group, cow within a group $\times$ monensin, period within a group, and cow $\times$ group $\times$ monensin $\times$ period. The different methods of calculating urinary Mg excretion were compared with output calculated with actual urine production and ICP (i.e., the standard method) using Dunnett's test. Several evaluations were conducted using regression (PROC REG). The root mean squared error (RMSE), which is the square root of the average of squared residuals, was used to evaluate the accuracy of the regressions.

Average milk yield, DMI, and BW of each cow during experimental periods of experiment 2 were analyzed using a mixed model that included the fixed effects of Mg (1 df), K (1 df), and the Mg $\times$ K interaction (1 df) and the random effect of block (7 df) and residual error. Blood and urine measures were analyzed with identical models but included the fixed effect of day (repeated) and all 2- and 3-way interactions among other fixed effects. The d 0 plasma Mg and PCV concentrations differed among treatments and were used as a covariate in their perspective models. The heterogeneous autoregressive covariance structure was used for repeated measures based on the lowest Bayesian information criterion. All degrees of freedom were calculated using the Kenward-Roger method. When time interactions were significant ($P < 0.10$), the SLICE option was used to determine ($P < 0.10$) which days treatment means

would be separated using a Fisher least significant difference test.

RESULTS

The colorimetric method yielded significantly lower urinary Mg concentrations than did ICP, and the regression had both a mean and slope bias (Figure 2). Perhaps more important, the underestimation caused the statistical interpretation of treatments on urinary Mg excretion to differ between ICP and colorimetric methods. For example, using ICP data, monensin did not affect urinary Mg concentration ($P < 0.14$), but with the colorimetric assay, the effect was significant ($P < 0.03$; data not shown). Because the regression departed from unity and statistical interpretation of treatment effects differed by analytical method, the succeeding mineral excretion data were from ICP-assayed values only.

Experiment 1

Complete production, digestibility and balance results of experiment 1 were presented previously (Tebbe et al., 2018). Briefly, cows fed MgSO₄ consumed about 1.1 kg/d less DM ($P < 0.01$), weighed less (711 vs. 722 kg; $P < 0.03$), and had a tendency for lower ($P < 0.09$) milk yields compared with cows fed MgO.

Feeding MgSO_4 with monensin decreased apparent absorption of Mg compared with diets without monensin (15.6 vs. 19.2%), but feeding MgO with monensin had greater apparent absorption of Mg (23.0%) than other treatments. This monensin $\times$ Mg interaction paralleled treatment effects on urinary excretion of Mg.

Urine production and nutrient excretion during total collection varied among cow-periods (Table 3). On average, measured urine volume was similar (method: $P < 0.96$) to estimated urine volume (Table 4); however, estimating urine reduced the standard error of the mean about 25%. When individual cow-period estimates of urine output were compared against actual urine production (Figure 3), the regression had an intercept and slope similar to 0 ($P < 0.15$) and to 1 ($P < 0.15$), respectively. The RMSE of the regression was high at 14% of the mean (RMSE = 4.8), which may have been a reason why no statistical effects on slope and the intercept were observed. Although treatment averages were not statistically different between actual and estimated volume of urine, some individual cow observations differed markedly (Figure 4).

Likewise, treatment-average excretion of N and minerals (K, Na, S, Mg) did not differ ($P \geq 0.66$) between urine methods, and no method $\times$ treatment interactions were significant (Table 4). However, statistical

Table 3. Descriptive statistics for measures taken during total collection of urine and feces of experiment 1 ($n = 34$ cow-period observations from 4 treatments¹)

Item	Mean	SD	10th–90th percentile
BW, kg	713	58.0	644–797
Milk, kg/d	40.1	6.77	32.2–47.4
Intake			
DM, kg/d	25.2	1.64	23.3–27.3
Free water, kg/d	111	10.7	102–126
Total water, kg/d	140	11.5	127–157
Urine excretion ²			
Volume, kg/d	33.3	6.39	25.7–44.8
Creatinine, g/d	20.8	3.24	16.7–25.2
N, g/d	215	30.7	173–253
K, g/d	340	50.4	267–407
Na, g/d	39.1	10.4	25.4–52.6
S, g/d	32.0	17.7	12.1–52.1
Mg, g/d	7.9	2.38	4.7–11.1
Urinary concentration ²			
Creatinine, mg/L	634	103	504–782
N, g/L	6.54	0.74	5.60–7.40
K, g/L	10.3	1.01	8.46–11.5
Na, g/L	1.18	0.27	0.87–1.59
S, g/L	1.01	0.61	0.34–1.73
Mg, mg/L	240	76.1	137–328

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO_4 .

