Validation of diagnostic tests for determination of magnesium status in horses with reduced magnesium intake

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Objective—To evaluate the diagnostic value of serum concentrations of total magnesium (tMg) and ionized magnesium (iMg), concentrations of magnesium (Mg) in muscle, intracellular Mg (icMg) concentrations, urinary Mg excretion (E_{Mg}), Mg clearance (C_{Mg}), and fractional clearance of Mg (FC_{Mg}) in horses fed diets with Mg content above and below National Research Council recommendations.

Animals—9 young female horses.

Procedures—6 horses were fed a reduced-Mg diet for 29 days followed by an Mg-supplemented diet for 24 days. Control horses (n = 3) were fed grass hay exclusively. Blood, urine, and tissue samples were collected, and an Mg retention test was performed before and after restriction and supplementation of Mg intake. Serum tMg, serum iMg, muscle Mg, icMg, and urine Mg concentrations were measured, and 24-hour E_{Mg}, C_{Mg}, and FC_{Mg} were calculated.

Results—Reductions in urinary 24-hour E_{Mg}, C_{Mg}, and FC_{Mg} were evident after 13 days of feeding a reduced-Mg diet. Serum tMg and iMg concentrations, muscle Mg content, and results of the Mg retention test were not affected by feeding the Mg-deficient diet. Spot urine sample FC_{Mg} accurately reflected FC_{Mg} calculated from 6- and 24-hour pooled urine samples. Mean ± SD FC_{tMg} of horses eating grass hay was 29 ± 8%, whereas mean FC_{tMg} for horses fed a reduced-Mg diet for 29 days was 6 ± 3%.

Conclusions and Clinical Relevance—The 24-hour E_{Mg} was the most sensitive indicator of reduced Mg intake in horses. Spot sample FC_{Mg} can be conveniently used to identify horses consuming a diet deficient in Mg. (*Am J Vet Res* 2004;65:422–430)

Potassium is the most common intracellular cation, and **magnesium (Mg)** is the second most common intracellular cation. Approximately 50% to 60% of total body Mg is distributed in bone, with 40% to 50% in soft tissues. Magnesium is an essential intracellular cation and cofactor for more than 300 enzymatic reactions involving ATP, including glycolysis and oxidative phosphorylation. It is also important in the function of the sodium-potassium-ATPase pump, membrane stabilization, nerve conduction, ion transportation, and calcium channel activity.[1,2] Clinical manifestations of severe hypomagnesemia include muscle weakness, muscle fasciculations, ventricular arrhythmias, seizures, ataxia, and coma.[3-5] Subclinical hypomagnesemia increases severity of the systemic inflammatory response syndrome; worsens the systemic response to endotoxins; and can lead to ileus, cardiac arrhythmias, refractory hypokalemia, and hypocalcemia.[1,2,6,7]

Abnormalities in serum Mg concentrations have gained attention in critical-care arenas in human and veterinary medicine. Low serum Mg concentrations have been reported in 65% of critically ill humans,[8,9] 39% to 46% of cats and dogs in intensive care units,[a,10] 49% of hospitalized horses,[11] 54% of equine surgical colic patients,[12] and 78% of horses with enterocolitis.[13]

Traditionally, only serum concentrations of **total Mg (tMg)** have been reported in prevalence studies of hypomagnesemia in hospitalized patients. Similar to calcium, serum tMg can be classified into 3 forms. The physiologically active (free) fraction is **ionized Mg (iMg)**, whereas the protein-bound and chelated fractions are unavailable for biochemical processes. In humans, serum iMg concentrations cannot be accurately calculated from serum tMg and albumin concentrations; therefore, serum iMg concentration should be determined by direct measurement.[14] Less than 1% of body Mg is contained within the extracellular fluid; therefore, it is speculated that serum Mg concentrations may not adequately reflect total body stores of Mg. Sensitive tests of Mg status are needed in clinical situations in which Mg deficiency may contribute to the disease condition in animals.

Magnesium is cleared by glomerular filtration, and renal homeostatic mechanisms will attempt to main-

Received February 18, 2003.
Accepted September 3, 2003.

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Supported by the Ohio State University College of Veterinary Medicine Equine Research Fund and a research grant from the American Association of Equine Practitioner's Foundation Incorporated.

Presented in part as a poster at the 21st American College of Veterinary Internal Medicine Forum, Charlotte, NC, June 2003; presented in part at the 9th International Veterinary Emergency and Critical Care Society Forum, New Orleans, La, September 2003; and presented in part at the 49th American Association Equine Practitioners Forum, New Orleans, La, December 2003.

The authors thank Lori Avila, Mathew Lovett, Sae Tsubakishita, Michael Suhie, Anne Weibe, Leia Hill, and Kelly Rourke for technical assistance.

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tain Mg balance. When the diet is deficient in Mg, renal tubular reabsorption increases to maintain serum concentrations of Mg within narrow physiologic limits.[15] Determination of urinary excretion may be an extremely sensitive indicator of dietary Mg deficiency, whereas decreases in serum concentrations of Mg are evident only after severe chronic deficiency or acute uncompensated redistribution.[13,16]

Intravenous administration of large doses of Mg can be safely used to concurrently diagnose and treat hypomagnesemia in humans. The IV Mg retention test has become the criterion-referenced standard to determine Mg status in human intensive care units.[17] After determination of basal Mg urinary excretion for a 24-hour period, a dose of Mg is infused and urinary excretion of Mg during the subsequent 24 hours is measured. Patients who excrete most of the infused Mg are considered magnesium-replete patients, whereas magnesium-depleted patients retain most of the administered Mg. The percentage of Mg retention that defines a normomagnesemic state is dependent on the amount of Mg infused and the equation used to describe the percentage of retention. In 1 study[17] in humans, investigators found that normomagnesemic patients excrete > 70% of the infused Mg, whereas Mg-deficient patients excrete < 70% of the total amount of administered Mg. In another study[18] in humans, retention of 40% to 50% of the administered Mg indicated Mg deficiency, whereas retention of < 20% indicated that Mg deficiency was unlikely. The IV Mg retention test has been validated for use in humans and dogs, but to our knowledge, it has not been investigated in horses or ponies.

