



Production, physiological response, and calcium and magnesium balance of lactating Holstein cows fed different sources of supplemental magnesium with or without ruminal buffer

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ABSTRACT

The objective of this study was to evaluate the effects of dietary replacement of magnesium oxide (MgO) with calcium-magnesium hydroxide [CaMg(OH)₂] and its interaction with ruminal buffer (sodium sesquicarbonate) supplementation on production, Ca and Mg balance, and overall physiological response of mid-lactation Holstein dairy cows. Sixty cows averaging 40.5 ± 7.0 kg of milk/d were used. Treatments were assigned following a 2 × 2 factorial arrangement: (1) MgO, (2) MgO + buffer, (3) CaMg(OH)₂, or (4) CaMg(OH)₂ + buffer. Diets were formulated to have 16.5% of crude protein, 1.82 Mcal/kg of net energy for lactation, 0.67% Ca, 0.39% P, and 0.25% Mg, all on a dry matter (DM) basis. Treatments were individually top dressed. Milk production, composition, and DM intake were evaluated. A subsample of 20 cows were randomly selected for the evaluation of Ca and Mg balance, blood gases, and electrolytes. Ruminal fluid was also collected for evaluation of pH and Ca and Mg solubility. Effects of Mg source, buffer, and the interaction Mg source × buffer were analyzed through orthogonal contrasts. An interaction of Mg source × buffer was found for DM intake and feed efficiency, in which cows fed CaMg(OH)₂ had a similar feed efficiency regardless of ruminal buffer inclusion; however, when cows were fed MgO, the inclusion of buffer reduced feed efficiency. No effects on body weight and milk yield were observed. Buffer

addition tended to increase the concentrations of fat, protein, and solids-not-fat, without affecting the yields of these milk components. Magnesium source and buffer did not affect ruminal fluid, blood, urine, or fecal pH; however, buffer supplementation increased urinary pH. Treatment with CaMg(OH)₂ increased blood concentration of HCO₃⁻, total CO₂, and base excess compared with cows fed MgO. No differences were observed in the ruminal solubility of Ca and Mg or on milk or urinary Ca and Mg excretion. Greater plasma Mg concentration was observed for animals fed MgO compared with cows fed CaMg(OH)₂; however, both sources were above the threshold recommended in the literature for dairy cows. Also, a reduction in fecal Mg excretion was observed in animals fed CaMg(OH)₂. In summary, we provide evidence that CaMg(OH)₂ could replace MgO without affecting performance, overall physiological response, or Ca and Mg balance of mid-lactating dairy Holstein cows.

Key words: acid-base balance, magnesium excretion, magnesium hydroxide, magnesium oxide

INTRODUCTION

Magnesium is an essential mineral for dairy cow metabolism as it is an enzymatic cofactor and vital for bone formation (Rude et al., 1999), muscle function (Gordon et al., 2000), and nerve function (Katz and Miledi, 1967). According to Storry and Rook (1963), the majority of Mg in the body of dairy cows is present in bone, which accounts for 60 to 70%. However, in times of deficit, bone is not a significant source of Mg that can be utilized; thus, dietary supplementation of Mg, which is ruminally absorbed, is required to maintain the normal plasma concentration of Mg (NASEM, 2021).

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Supplementation of dietary Mg can increase plasma Mg concentration of dairy cows; however, the chemical and physical characteristics of the mineral's source could affect its bioavailability (Thomas et al., 1984). For instance, the chemical structure of magnesium oxide (MgO) and carbonate sources can have different solubility (Arce-Cordero et al., 2021), possibly affecting mineral balance and animal performance (Bach et al., 2018). Also, physical characteristics, such as particle size, affect the reactivity of the mineral and its status in the body (Thomas et al., 1984).