²All values were measured using creatinine colorimetric assay, N by the Kjeldahl method, minerals with inductively coupled plasma emission spectroscopy, and actual volume.

Figure 2. The relationship between Mg concentration measured using inductively coupled plasma (ICP) emission spectroscopy versus using colorimetric assay: $Y = 1.12 (\pm 0.04) X + 43.4 (\pm 9.02)$; root mean squared error = 49.8; $P < 0.01$. The Y-intercept and slope were different from 0 ($P < 0.01$) and 1 ($P < 0.01$), respectively. The dashed line is the linear regression, and the solid line is $Y = X$. Each symbol represents a cow-period ($n = 34$ cow-periods) from experiment 1 [MgO = (gray) squares; MgSO_4 = (red) triangles; no monensin = open (gray) shape; monensin = solid (red) shape] and a cow-day ($n = 128$) experiment 2 [basal = solid (blue) diamonds; HiK = solid (orange) circles; HiMg = open (blue) diamond; HiK+Mg = open (orange) circle]. Basal = no supplemental Mg and K (total diet Mg and K: 0.2 and 1.6%, respectively); HiK = supplemental K_2CO_3 [total diet K: 2.6% of DM, 40% from K_2CO_3 (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ)]; HiMg = supplemental MgO [total diet Mg: 0.4% of DM, 50% as MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD)]; HiK+Mg = supplemental K_2CO_3 and MgO (total dietary K and Mg: 2.6 and 0.4% of DM, respectively; 40% from K_2CO_3 and 50% from MgO). Color version available online.

Table 4. Effect of dietary treatment and urine method on nutrient excretion in experiment 1¹

Item	Treatment ²				SEM ³	<i>P</i> -value		
	Control		Monensin			Monensin	Mg	Monensin × Mg
	MgO	MgSO ₄	MgO	MgSO ₄				
Cows, no.	9	9	7	9				
Volume, L/d	M: <i>P</i> < 0.96		M × Mon: <i>P</i> < 0.99				M × Mg: <i>P</i> < 0.98	
Actual	35.8	30.2	36.1	31.8	2.51	0.69	0.01	0.68
Estimated	35.9	30.2	35.7	31.9	1.93	0.70	0.01	0.52
N, g/d	M: <i>P</i> < 0.92		M × Mon: <i>P</i> < 0.93				M × Mg: <i>P</i> < 0.83	
Actual	231	205	214	210	12.1	0.63	0.06	0.18
Estimated	232	204	214	209	10.5	0.50	0.01	0.08
K, g/d	M: <i>P</i> < 0.91		M × Mon: <i>P</i> < 0.98				M × Mg: <i>P</i> < 0.86	
Actual	354	317	350	338	20.5	0.67	0.08	0.40
Estimated	355	318	352	336	17.6	0.64	0.01	0.30
Na, g/d	M: <i>P</i> < 0.88		M × Mon: <i>P</i> < 0.79				M × Mg: <i>P</i> < 0.71	
Actual	41	38	40	37	4.4	0.76	0.42	0.98
Estimated	42	39	38	38	4.7	0.50	0.62	0.61
S, g/d	M: <i>P</i> < 0.84		M × Mon: <i>P</i> < 0.67				M × Mg: <i>P</i> < 0.99	
Actual	15	41	13	49	1.5	0.86	0.01	0.17
Estimated	15	48	13	49	2.3	0.62	0.01	0.34
Mg, g/d	M: <i>P</i> < 0.66		M × Mon: <i>P</i> < 0.87				M × Mg: <i>P</i> < 0.99	
Actual	8.0	8.8	8.1	6.7	0.92	0.33	0.52	0.04
Estimated	8.1	8.9	8.0	6.8	0.97	0.28	0.58	0.04

¹Urine production was either measured from total collection of urine (actual) or estimated from urinary creatinine concentration in a 4-d composite sample (estimated) and BW [volume = 29 × BW (kg)/creatinine (mg/L)]. Excretion of nutrients (g/d) was then calculated using either actual or estimated urine volume. Urinary N was measured using the Kjeldahl method, and minerals were measured using inductively coupled plasma emission spectroscopy. M = method; Mon = monensin.

²Control = 0 mg/kg of monensin and Mg from MgO or MgSO₄; monensin = 14 mg/kg of monensin and Mg from MgO or MgSO₄.