Hypomagnesemia in horses has rarely been reported,[3,5,19] but it can be induced experimentally by feeding an Mg-deficient diet.[4,16,20,21] The **National Research Council (NRC)** recommends that horses receive > 13 mg/kg/d or 1,600 mg/kg of feed.[20] Lactating and growing animals have higher requirements for Mg. Subclinical hypomagnesemia was detectable in adult ponies fed a diet deficient in Mg (370 mg/kg of feed) for 18 days.[16]

Our hypothesis for the study reported here was that feeding an Mg-deficient diet to young horses would induce Mg deficiency evident as hypomagnesemia; reductions in Mg concentrations in muscle tissues and sublingual epithelial cells; and decreases in **urinary Mg excretion (E_{Mg})**, **clearance of Mg (C_{Mg})**, and **fractional clearance of Mg (FC_{Mg})**. Furthermore, we hypothesized that results of the IV Mg retention test would reflect the reduced dietary intake of Mg. Our objectives were to validate diagnostic tests for the identification of reduced Mg intake in horses by comparing serum tMg and iMg concentrations, muscle concentrations of Mg, sublingual epithelial **intracellular Mg (icMg)** concentrations, E_{Mg}, C_{Mg}, and FC_{Mg} in horses fed grass hay, with values for horses fed Mg-reduced and Mg-supplemented diets. We also investigated the use of spot FC_{Mg}, compared with 6- and 24-hour FC_{Mg}, thereby possibly providing a reference range for FC_{Mg} and alleviating the necessity for cumbersome and time-consuming volumetric urine collections in hospitalized horses.

Materials and Methods

Animals—Nine female horses of various breeds were used in the study. Horses ranged from 8 to 12 months of age and weighed between 219 and 448 kg. Horses were housed separately in box stalls and exercised daily by hand-walking and lunging. All horses were fed the same grass hay diet ad libitum during a 6-week quarantine period. All horses were accustomed to handling procedures, including conditioning to prolonged periods of being restrained in a cross-tie. Horses were dewormed[b] and vaccinated[c] before entering the study. No drugs were administered to any horses during the 16-day baseline period. All horses were considered to be healthy on the basis of results of physical examination, culture for *Streptococcus equi* var *equi* of swab specimens obtained from the nasopharyngeal area, endoscopy of the nasopharynx and diverticulum of the auditory tubes (ie, guttural pouches), CBC counts, serum biochemical analyses, plasma fibrinogen concentration, urinalysis, and quantitative microbial culture of a urine sample. The study was approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

Diets—The control diet consisted of unsupplemented mixed grass hay. Horses had unrestricted access to tap water. One batch of hay was fed to all horses during the baseline period, and a second batch was fed to the control horses for the remainder of the study. A reduced-Mg diet was prepared.[d] The diet was formulated to contain Mg (600 mg/kg of feed), as determined on the basis of a ration (ie, 350 mg/kg of feed) described elsewhere (**Appendix 1**).[16] A representative sample of each batch of hay and 3 random samples of the reduced-Mg diet were independently analyzed by a commercial laboratory[e] (**Appendix 2**). We suspected an error in the production of the reduced-Mg diet because major discrepancies were evident in the protein, calcium, phosphorus, potassium, and sodium contents between the formulated diet and the diet that was prepared for consumption. The reduced-Mg diet was a powder, which was moistened to achieve a crumbly consistency for feeding. Feed and water were offered ad libitum, and we did not attempt to quantitate the amounts consumed or provide isocaloric amounts of the formulated diet or grass hay. An Mg-supplemented diet was also formulated and prepared,[d] consisting of the reduced-Mg diet with Mg oxide added at the rate of 40 mg/kg/d.

Experimental protocol—Horses were randomly assigned to 2 groups (6 horses in the treatment group and 3 horses in the control group). Control horses were fed grass hay throughout the study, whereas treated horses were fed diets corresponding to 3 dietary phases (phase 1, baseline diet; phase 2, low-Mg diet; and phase 3, Mg-supplemented diet). All horses were used in the study concurrently. Horses were monitored daily, and results of daily physical examinations were recorded. Body weight was measured before each 24-hour urine collection.

During phase 1 (baseline; days 1 to 16), all horses were fed the control grass hay diet and had ad libitum access to tap water. Urine and blood samples were collected from all horses on day 14, and a muscle biopsy specimen was obtained from each horse on day 15. On day 16, the Mg retention test was performed on all horses.

During phase 2 (Mg restriction, days 17 to 53), horses in the treatment group were fed a reduced-Mg diet. On days 17 to 24, treatment-group horses were gradually fed increasing amounts of the low-Mg ration and decreasing quantities of grass hay. For the 29-day period from days 24 to 53, horses in the treatment group were housed separately in stalls with bare rubber floors and fed the low-Mg diet exclusively. Furthermore, treatment-group horses were allowed to drink only distilled water[f] during feeding of the low-Mg diet. Blood

and urine samples were collected from all 9 horses on days 37 and 51. A muscle biopsy specimen was obtained from each horse on days 38 and 52. On day 53, the Mg retention test was performed on all horses.

During phase 3 (23-day period from days 54 to 77), horses in the treatment group continued to receive the low-Mg ration, but it was supplemented with Mg oxide at the rate of 40 mg/kg/d (corresponding to 24.1 mg of elemental Mg/kg/d)[22]; treatment-group horses were again allowed ad libitum access to tap water. On day 75 (21 days of feeding the Mg-supplemented diet to the treatment horses), urine and blood samples were collected from all 9 horses. A muscle biopsy specimen was obtained from each horse on day 76, and the Mg retention test was performed on all horses on day 77.

Collection of urine samples—Twenty-four hour urine collections were performed on days 14, 16, 37, 51, 53, 75, and 77. After wrapping the tail and washing the perineal region with antiseptic soap, a sterile Foley catheter[g] was aseptically inserted into the bladder of each horse. Each horse was then cross-tied in a box stall in a manner that allowed access to feed and water. The bladder of each horse was emptied, and urine was then collected into 5-L sterile plastic containers for the next 24 hours via continuous flow by use of laboratory-grade latex rubber tubing.[h] At 0, 6, 12, 18, and 24 hours after initiation of urine collection, the volume of urine collected during each 6-hour period was quantified; the urine for that 6-hour period was thoroughly mixed, and a representative 20-mL sample was obtained. The remaining urine was then pooled with the other 6-hour collections; the urine for the 24-hour collection was thoroughly mixed, and a pooled 24-hour sample was obtained. On day 14, an additional 20-mL spot urine sample was collected by direct aspiration from the Foley catheter every 6 hours (corresponding to the time at which blood samples were collected). All urine samples were frozen at –20°C until analyzed to measure Mg and creatinine concentrations, which allowed us to calculate E_{Mg}, C_{Mg}, and FC_{Mg} for each 6-hour interval.