The most used source of Mg in dairy cow nutrition is MgO, which has alkalizing properties that may help with ruminal pH control (Goff, 2018; NASEM, 2021). However, dairy operations may benefit from alternative sources of Mg with alkalizing potential, such as calcium-magnesium carbonate [$\text{CaMg}(\text{CO}_3)_2$] and calcium-magnesium hydroxide [$\text{CaMg}(\text{OH})_2$] (Rauch et al., 2012; Bach et al., 2018; Arce-Cordero et al., 2020, 2021). According to Arce-Cordero et al. (2021), a blend of $\text{CaMg}(\text{CO}_3)_2$ and $\text{CaMg}(\text{OH})_2$ had 220% of the reactivity of MgO in a acetic acid reactivity test. The carbonate Mg source is an alternative to replace the oxide source; however, Arce-Cordero et al. (2020) reported that the carbonate source of Mg was less efficient for controlling ruminal pH than the oxide and hydroxide sources. A few studies have evaluated the effects of $\text{Mg}(\text{OH})_2$ on performance and physiological parameters of dairy cows; however, to the best of our knowledge, this is the first time that $\text{CaMg}(\text{OH})_2$ has been evaluated *in vivo*.

Ruminal buffers, such as sodium bicarbonate and sodium sesquicarbonate, are often used as strategies to promote pH control and improve milk fat synthesis (Solorzano et al., 1989; Rauch et al., 2012; Iwaniuk and Erdman, 2015). However, it is still not well established if the combined supplementation of ruminal buffer and Mg sources with alkalizing potential would positively affect milk production and the overall physiological response of Holstein dairy cows (Erdman et al., 1980, 1982). Also, there is a knowledge gap regarding potential interactions between Mg sources and buffer supplementation. Therefore, our experiment aimed to investigate the effects of the interaction of 2 Mg sources, MgO and $\text{CaMg}(\text{OH})_2$, with buffer supplementation on the production, overall physiological response, and Ca and Mg balance of mid-lactation Holstein dairy cows. We hypothesized that MgO could be safely replaced by $\text{CaMg}(\text{OH})_2$ without major effects on the production, physiological response, or Ca and Mg balance. We also hypothesized that buffer supplementation would have a similar effect on the physiological response of the dairy cow regardless of Mg source.

MATERIALS AND METHODS

The project was approved by the Institutional Animal Use and Care Committee at the University of Florida. The experiment was conducted at the University of Florida Dairy Unit, Alachua, Florida, between August and October 2020.

Cows, Diet, and Experimental Design

All cows used in this trial were trained to use the Calan gate system (Calan Broadbent feeding system, American Calan Inc.) before the start of the trial, which allowed the measurement of individual feed intake. Animals were maintained in the same freestall barn with free access to sand beds and drinking water. Fans and misters were placed in the barn to improve heat abatement. Treatments were offered individually as a top-dress, where the treatment was split into 2 and placed directly on top of the feed in the feedbunk and manually homogenized to the top one-third of the feed at each feeding time. Animals were fed the experimental diets for 60 d, with the first 20 d used as an adaptation to the diet and the following 40 d for sample and data collection. Before the morning feeding, individual feed bunks were emptied, and the weight of the orts was recorded. The DMI, milk production, and milk composition of the last 5 d of the adaptation period were used to measure baseline levels for the true covariate of these parameters. Similarly, for physiological response parameters and Ca and Mg balance, d 19 and 20 of the adaptation were used as a true covariate. The collection period of 40 d was split into 4 periods of 10 d, in which ruminal fluid, feces, feed, and blood samples were collected and analyzed as repeated measures in the statistical model. For DMI, milk production, and composition, a weekly average was produced and analyzed as repeated measures as well.

For this experiment, a group of 60 high-producing, lactating Holstein dairy cows were assigned to blocks based on parity and DIM. A total of 15 blocks of 4 animals were established. Each animal, within a block, was randomly assigned to 1 of the 4 experimental treatments. Dry matter intake, milk yield, and milk composition were measured from all 60 animals (15 blocks). A subsample of cows was randomly selected ($n = 20$ cows; 5 from each treatment) for the analysis of the physiological parameters and Ca and Mg balance. Overall, the cows used for the experiment consisted of 32 multiparous and 28 primiparous cows with an initial average of 100 ± 26 DIM, BW of 630 ± 68 kg, and milk production of 40.5 ± 7.3 kg/d.