³Highest SEM because of unbalanced data.

inferences of treatments changed for N and K excretion, likely because the standard error of the mean decreased when estimated urine output was used. The regressions of actual versus estimated excretion of Mg (Figure 5), N, K, and Na departed from unity (Table 5). The regression for S excretion (Figure 6) was the only element that did not differ from unity, but the regression also had the greatest RMSE as a percentage of the mean.

Experiment 2

Adding supplemental K or Mg did not affect DMI, BW, or yield of milk during the 7-d experiment (Table 6), but because of the short duration of the experiment, production data may not accurately reflect treatments. Plasma Mg was greater (Mg; *P* < 0.04) for cows receiving supplemental Mg compared with unsupplemented cows (1.03 vs. 0.99 mM). Supplementing K increased (*P* < 0.01) PCV and decreased (*P* < 0.01) urinary creatinine concentrations compared with basal K diets, but no interactions were observed (*P* > 0.17). Esti-

mated urine output was about 9 kg/d greater for the supplemental K diets (*P* < 0.01).

Concentration and estimated urinary excretion of Mg was not different between treatments on d 0 (*P* > 0.68) and averaged 194 mg/L and 5.7 g/d, respectively. Urinary Mg concentration had K × day (*P* < 0.03) and Mg × day (*P* < 0.01) interactions, but no 3-way interaction was observed (*P* > 0.63; Figure 7a). When supplemental Mg was fed concentrations increased over time, whereas concentrations did not change greatly over time for cows fed no supplemental Mg. No discernable pattern is evident describing the K × day interaction. Urinary Mg output (Figure 7b) had a K × Mg interaction (*P* < 0.10). When diets contained supplemental Mg, the addition of K did not affect urinary Mg output, but when diets contained no supplemental Mg, the addition of K decreased urine Mg output. Supplementing Mg also interacted with time (Mg × day; *P* < 0.01); urinary Mg output increased over the 7-d period when cows were fed supplemental Mg but was essentially unchanged or decreased for cows not fed supplemental Mg.

Figure 3. Relationship between actual and estimated urine output using creatinine as a marker (experiment 1): $Y = 0.77 (\pm 0.15) X + 7.59 (\pm 5.13)$; root mean squared error = 4.8; $P < 0.01$. The Y-intercept and slope were not different from 0 ($P > 0.15$) and 1 ($P > 0.15$), respectively. The dashed line is the linear regression, and the solid line is $Y = X$. Each symbol represents a cow-period ($n = 34$ cow-periods). MgO = (gray) squares; MgSO_4 = (red) triangles; no monensin = open shape; monensin = solid shape. Color version available online.

Figure 4. Histogram of deviations between actual and estimated urine output for individual observations: $(\text{actual urine volume} - \text{estimated urine volume}) / \text{actual urine volume} \times 100$ ($n = 34$ cow-periods). Each bin contains the number of cow-periods within ± 2.5 percentage units of the value shown on the axis. The percentage of total observations is above each bar.

Table 5. Actual versus estimated regressions for urine volume and the urinary excretion of nutrients in experiment 1¹

Dependent variable (actual)	Intercept	SE	<i>P</i> -value ²	Slope (estimated)	SE	<i>P</i> -value ³	RMSE ⁴	
							Estimate	% of mean
Urine, kg/d	7.6	5.13	0.15	0.77	0.15	0.14	4.83	14.4
N, g/d	121	43.5	0.01	0.44	0.20	0.01	29.1	13.5
K, g/d	181	65.5	0.01	0.47	0.19	0.01	47.0	13.8
Na, g/d	7.3	3.85	0.07	0.81	0.09	0.05	5.84	15.0
S, g/d	2.2	2.07	0.29	0.93	0.06	0.19	5.83	18.2
Mg, g/d	1.31	0.66	0.05	0.82	0.08	0.03	1.16	14.7

¹Urine production was either measured from total collection of urine (actual) or estimated from urinary creatinine concentration in a 4-d composite sample (estimated) and BW [volume = $29 \times \text{BW (kg)}/\text{creatinine (mg/L)}$]. Excretion of nutrients (g/d) was then calculated using either actual or estimated urine volume. Urinary N was measured using the Kjeldahl method, and minerals were measured using inductively coupled plasma emission spectroscopy.

²*P*-value for rejecting intercept = 0.

³*P*-value for rejecting slope = 1.

⁴Root mean squared error.