Collection of blood samples—On days 14, 37, 51, and 75, a catheter[i] was aseptically inserted in each jugular vein of all horses. The catheter in the left jugular vein was used to obtain blood samples for analysis, and the catheter in the right jugular vein was used to administer the Mg infusion on days 16, 53, and 77. Blood samples were collected at 0, 6, 12, 18, and 24 hours during the 24-hour urine collections. Blood samples were allowed to clot for 30 minutes at 25°C; samples were then centrifuged in a refrigerated centrifuge, and serum was then harvested. Serum iMg concentrations were measured within 1 hour after collection of samples, and the remaining serum was frozen at –20°C until subsequent analysis.

Collection of muscle biopsy specimens—Muscle biopsies were performed on each horse on days 15, 38, 52, and 76. Horses were sedated by administration of xylazine hydrochloride[j] (150 mg, IV), and the skin and subcutaneous tissue over the middle gluteal muscle were infiltrated locally with an anesthetic.[k] Muscle specimens were collected percutaneously from the middle gluteal muscle by use of a needle-biopsy technique.[23] Muscle specimens were immediately placed in tissue cassettes, wrapped in aluminum foil, snap-frozen in liquid nitrogen, and stored at –70°C until subsequent analysis.

Collection of sublingual epithelial cells—On days 15, 38, 52, and 76, sublingual epithelial cells were collected from each horse by firmly scraping tissue adjacent to the frenulum with a disposable wooden spatula. The cells were applied to low-background carbon slides, dehydrated by application of a standard cytologic fixative,[l] and air-dried prior to shipment to a laboratory for determination of intracellular electrolyte concentrations.

IV Mg retention test—On days 16, 53, and 77, Mg sulfate[m] (100 mg of $MgSO_4 \bullet 7H_2O$ solution/kg, which was equivalent to 10 mg of elemental Mg/kg) in the form of a 5% solution in saline (0.9% NaCl) solution[24] was infused IV during a 1-hour period into the right jugular catheter. Volumetric urine collection was performed for 24-hours beginning at the start of the Mg infusion. Serum and pooled urine samples were collected 0, 6, 12, 18, and 24 hours after commencement of the Mg infusion.

Hematologic and serum biochemical analyses—Serum iMg concentrations were measured by use of an ion-specific electrode[n] within 1 hour after anaerobic collection of samples. Serum tMg concentrations were determined by use of an automated analyzer.[o] Serum creatinine concentrations were determined by use of colorimetric reactions in an automated analyzer.[p]

Urine biochemical analysis—Urine creatinine concentrations were measured in uncentrifuged nonacidified samples by use of an automated analyzer,[p] as described for measurement of serum creatinine concentrations. For measurement of urine Mg concentrations, 4.0 mL of well-mixed uncentrifuged urine was diluted in 5 mL of distilled water and digested in 5 mL of 20% nitric acid and 1.0 mL of hydrogen peroxide via a microwave system.[25,q] The samples were then diluted to a final volume of 25 mL with distilled water and analyzed for Mg concentration by use of **inductively coupled plasma-atomic emission spectrophotometry (ICP-AES)**.[r]

Values for E_{Mg} were calculated by use of the following equation:

$$E_{Mg} = (U_{Mg} \bullet V)/BW$$

where U_{Mg} is the urine Mg concentration, V is urine volume in mL, and BW is body weight in killigrams.

Values for C_{Mg} were calculated by use of the following equation[26]:

$$C_{Mg} = (U_{Mg}/tMg) \bullet ([V/T]/BW)$$

where tMg is the serum tMg concentration, and T is the time interval in minutes.

Values for **fractional clearance of tMg (FC_{tMg})** were calculated by use of the following equation[27]:

$$FC_{tMg} = ([U_{Mg}/tMg] \bullet [S_{Cr}/U_{Cr}]) \bullet 100$$

where S_{Cr} is the serum creatinine concentration, and U_{cr} is the urine creatinine concentration. Concentrations were measured in concurrently obtained serum and urine samples.

Values for **fractional clearance of iMg (FC_{iMg})** were calculated by use of the following equation[7]:

$$FC_{iMg} = ([U_{Mg}/iMg] \bullet [S_{Cr}/U_{Cr}]) \bullet 100$$

where iMg is the serum iMg concentration. Concentrations were measured in concurrently obtained serum and urine samples.

The E_{Mg}, C_{Mg}, and FC_{Mg} values were calculated by use of urine Mg concentrations measured in each of the 6-hour-interval aliquots of urine as well as from the 24-hour urine sample. Values for FC_{Mg} were also calculated by use of Mg concentrations measured in spot samples of urine that were collected every 6 hours on day 14.

The **percentage of Mg retained (% Mg Ret)** after IV infusion of the Mg solution was calculated for each of 3 methods, as reported in other studies.[17,28-32] For method 1, the amount of Mg excreted was divided by the amount of

Mg infused, as determined by use of the following equation[29,30]:

$$\% \text{ Mg Ret} = (1 - [\text{24-hour } E_{Mg} \text{ after infusion}]/[\text{amount of elemental Mg infused}]) \bullet 100.$$

Method 2 required consideration of the basal 24-hour E_{Mg} value obtained before infusion. The basal 24-hour E_{Mg} was subtracted from the E_{Mg} measured after infusion, and the difference was then divided by the amount of elemental Mg infused.[28] The basal E_{Mg} was determined from the previous 24-hour urine collection for each horse. Values for method 2 were determined by use of the following equation[28]:

$$\% \text{ Mg Ret} = ([1 - \{\text{24-hour } E_{Mg} \text{ after infusion} - \text{24-hour } E_{Mg} \text{ before infusion}\}]/\text{amount of elemental Mg infused}) \bullet 100.$$

Method 3 used the serum creatinine concentration to correct for the urine concentration by use of the following equation:

$$\% \text{ Mg Ret} = (1 - [EU_{Mg} - \{R \bullet UE_{Cr}\}]/\text{amount of elemental Mg infused}) \bullet 100$$

where EU_{Mg} is the 24-hour E_{Mg} after infusion, R is the 24-hour urine Mg-to-urine creatinine ratio (ie, $[U_{Mg}]/[U_{Cr}]$) before infusion, and UE_{Cr} is the 24-hour urine creatinine concentration after infusion.[28,31,32]

Intracellular concentrations of electrolytes in sublingual epithelial cells—Intracellular electrolyte concentrations were determined by use of energy-dispersive x-ray analysis.[s] This assay was performed by use of a specially configured scanning electron microscope to irradiate the cells with a focused electron beam and subsequently measure radiographic fluorescence.