Diets were formulated to be isonitrogenous and isocaloric, and to have the same concentration of macro- and

Table 1. Ingredient and chemical composition of experimental diets fed to mid-lactation dairy cows (n = 60) fed either MgO or CaMg(OH)₂ with or without buffer

Item	Treatment ¹			
	MgO	MgO ⁺	CaMgOH	CaMgOH ⁺
Ingredient, % of DM				
Corn silage ²	44.4	44.4	44.4	44.4
Ground corn grain	21.0	21.0	21.0	21.0
Soybean meal 48% CP	16.0	16.0	16.0	16.0
Citrus pulp	8.00	8.00	8.00	8.00
Cottonseed	7.00	7.00	7.00	7.00
Mineral premix (no Ca or Mg source added) ³	3.60	3.60	3.60	3.60
CaCO ₃	0.74	0.74	0.51	0.51
Magnesium oxide	0.14	0.14	—	—
CaMgOH	—	—	0.31	0.31
Sodium sesquicarbonate	—	0.60	—	0.60
Chemical composition, % of DM				
CP	16.3	16.3	16.3	16.3
NDF	26.8	26.8	26.8	26.8
Starch	31.8	31.8	31.8	31.8
NE _L , Mcal/kg DM	1.73	1.73	1.73	1.73
S	0.07	0.07	0.07	0.07
Ca	0.70	0.70	0.70	0.70
P	0.46	0.46	0.46	0.46
Mg	0.27	0.27	0.27	0.27
Na	0.16	0.29	0.16	0.29
Cl	0.37	0.37	0.37	0.37
K	1.19	1.19	1.19	1.19
DCAD, mEq/100 g	22.6	28.3	22.6	28.3

¹MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

²Composition on a DM basis: CP = 7.95%; ether extract = 3.48%; NDF = 40.0%; ADF = 23.8%; starch = 33.9%; NE_L = 1.65 Mcal/kg; Ca = 0.22%; P = 0.23%; Na = 0.03%; Mg = 0.16%; K = 1.06%; Cl = 0.29% Na = 0.03%.

³Mineral composition: DM = 89.33%; CP = 24.63%; ether extract = 3.08%; NDF = 11.13%; ADF = 4.65%; starch = 23.51%; NE_L = 1.51 Mcal/kg; Ca = 2.67%; P = 3.45%; Na = 3.56%; Mg = 0.26%; K = 1.17%; Cl = 5.33% Na = 8.89%; S = 0.50%; Co = 21.270 mg/kg; Cu = 207.10 mg/kg; I = 11.64 mg/kg; Fe = 1,566.71 mg/kg; Mn = 1,119.46 mg/kg; Se = 6.91 mg/kg; Zn = 1,362.38 mg/kg; biotin = 2.398 mg/kg; vitamin A = 176.49 × 1,000 IU/kg; vitamin D3 = 58.87 × 1,000 IU/kg; vitamin E = 506.02 IU/kg; metabolizable lysine = 1.28%; metabolizable methionine = 0.37%; monensin = 391.81 g/ton.

microminerals, following the recommendations of NRC (2001) for mid-lactation cows with similar BW and milk production of the group used. The basal diet was composed of 44% forage and 56% concentrate (Table 1) and fed twice daily as a TMR in individual bunks. The daily diet offered was adjusted to allow at least 10% orts. The treatments were arranged in a 2 × 2 factorial design, in which the first factor was the source of Mg and the second factor was the supplementation of ruminal buffer (Na sesquicarbonate). The arrangement of treatments was (1) MgO, (2) MgO + Na sesquicarbonate, (3) CaMg(OH)₂, and (4) CaMg(OH)₂ + Na sesquicarbonate (Table 1). Chemical characterization of the 2 Mg sources was determined; MgO had 2.4% Ca and 47.8% Mg, and CaMg(OH)₂ had 35.4% Ca and 21.3% Mg. The physical characterization of relative particle size of Mg sources was determined using a W. S. Tyler shaker (RX-812) and sieves of 1.7, 1.2, 0.6, and 0.3 mm (Hogentogler & Co. Inc.). The analysis was carried out in triplicate and the shaker was set to a 10-min cycle.