DISCUSSION

The method of measuring Mg concentration affected Mg excretion (Figure 2). The reason for the difference between assay methods is unknown, but the colorimetric assay was developed for measuring Mg in human urine, which has Mg concentrations that are approximately 10 to 20 times less than those of the average cow in this study (average: 224 mg/L).

Plasma Mg concentrations did not respond to abrupt diet changes (i.e., no treatment $\times$ day interactions)

and did not change when supplemental K was fed. In contrast, Schonewille et al. (2000) reported a significant decrease in plasma Mg within 2 d of feeding additional K. The source of Mg also did not affect plasma Mg, although apparent absorption differed (experiment 1). Both observations suggest that plasma Mg is not a sensitive marker of Mg supply.

Urinary Mg concentrations were more responsive to dietary changes than blood data; however, if treatment affects urine output, concentration data can be misinterpreted. In experiment 1, the urine Mg concentration

Figure 5. Relationship between urinary Mg excretion determined using actual or estimated (via creatinine) urine output and Mg measured using inductively coupled plasma emission spectroscopy (experiment 1): $Y = 0.82 (\pm 0.08) X + 1.31 (\pm 0.66)$; root mean squared error = 1.16; $P < 0.01$. The Y-intercept and slope were different from 0 ($P < 0.05$) and 1 ($P < 0.03$), respectively. The dashed line is the linear regression, and the solid line is $Y = X$. Each symbol represents a cow-period ($n = 34$ cow-periods). MgO = (gray) squares; MgSO₄ = (red) triangles; no monensin = open shape; monensin = solid shape. Color version available online.

Table 6. Effect of supplemental Mg and K on production measures, blood measures, and urinary excretion (experiment 2)

Item	Treatment ¹				SEM	P-value		
	Basal	HiK	HiMg	HiK+Mg		K	Mg	K × Mg
Cows, no.	8	8	8	8				
DMI, kg/d	22.8	22.7	24.2	22.8	1.04	0.30	0.34	0.34
BW, kg	726	732	772	742	25.3	0.50	0.12	0.31
Milk yield, kg/d	29.2	30.5	30.7	30.8	2.75	0.57	0.45	0.62
Intake, g/d								
K	365	570	378	597				
Mg	46	41	102	98				
Blood ²								
Plasma Mg, mM	1.00	1.00	1.02	1.04	0.02	0.88	0.04	0.43
PCV, %	28.7	30.7	29.6	30.5	0.44	0.35	0.01	0.17
Urine ³								
Estimated volume, L/d	31.9	41.8	29.5	37.2	3.06	0.01	0.26	0.72
Creatinine, mg/L	704	584	788	635	53.7	0.02	0.22	0.76
Mg, ^{4,5} mg/L	215	125	276	222	21.6	0.01	0.01	0.41
Estimated Mg excretion, ⁵ g/d	6.4	4.3	7.9	7.7	0.60	0.04	0.01	0.10

¹Basal = no supplemental Mg and K (total diet Mg and K: 0.2 and 1.6%, respectively); HiK = supplemental K₂CO₃ [total diet K: 2.6% of DM, 40% from K₂CO₃ (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ)]; HiMg = supplemental MgO [total diet Mg: 0.4% of DM, 50% as MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD)], HiK+Mg = supplemental K₂CO₃ and MgO (total dietary K and Mg: 2.6 and 0.4% of DM, respectively; 40% from K₂CO₃ and 50% from MgO).

²Plasma Mg and packed cell volume (PCV) are covariate adjusted means of blood draw on d 3 and 7. Day was significant (average concentration on d 0, 3, and 7 was 1.01, 1.03, and 0.99 mM, respectively; $P = 0.03$), but no interactions of fixed effects and day were found ($P > 0.15$).

³Means of urine spot samples from d 0, 2, 4, and 7. Urine Mg concentration was measured using inductively coupled plasma emission spectroscopy, and urine volume (L/d) was estimated [$29 \times \text{BW (kg)}/\text{urinary creatinine concentration (mg/L)}$] for measuring Mg excretion (g/d).

⁴K × day interaction ($P < 0.10$).

⁵Mg × day interaction ($P < 0.10$).