Analysis of mineral concentration in muscle tissues—Muscle biopsy specimens were thawed at 22°C and then dehydrated at 75°C for 7 hours in a commercial desiccator.[t] Dehydration was considered complete when there was no weight change for a 60-minute period. Mineral analysis of digested muscle specimens[u] was performed by use of ICP-AES, similar to analysis of urine samples.

Statistical analysis—All assumptions of normalcy were met as determined by use of the Kolmogorov-Smirnoff test. A 1-way ANOVA with repeated measures was used to detect differences in mean serum tMg, serum iMg, muscle Mg, icMg, and urine Mg concentrations; 24-hour E_{Mg}, C_{Mg}, FC_{tMg}, and FC_{iMg}; and % Mg Ret after infusion of Mg in horses in the treatment group. A repeated-measure ANOVA was used to compare consecutive E_{Mg}, C_{Mg}, and FC_{tMg} values calculated for samples obtained at 6-hour intervals (6, 12, 18, and 24 hours) to determine variability of these variables over time and validity of the use of 6-hour-interval E_{Mg}, C_{Mg}, and FC_{tMg} to estimate 24-hour E_{Mg}, 24-hour C_{Mg}, and 24-hour FC_{tMg}, respectively. Fractional clearance calculations were performed for spot urine samples obtained at the end of phase 1 (day 14), and these values were compared by use of a repeated-measure ANOVA with FC_{tMg} calculated for urine samples obtained at 6-hour intervals (6, 12, 18, and 24 hours) and with FC_{tMg} calculated for the 24-hour pooled urine sample. This was used to determine significant differences in spot FC_{tMg}, 6-hour-interval FC_{tMg}, and 24-hour FC_{tMg} and, therefore, determine the reliability of the use of spot urine samples to estimate 24-hour clearance. Specific differences in means were identified with a post hoc Student-Neuman-Keuls multiple-comparison test. The Friedman nonparametric test for 1-factor repeated measure was used to determine differences over time in the control horses. Significance of each test was set at $P < 0.05$. Results were reported as mean ± SD and 95% **confidence interval (CI)**. The 95% CI was calculated as follows: 95% CI = mean ± $(1.96 \times [SD/\sqrt{n}])$.

Results

Horses—Results of physical examinations in all horses before and throughout the duration of the study were unremarkable. The treatment diets were accepted well by the horses in the treatment group. Wasted feed was rapidly mixed with fecal material because the horses were not confined in metabolism cages. Wasted feed was removed 3 times daily when stalls were cleaned with water from a hose. Weighing the discarded feed and determination of the amount consumed were impossible. Initial body weight of the horses ranged from 203 to 426 kg (mean ± SD, 271 ± 73 kg) on day 14 (ie, baseline). All horses gained weight during the study period. Mean weight gain from the beginning to the end of the study was similar between control (8.1 ± 3.5 kg) and treatment (6.7 ± 3.5 kg) groups.

Diets—The analysis performed after the reduced-Mg diet was prepared revealed substantial differences between the formulated diet and the initial preliminary sample prepared for consumption. Calcium, phosphorus, sodium, and potassium concentrations were all below NRC recommendations. Additionally, the calcium-to-phosphorus ratio was inappropriate. This precluded any conclusions on the effect of a reduced-Mg diet on other electrolytes or parathyroid hormone.

Control and treatment groups—We did not identify detectable changes in any of the variables over time for the control horses. Results differed between the 3 phases of the study for treatment horses (**Table 1**).

Serum tMg concentration—Serum tMg concentrations in the treatment group did not differ significantly between baseline (phase 1) and the end of reduced Mg intake (phase 2). However, serum tMg concentration increased significantly ($P = 0.01$) during feeding of the Mg-supplemented diet (phase 3), compared with the baseline tMg concentration.

Serum iMg concentration—Serum iMg concentrations did not differ significantly between baseline and the end of reduced Mg intake (phase 2). Serum iMg concentrations increased significantly ($P = 0.006$) during phase 3 (feeding of Mg-supplemented diet), compared with the baseline concentration. After feeding the low-Mg diet for 2 weeks, the percentage of Mg in the ionized state increased significantly ($P = 0.001$), compared with the baseline value. After 2 weeks of feeding the diet supplemented with Mg oxide, the percentage of Mg in the ionized state was not significantly different from the baseline value.

Urine volume—At baseline, the volume of urine produced in 24 hours by each of the 9 horses ranged from 10.6 to 34.2 mL/kg/d (mean, 17.8 ± 7.2 mL/kg/d). Although the mean urine volume in horses in the treatment group increased after consumption of the experimental diet, this change was not significant. One

Table 1—Mean ± SD and 95% confidence interval (CI) values for variables measured in 6 female yearling horses fed grass hay, a reduced-magnesium (Mg) diet, and an Mg-supplemented diet

