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

A. W. Tebbe and W. P. Weiss[1]
Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

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. $MgSO_4$; 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 K_2CO_3; 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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[1]Corresponding author: weiss.6@osu.edu

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 $MgSO_4$ (Magriculture; Giles Chemical, Waynesville, NC) or (2) $MgSO_4$ 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 K_2CO_3) 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 K_2CO_3 (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 K_2CO_3 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 × 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