Figure 6. Relationship between urinary S excretion determined using actual or estimated (via creatinine) urine output and Mg measured using inductively coupled plasma emission spectroscopy (experiment 1): $Y = 0.93 (\pm 0.06) X + 2.2 (\pm 2.07)$; root mean squared error = 5.83; $P < 0.01$. The Y-intercept and slope were similar to 0 ($P < 0.29$) and 1 ($P < 0.19$), respectively. The dashed line is the linear regression of all cow-periods, the solid line is $Y = X$, and the dot-dashed lines are regressions for each source (MgO = gray, line on the left; MgSO₄ = red, line on the right). For MgO treatment (or 0.2% S), the slope was 0.34 (SE = 0.22; $P < 0.15$), and for the MgSO₄ treatment (0.4% S), the slope was 0.03 (SE = 0.17; $P < 0.85$). Each symbol represents a cow-period ($n = 34$ cow-periods). MgO = (gray) squares; MgSO₄ = (red) triangles; no monensin = open shape; monensin = solid shape. Color version available online.

data indicated the MgSO_4 treatment without monensin had about 30% greater Mg absorption than other treatments (288 vs. 218 mg/L); however, this diet had statistically similar urinary Mg output as the MgO diets because Mg source affected urine volume. Expressing Mg output as a ratio of Mg to creatinine is also inappropriate because creatinine excretion is proportional to body mass (Schroeder et al., 1990), which inherently varies between cows. Therefore, determining Mg status or evaluating bioavailability requires measuring or estimating urine volume.

Method of Evaluating Urinary Excretion

Measuring BW and creatinine to estimate urine output accurately estimated treatment-average urinary output of all nutrients evaluated (N, K, Na, S, Mg). Our calculated creatinine excretion (mg/kg of BW) was identical to a previous estimate for lactating Holsteins (Figure 1; Valadares et al., 1999); however, the lack of fit was high (RMSE = 2,980; 14% of mean; Figure 3), indicating that the rate of creatinine excretion per unit of BW varies considerably among cows. When a con-

Figure 7. Effect of day after an abrupt diet change (experiment 2) on urinary Mg (A) concentration ($K \times \text{day}$, $P < 0.03$; $Mg \times \text{day}$, $P < 0.01$; $K \times Mg \times \text{day}$, $P < 0.63$) and (B) estimated excretion ($K \times \text{day}$, $P < 0.22$; $Mg \times \text{day}$, $P < 0.01$; $K \times Mg \times \text{day}$, $P < 0.50$). Cows were fed diets varying in Mg (0.20 vs. 0.42% of DM) and K (1.60 vs. 2.57% of DM). Urine Mg output was estimated using spot samples and measuring creatinine and Mg concentration with inductively coupled plasma emission spectroscopy. Bars are the SEM across treatments and days (panel A: SEM = 30 mg/L; panel B: SEM = 0.77 g/d), and each symbol represents the treatment average $\times$ day. Basal = solid (red) diamonds; HiK = solid (gray) circles; HiMg = open (red) diamonds; HiK+Mg = open (gray) circles. Basal = no supplemental Mg and K (total diet Mg and K: 0.2 and 1.6%, respectively); HiK = supplemental K_2CO_3 [total diet K: 2.6% of DM, 40% from K_2CO_3 (DCAD Plus; Church & Dwight Co. Inc., Piscataway, NJ)]; HiMg = supplemental MgO [total diet Mg: 0.4% of DM, 50% as MgO (Animag Prilled 30/100; Martin Marietta Magnesia Specialties LLC, Baltimore, MD)]; HiK+Mg = supplemental K_2CO_3 and MgO (total dietary K and Mg: 2.6 and 0.4% of DM, respectively; 40% from K_2CO_3 and 50% from MgO). Color version available online.

stant rate is assumed for urine volume calculations, that variation was ignored and resulted in an artificially low standard error of the mean for estimated excretion of urine, N, and K compared with measured urine outputs (Table 4). Inaccurate error variance for estimated urine output inflated the Type I error rate (i.e., increased probability of incorrectly rejecting null hypothesis) and produced several incorrect statistical inferences (i.e., N and K excretion). The lack of fit was apparent even though the samples came from total output of urine over a 4-d period. Studies that spot sample urine may introduce more variation than we observed because of diurnal variation (Shingfield and Offer, 1998). Day-to-day variation in urine volume, which averaged about 16% during total collection (data not shown), could also affect accuracy and precision of spot sampling if samples are not taken over enough days.