	Baseline		Middle of feeding reduced-Mg diet		End of feeding reduced-Mg diet		End of feeding Mg-supplemented diet	
Variable	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**
Serum total Mg (mg/dL)	1.4 ± 0.2	1.3–1.6	1.4 ± 0.1[a]	1.3–1.5	1.5 ± 0.1[a]	1.4–1.6	1.8 ± 0.2[b]	1.7–1.9
Serum ionized Mg (mg/dL)	1.1 ± 0.1	1.0–1.1	1.1 ± 0.1[a]	1.1–1.1	1.2 ± 0.1[a]	1.2–1.3	1.4 ± 0.1[b]	1.3–1.5
Serum Mg in ionized form (%)	73.8 ± 4.4	70.3–77.3	79.1 ± 4.16[a]	77.4–80.8	83.3 ± 2.8[a]	82.4–84.2	77.4 ± 3.9	74.3–80.5
Urine volume (L)	18.6 ± 8.6	11.7–25.5	48.6 ± 54.8	47.0–92.5	42.5 ± 40.8	9.8–75.2	39.5 ± 30.6	15.0–64.0
Urine Mg concentration (mg/dL)	60.1 ± 41.7	26.7–93.5	10.9 ± 13.0[b]	0.5–21.3	11.0 ± 12.6[b]	0.9–21.1	32.7 ± 20.6[b]	16.2–49.2
24-hour urine excretion of Mg (mg/kg/d)	9.3 ± 3.1	6.8–11.8	2.2 ± 1.4[a,b]	1.1–3.3	2.0 ± 1.3[a,b]	1.0–3.1	7.1 ± 3.5	4.3–9.9
24-hour urine clearance of Mg (mL/kg/min)	0.4 ± 0.1	0.4–0.5	0.1 ± 0.06[a,b]	0.05–0.15	0.1 ± 0.06[a,b]	0.05–0.15	0.3 ± 0.1[b]	0.2–0.4
24-hour fractional clearance of Mg (%)	29.3 ± 8.5	22.5–36.0	6.2 ± 3.3[a,b]	3.6–8.8	6.1 ± 3.3[a,b]	3.5–8.7	15.3 ± 3.9[b]	12.2–18.4
24-hour fractional clearance of ionized Mg (%)	41.8 ± 12.8	31.6–52.0	8.7 ± 5.5[a,b]	4.3–13.1	8.8 ± 5.0[a,b]	4.8–12.8	19.5 ± 4.5[b]	15.9–23.1
Muscle Mg content (mg/kg of dry weight)	799 ± 165	667–931	769 ± 46	732–806	813 ± 71	756–87	753 ± 87	684–823
Intracellular Mg concentration (mg/kg)	ND	ND	60.3 ± 6.9	54.8–65.8	54.4 ± 2.7[c]	52.2–56.6	56.1 ± 3.7	53.1–59.1

Samples for measurement of variables were obtained on day 14 (baseline), day 37 (middle of feeding reduced-Mg diet), day 51 (end of feeding reduced-Mg diet), and day 75 (end of feeding Mg-supplemented diet), except for the muscle Mg content and intracellular Mg concentration, which were obtained on days 15, 38, 52, and 76.

[a]Value differs significantly ($P < 0.05$) from value after feeding Mg-supplemented diet for 4 weeks. [b]Value differs significantly ($P < 0.05$) from baseline value. [c]Value differs significantly ($P > 0.05$) from value after feeding Mg-supplemented diet for 2 weeks. ND = Not determined.

horse was diagnosed with psychogenic polydipsia, which skewed urine volume results.

Urine Mg concentration—Compared with baseline concentration, urine Mg concentration decreased significantly ($P = 0.002$) by day 37; there was no further decrease through day 51. There was a small increase in urine Mg concentration after the start of the Mg-supplemented diet, but this value was still significantly ($P = 0.007$) less than the baseline concentration.

Urinary Mg excretion—We did not detect diurnal variation in E_{Mg}, and there were no differences among the 4 E_{Mg} values calculated at 6-hour intervals (6, 12, 18, and 24 hours) on days 14, 37, 51, and 67. We did not detect a difference between the 24-hour E_{Mg} (calculated by use of the pooled 24-hour urine sample), compared with the total for the 4 consecutive 6-hour-interval E_{Mg} values (6, 12, 18, and 24 hours). On the basis of the 24-hour pooled urine sample, the 24-hour E_{Mg} at day 37 was significantly ($P < 0.001$) decreased from the baseline 24-hour E_{Mg}. We did not detect a further reduction in the 24-hour E_{Mg} at day 51 (end of phase 2). Restoration of renal 24-hour E_{Mg} was observed after feeding the Mg-supplemented diet, compared with the baseline 24-hour E_{Mg} **(Fig 1)**.

Urinary Mg clearance—We did not detect diurnal variation over time in C_{Mg}, and there were no detectable differences among the 4 mean 6-hour-interval (6, 12, 18, and 24 hours) C_{Mg} on days 14, 37, 51, and 67. This validated the use of 6-hour-interval C_{Mg} as an estimate of 24-hour C_{Mg}. There was no significant difference between C_{Mg} calculated on the basis of the pooled 24-hour urine sample, compared with the mean value for clearance calculated from pooled 6-hour-interval urine samples. The 24-hour C_{Mg} was significantly ($P < 0.001$) decreased by day 37, compared with the baseline value. We did not detect a further reduction at day 51. After feeding the Mg-supplemented diet, the 24-hour C_{Mg} increased from the value for the Mg-restriction phase, but it was still significantly ($P = 0.002$) less than the baseline 24-hour C_{Mg}.

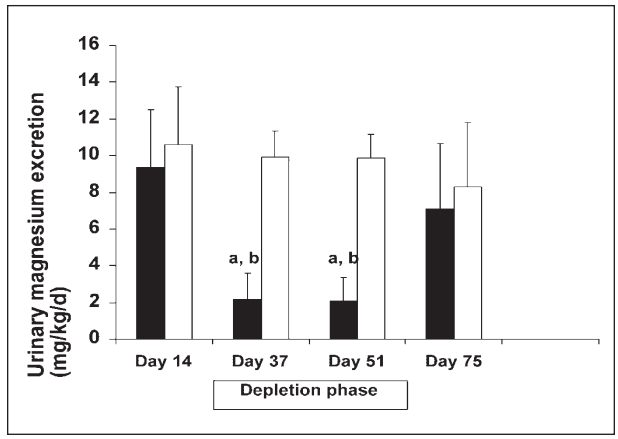

Figure 1—Mean ± SE urinary excretion of magnesium (E_{Mg}) at baseline (day 14), at the middle (day 37) and end (day 51) of feeding a reduced-magnesium (Mg) diet, and at the end of feeding an Mg-supplemented diet (day 75) to 6 horses (treatment group; black bars) and for 3 control horses fed a grass hay diet throughout the study (white bars). a—Within a group, value differs significantly ($P < 0.05$) from baseline value. b—Within a group, value differs significantly ($P < 0.05$) from value for the Mg-supplemented phase (ie, day 75).