Disregarding lack of fit variation in creatinine excretion rate did not reduce the standard error of the mean of all excretion estimates (i.e., Na, S, and Mg; Table 4). Upon further investigation, urinary concentration of Na and Mg was more negatively correlated with actual urine volume than with estimated volume (Mg: $r = -0.32$ vs. -0.16 ; Na: $r = -0.13$ vs. -0.04). This means that variation in actual urine excretion of those nutrients was reduced as urine volume increased, but not so with estimated urine output. The reason for the different correlations is unknown, but if real, it may increase differences between actual and estimated output at either low or high excretion rates of urine. Although regression analysis indicated that estimated excretion of S was accurate, the regression analysis must be evaluated carefully (Figure 6). By design, concentration of dietary S differed between Mg source treatments (0.2 vs. 0.4%), and when regressions were performed within each Mg treatment, the slope relating estimated to actual urinary excretion of S was not significant. For MgO treatment (or 0.2% S), the slope was 0.34 (SE = 0.22; $P < 0.15$), and for the MgSO₄ treatment (0.4% S), the slope was 0.03 (SE = 0.17; $P < 0.85$). This suggests that if dietary S does not vary greatly among diets, estimating excretion with creatinine may not be accurate.

Variable rates of creatinine excretion per kilogram of BW also caused individual cow urine output to differ substantially from estimated output (Figure 4). Estimated urine volume differed from actual urine output by more than 15% in 13 of the 34 cow-periods (38% of observations) and by more than 20% in 15% of the observations. These large differences indicate that an adequate number of cows is needed to obtain accurate results; however, we cannot determine the number needed from this experiment.

Time Course of Mg Excretion

For valid comparisons, Mg urinary excretion must be at steady state (Van Ravenswaay et al., 1992). For diets with supplemental K (i.e., HiK and HiK+Mg), the decrease in estimated Mg output was stable by d 2 (Figure 7b). In dry cows abruptly switched to a high-K diet (2.6–5.0% of DM), urinary Mg excretion required 4 to 6 d to stabilize (Schonewille et al., 2000). Absorption of Mg occurs in the rumen, and because K₂CO₃ is rapidly solubilized in the rumen, the antagonistic effect of K on Mg absorption should develop quickly upon increased K intakes. Faster time to steady state in lactating cows is likely a consequence of higher Mg demand and faster depletion of blood Mg compared with dry cows. Based on the NRC (2001) Mg requirement, a nonpregnant, nonlactating cow weighing the same as the average lactating cow of experiment 2 (Table 6) requires about 3-fold less Mg (2.1 vs. 6.1 g/d) than the average cow in this experiment. Because healthy cows may have only 3 to 4 g of labile Mg (Storry, 1961; Fach, 2015), urinary homeostasis (and hypomagnesemia) likely occurs more quickly for lactating cows after abrupt increases in dietary K.

When dietary Mg intake was doubled (HiMg and HiK+Mg diets), urinary output of Mg did not appear to reach steady state by d 7. In sheep, urinary excretion of Mg was consistently stable 4 to 5 d after a large change in dietary Mg concentration and Mg source (Van Ravenswaay et al., 1989, 1992). The reason why urinary Mg output did not reach steady state for cows fed supplemental Mg could not be determined from this study. Spot sampling frequency may have limited our ability to detect steady state (i.e., no sample between d 4 and 7). Failing to reach steady state in 7 d could partly be a consequence of low ruminal solubility of Mg (Horn and Smith, 1978), which is the rate-limiting step to absorption (Schweigel and Martens, 2000). Because lactating dairy cattle have faster passage rates, larger rumen volumes, and greater Mg demands compared with sheep or dry cows, the time for soluble Mg concentrations to reach a maximum may have been longer than the duration of this experiment.

When both Mg and K were supplemented, urinary Mg output did not rapidly decrease such as with the HiK diet, which was contrary to our original hypothesis. Using estimates of Weiss (2004), these cows consumed about 39 g/d excess Mg relative to the dietary K concentration. The lack of a decrease in urine Mg output could have been an effect of mass action from the large amount of Mg consumed, which overwhelmed the antagonist effect of K.

CONCLUSIONS

Measuring BW and urinary creatinine was an accurate method for estimating treatment-average excretion of urine, urinary minerals, and N, but lack of fit error was high. Accuracy was poor for many individual observations; future studies using creatinine as a urine marker need to include additional replication to account for both prediction and cow-to-cow variation. Using a fixed rate of creatinine excretion does not include lack of fit variation associated with estimated urine production and affected the statistical interpretation of treatments. Excretion of urinary Mg reached steady state rapidly after abruptly increasing dietary K, whereas adding supplemental Mg gradually increased excretion over time, and excretion did not reach steady state after 7 d. The different methodology for measuring urine and timeline of Mg excretion can be used for future balance and Mg bioavailability studies.

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