Urinary fractional clearance of tMg—At baseline, we did not detect significant differences among FC_{tMg} calculated on the basis of results for aliquots of urine representative of a 24-hour period (24-hour FC_{tMg}), each 6-hour period (6-hour-interval FC_{tMg}), or spot urine samples that were collected every 6 hours (spot

Table 2—Mean ± SD and 95% CI values for urine indices measured after an Mg retention test in 6 female yearling horses fed grass hay, a reduced-Mg diet, and an Mg-supplemented diet

	Baseline		End of feeding reduced-Mg diet		End of feeding Mg-supplemented diet	
Variable	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**
24-hour urine excretion of Mg (mg/kg/d)	13.8 ± 3.5	11.0–16.6	8.8 ± 3.7	5.8–11.7	12.0 ± 6.9	6.5–17.5
24-hour urine clearance of Mg (ml/kg/min)	0.7 ± 0.2	0.5–0.9	0.3 ± 0.1[a]	0.2–0.4	0.42 ± 0.26[a]	0.2–0.6
24-hour fractional clearance of Mg (%)	61.2 ± 19.9	45.3–77.1	18.8 ± 7.1[a]	13.1–24.5	28.7 ± 12.2[a]	18.9–38.5
24-hour fractional clearance of ionized Mg (%)	90.7 ± 36.8	61.3–120.0	26.7 ± 9.7[a]	18.9–34.5	35.8 ± 19.2[a]	20.4–51.2
Mg retention (%)						
Method 1	–37.9 ± 34.6	–65.6–(–10.2)	12.1 ± 36.9	–17.4–41.6	–20.4 ± 69.5	–48.8–8.0
Method 2	28.0 ± 16.6	14.0–34.8	28.4 ± 24.5	8.8–48.0	33.3 ± 25.6	–12.8–53.8
Method 3	20.4 ± 30.7	7.9–32.9	51.0 ± 50.6	10.5–91.5	47.4 ± 45.9	10.7–84.1

Samples for measurement of variables were obtained on day 16 (baseline), day 53 (end of feeding reduced-Mg diet), and day 77 (end of feeding Mg-supplemented diet).
[a]Value differs significantly ($P < 0.05$) from baseline value.

FC_{tMg}). We did not detect diurnal variation in the 4 consecutive 6-hour-interval FC_{tMg} collected on days 14, 37, 51, and 75. There was no detectable difference between the final 6-hour-interval FC_{tMg} (from 18 to 24 hours), compared with the 24-hour FC_{tMg}, on days 14, 37, 51, and 75. The 24-hour FC_{tMg} was significantly ($P < 0.001$) decreased by day 37, compared with the baseline 24-hour FC_{tMg}. We did not detect a further reduction at day 51. After feeding the Mg-supplemented diet, the 24-hour FC_{tMg} had increased from the value for the Mg-restriction phase, but it was still significantly ($P = 0.002$) less than the baseline 24-hour FC_{tMg}. Similar results were seen for analysis of the 6-hour-interval FC_{tMg}.

Urinary fractional clearance of iMg—The 24-hour FC_{iMg} was decreased significantly ($P < 0.001$) by day 37, compared with the baseline value. We did not detect a further reduction at day 51. After feeding the Mg-supplemented diet (phase 3), the 24-hour FC_{iMg} increased from the value for phase 2, but it was still significantly ($P < 0.001$) less than the baseline 24-hour FC_{iMg}.

Mg concentration in muscle biopsy specimens—We did not detect changes in muscle Mg content in either group of horses during the study.

Concentration of icMg in sublingual cells—Baseline results for sublingual epithelial icMg were not available on day 14 because of poor cell viability that was attributed to time lapse until fixation and failure to scrape the sublingual epithelium with sufficient force. There was a significant ($P = 0.03$) decrease in icMg concentration in the treatment horses between the middle (day 38) and end (day 52) of phase 2.

Urinary Mg excretion after the IV Mg retention test—The 24-hour E_{Mg} after IV infusion of Mg solution was not significantly different between baseline, Mg-reduced, or Mg-supplemented phases (**Table 2**).

Urinary Mg clearance after the IV Mg retention test—The 24-hour C_{Mg} after the IV Mg retention test was significantly ($P = 0.008$) decreased after feeding the Mg-reduced diet for 4 weeks, compared with the baseline value. After feeding the Mg-supplemented diet, the 24-hour C_{Mg} increased from the value for the Mg-reduced phase, but it was still significantly ($P = 0.004$) less than the baseline 24-hour C_{Mg}.

Urinary fractional clearance after the IV Mg retention test—The 24-hour FC_{tMg} after the IV Mg retention test was significantly ($P < 0.001$) decreased after feeding the Mg-reduced diet for 4 weeks, compared with the baseline value. After feeding the Mg-supplemented diet, the 24-hour FC_{tMg} increased from the value for the Mg-reduced phase, but it was still significantly ($P = 0.005$) less than the baseline 24-hour FCt_{Mg}. The 24-hour FC_{iMg} after the IV Mg retention test was also significantly ($P = 0.006$) decreased after feeding the Mg-reduced diet for 4 weeks, compared with the baseline value. After feeding the Mg-supplemented diet, the 24-hour FC_{iMg} increased from the value for the Mg-reduced phase, but it was still significantly ($P = 0.008$) less than the baseline 24-hour FC_{iMg}.

Value for % Mg Ret after the IV Mg retention test—We did not detect significant changes throughout the study for % Mg Ret after the IV Mg retention test, regardless of the method used to calculate values.

Discussion

A diet containing Mg (600 mg/kg of feed) induced detectable urinary conservation of Mg by 13 days after onset of feeding in yearling horses, with a significant reduction of urine Mg concentration, E_{Mg}, C_{Mg}, FC_{tMg}, and FC_{iMg}. In the study reported here, serum tMg, serum iMg, and muscle Mg concentrations were not changed by the end of the Mg-restriction phase; however, a decrease in icMg concentration in sublingual epithelial cells was detectable. Our study validates the use of 6-hour-interval E_{Mg} as an estimate of 24-hour E_{Mg} and the use of spot sample FC_{tMg} to detect reduced urinary excretion of Mg. Spot sample collection provides a more convenient indicator of Mg status, compared with Mg concentrations for volumetric urine collections and calculation of 6- or 24-hour E_{Mg}. Calculation of % Mg Ret by methods 1 and 2 (adjusting for basal E_{Mg}) or 3 (adjusting for urine creatinine concentration) failed to

identify horses fed the low-Mg diet. In this study, diagnostic tests for the detection of reduced Mg intake in horses were validated. The E_{Mg} was considered the most sensitive indicator of Mg intake.

The diet used in this study was accepted well by the horses, and all horses gained weight and remained healthy during the study. Unfortunately, an error during preparation resulted in the low-Mg diet being unbalanced with regard to NRC recommendations.[33] The diet was moderately low in digestible energy, crude protein, phosphorus, and potassium and extremely low in calcium, with an inappropriate calcium-to-phosphorus ratio. Because the diet was not balanced, we cannot make conclusions of the effect of a low-Mg diet on serum, urine, or tissue concentrations of other electrolytes. Parathyroid hormone concentration is influenced by and subsequently affects calcium, phosphorus, and Mg homeostasis. The calcium and phosphorus concentrations in the diet were low; thus, parathyroid hormone may have influenced the Mg status, and the interrelationship of these components on Mg homeostasis could not be investigated. Therefore, this study was confined to describing the diagnostic tests for Mg status. Control horses were fed unsupplemented grass hay. Both batches of hay were extremely low in digestible energy, crude protein, and sodium and moderately low in phosphorus, and batch 2 was extremely low in calcium. Control horses were used as an environmental control without variations in any of the variables measured during the study.

The Mg concentration of our experimental diet was higher than that in other studies[16,34] of dietary-induced hypomagnesemia in horses, but as intended, we did not induce clinical signs of Mg depletion. Serum Mg concentration did not decrease in the horses subjected to this method of reduced Mg intake. A severely Mg-deficient (7 mg/kg) diet fed to foals during a period of 150 days resulted in a decrease in serum tMg concentrations.[34] That extreme dietary Mg deficiency induced severe clinical signs of muscular tremors, ataxia, collapse, and seizures in 2 of 11 foals, with death in 1 of the affected foals.[34] When fed a diet containing 370 mg of Mg/kg, serum tMg concentrations in Shetland ponies decreased after 18 days.[16] It is not known whether serum hypomagnesemia would have developed if our deficiency period had exceeded 28 days. Such a moderate dietary restriction of Mg may be compensated by a reduction in urinary and fecal excretion with no reduction in total body Mg content. Reduction in serum Mg content is only likely when Mg depletion is too severe to be successfully compensated by renal and intestinal mechanisms and release of Mg from skeletal stores.

Severe chronic Mg deficiency or acute Mg redistribution is required to alter serum Mg concentrations because homeostatic mechanisms maintain intracellular and serum concentrations within narrow limits. In humans, serum Mg concentrations can be within the reference range in the face of total body Mg depletion[35,36] identified by use of an IV Mg retention test[6,37]; evaluation of muscle biopsy specimens; and measurement of intracellular concentrations in RBCs,[6,31] leukocytes,[2,38] or sublingual epithelial cells.[39] Measurement of serum tMg concentrations is inexpensive and convenient but generally provides an insensitive indicator of total body Mg. Serum tMg and iMg concentrations were slightly increased in the study reported here after feeding an Mg-supplemented diet that contained Mg in excess of daily requirements.[20,21,33] Other studies[16,21] in which investigators fed excess dietary Mg also resulted in increases in serum tMg concentrations.

Baseline muscle Mg content of the yearling horses of our study was lower than that of pastured adult horses,[40] which could have been attributable to geographic differences in the mineral content of feed, growth status, or age. We did not detect any change in muscle Mg content with dietary manipulation, a finding supported by results of another study.[34]

Measurement of E_{Mg} was considered the most sensitive indicator of Mg status in our horses. In humans, E_{Mg} can decrease from 1.7 to 0.34 mg/kg/d in states of Mg deficiency.[41] In comparison, we found baseline E_{Mg} to be much higher in horses (9.8 mg/kg/d). Our results are comparable to those for a study[42] of dietary cation-anion balance in adult horses in which E_{Mg} varied between 5 and 9 mg/kg/d in horses fed diets of differing dietary cation-anion balance containing 2,200 to 2,400 mg of Mg/kg of feed. A study[43] of 11 geldings revealed a much higher mean E_{Mg} of 21 ± 8 mg/kg/d.

Volumetric urine collection is required for the determination of 24-hour E_{Mg}. Volumetric urine collection is labor-intensive and tedious. We compared successive 6-hour-interval E_{Mg} at each phase of dietary Mg manipulation and found no diurnal variation, thereby validating the use of 6-hour-interval E_{Mg} to estimate 24-hour E_{Mg}.

The typical dietary intake of Mg in humans is much less than the daily requirement in horses. Herbivorous diets generally provide excess Mg; therefore, horses are less likely to develop chronic dietary Mg deficiency and subsequent clinical signs of hypomagnesemia. We found that E_{Mg} was stable between days 37 and 51 of the study (corresponding to days 13 to 28 of exclusive feeding of the Mg-deficient diet). We believe that equilibrium between Mg intake, metabolic requirements, and excretion was achieved with minimal effects on total body Mg status. By extrapolating a linear equation of urinary Mg excretion versus Mg intake, other investigators[20,21] estimated the endogenous E_{Mg} to be 3.93 mg/kg/d when there was no Mg intake. This theory was disproved in another study[16] in which it was documented that for extremely low Mg intake, the aforementioned equation was no longer linear and E_{Mg} was reduced to 0. The kidneys and gastrointestinal tract attempt to maintain Mg homeostasis in the face of Mg depletion by reducing urinary and fecal excretion of Mg. Once E_{Mg} reaches a minimal amount, additional reductions in Mg intake cannot be compensated, resulting in decreases in serum and tissue Mg concentrations. With more severe restriction of Mg intake than was used in our study, we hypothesize that E_{Mg} would decrease to less than the observed 2.0 mg/kg/d.

Analysis of our results did not reveal differences among any of the 6-hour-interval C_{Mg} or E_{Mg} on each of 4 days of sample collection. Because of the lack of diurnal variation, 6-hour-interval C_{Mg} and E_{Mg} provide valid esti-

mates of 24-hour C_{Mg} and E_{Mg} in horses. The accuracy of spot sample FC, as a representation of 24-hour-interval clearance, is controversial.[26,42,44,45] Although there is considerable individual variation in FC_{Mg} among horses, we did not detect variation among mean 24-hour, 6-hour-interval, and spot sample FC_{Mg} during a 24-hour period. Therefore, the spot sample FC_{Mg} adequately reflected FC_{Mg} calculated for volumetric urine collection. Our spot samples were obtained via aspiration from the indwelling catheter that was maintaining bladder evacuation, rather than via catheterization of a full or partially full bladder in which there may be ventral settling of crystalline material. The use of single random-sample spot FC_{Mg} eliminates the necessity of volumetric urine collection, but we postulate that there may be differences in mineral concentrations from samples aspirated from the top of a full bladder, compared with concentrations in midstream samples obtained from the beginning, middle, or end of urination. Acidification of urine to dissolve urinary crystals is considered essential for complete Mg quantification.[44,45]

Although not described in the veterinary literature, FC_{iMg} has been advocated as a test of Mg excretion because only ionized or free serum Mg is filtered across the glomerulus, whereas the protein-bound serum Mg will not be filtered.[7] Use of serum iMg in the fractional clearance calculation allows investigators to calculate FC_{iMg}. In the study reported here, we have provided reference ranges for FC_{iMg} in clinically normal horses fed a typical diet or a reduced-Mg diet.

The Mg retention test is considered the most sensitive index of total body Mg stores in humans.[24,31,37] The dosage of infused Mg varies between 1.7 and 10 mg/kg.[18,24,31] We performed our Mg retention test by use of a dosage of 10 mg/kg without adverse effects 3 times in each of 9 horses. The Mg retention test does not appear to be a sensitive indicator of Mg status in horses, at least in our depletion model. It is not known whether the Mg retention test would be a more sensitive indicator of Mg status in horses subjected to a more severe method of Mg depletion. The typical diet of horses has a high Mg content that results in an endogenous E_{Mg} approximately 10 times greater than the E_{Mg} of humans.[16,41-43] In humans, the amount of Mg infused for the retention test is 1 to 30 times greater than the endogenous E_{Mg}.[18,24,31,41] The horses in the study reported here had an endogenous E_{Mg} similar to the amount of Mg infused for the retention test. Although the dose of Mg sulfate used in this study was equal to the maximum dose used in humans, a potentially higher dose may be required in horses. In humans, most of the excessive Mg is excreted via the urine,[31] whereas in horses, most of the excessive Mg is excreted in the feces.[16,42] However, we did not analyze Mg content of fecal material in our study. The Mg retention test can be safely performed in horses, but its ability to successfully indicate Mg depletion in horses requires further investigation.

[a]Summers AJ, Chew DJ, Buffington CT. Serum ionized magnesium and calcium concentrations in a population of sick dogs and cats (abstr), in *Proceedings*. Purina Nutrition Forum 1998;54.
[b]Eqvalan paste 1.87%, Merck & Co Inc, Rahway, NJ.
[c]Prestige with havlogen encephalomyelitis-rhinopneumonitis-influenza vaccine, Eastern and Western killed virus, Tetanus toxoid, Intervet Inc, Millsboro, Del.
[d]Ohio Agriculture Research Development Center, Wooster, Ohio.
[e]Holmes Laboratory Inc, Millersburg, Ohio.
[f]Water Deionizer, US Filter Service Deionization, Lowell, Mass.
[g]24-F 30-mL balloon, series 6000, Akron Catheter Inc, Arkon, Ohio.
[h]4.7-mm ID, 1.6-mm wall thickness, VMR Scientific, Columbus, Ohio.
[i]14- gauge, 5.25-inch PEP polymer, Angiocath, Becton-Dickinson & Co, Sandy, Utah.
[j]Xylazine HCL injection, Prolabs Ltd, St Joseph, Mo.
[k]Carbocaine (mepivacaine hydrochloride USP 2% sterile aqueous solution) Pharmacia & Upjohn Co, Kalamazoo, Mich.
[l]Cytology fixative, 2.5% Carbowax, 95% ethanol, Medical Packaging Corp, Camarillo, Calif.
[m]50% magnesium sulfate, Abbott Laboratories, North Chicago, Ill.
[n]Nova 8 calcium analyzer, Nova Biomedical, Waltham, Mass.
[o]Vitros TT60II chemistry system, Ortho-Clinical Diagnostics, Rochester, NY.
[p]Boehringer Mannheim/Hitachi 911 system, Boehringer Mannheim Corp, Indianapolis, Ind.
[q]MARS 5 microwave unit, CEM Corp, Matthews, NC.
[r]ES3000 ICP, Leeman Labs Inc, Hudson, NH.
[s]Exatest, Intracellular Diagnostics Inc, Foster City, Calif.
[t]Isotemp oven, Model 630F, Fisher Scientific, Pittsburgh, Pa.
[u]STAR Lab, Ohio Agriculture Research Development Center, Wooster, Ohio.

Appendix 1

Composition of the reduced-magnesium diet

Component	%
Fine wood shavings (cellulose)	31.3
Chopped corn	25.2
Sugar	10.1
Starch	10.1
Casein	7.9
Soybean fiber	8.1
Soybean oil	3.0
Mineral-and-vitamin supplement	2.1

Appendix 2

Results of analysis of the diets fed to the 2 groups of horses

Variable	Requirements* for growing horses[33]	Grass hay Batch 1	Grass hay Batch 2	Reduced-magnesium diet†‡
Dry matter (%)	NA	88.9	86.6	90.1
Crude protein (%)	12.6	5.6	6.8	9.9
ADF (%)	NA	41.9	40.6	38.1
NDF (%)	NA	64.2	61.6	44.1
Lignin (%)	NA	4.0	2.7	0.3
Estimated crude fat (%)	NA	2.4	2.4	3.5
Ash (%)	NA	7.9	8.9	1.7
DE (Mcal/kg)	2.8	1.7	1.8	2.3
Calcium (%)	0.43	0.50	0.37	0.11
Magnesium (%)	0.16	0.24	0.22	0.06
Sodium (%)	0.100	0.007	0.008	0.240
Potassium (%)	0.3	1.2	1.6	0.2
Phosphorus (%)	0.24	0.22	0.22	0.15

Values are reported on a dry-matter basis.
*Recommendations of the National Research Council. †Three samples of the reduced-magnesium diet were analyzed, and the means of the results are reported. ‡Horses in the treatment group were also fed a magnesium-supplemented diet, which was the same formulation as the reduced-magnesium diet but with the addition of magnesium oxide at a rate of 40 mg/kg/d.
NA = Not applicable. ADF = Acid detergent fiber. NDF = Neutral detergent fiber. DE = Digestible energy.

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