The relative separation of the particle size is presented in Figure 1; the median particle size of MgO was 300 μm, and >99% of the particles of CaMg(OH)₂ were able to pass the 300-μm sieve.

Data and Sample Collection

Cows were milked twice daily (1100 and 2300 h) and milk production was recorded individually for each milking time using electronic flow meters (AfiMilk, Afikim Ltd.). A representative sample of milk was collected from each cow by a sample collector attached to the milking system. The collector retained a small portion of the milk produced for the cow throughout the milking process. Collection was carried out twice a week at both milking times throughout the experiment, and a portion of the milk collected was transferred to a tube containing 2-bromo-2-nitropropane-1,3-diol. Samples were sent to Southeast Milk Inc. (Bellevue, FL) for SCC and analysis of MUN, fat, true protein,

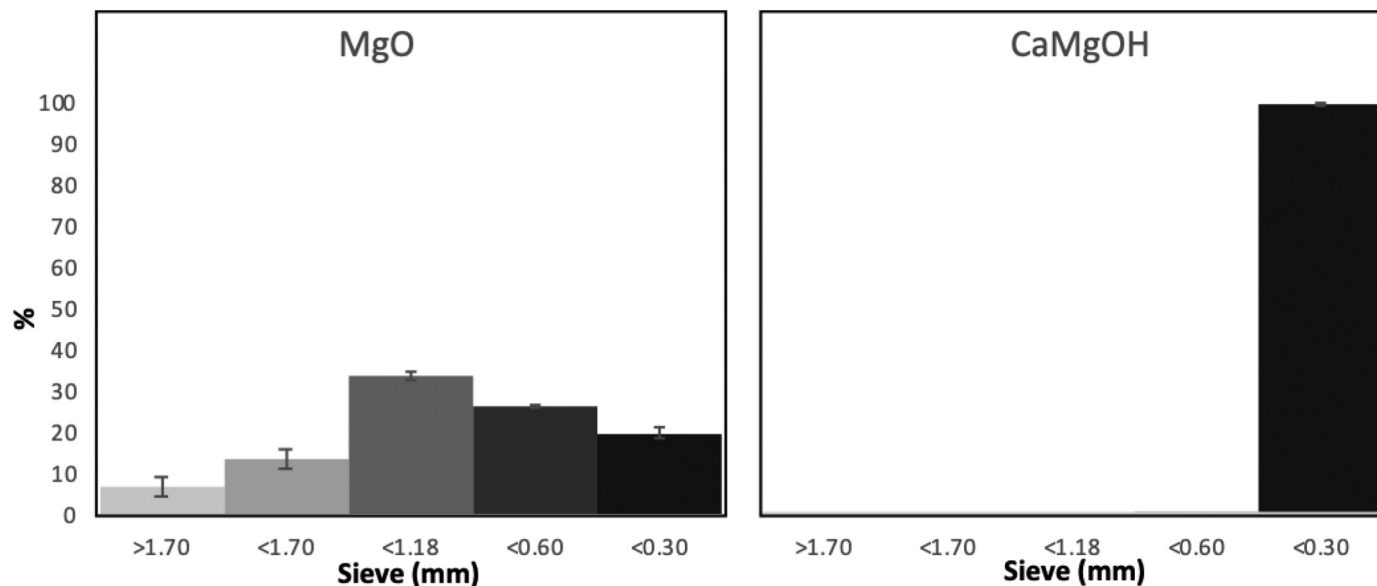


Figure 1. Relative particle retention of MgO and CaMg(OH)₂. The bars represent the relative retention of the mineral source, and the error bars represent the standard deviation.

lactose, and SNF by infrared spectroscopy. At the same time, in a separate 2-mL microcentrifuge tube, a milk sample from each of the 20 selected cows was collected and stored at -20°C for Ca and Mg analysis.

Collection of feces and urine from the 20 selected cows was carried out by spot urine and fecal sampling at 0400, 1200, and 2000 h on d 19, 28, 38, 48, and 58, and at 0800, 1600, and 2400 h on d 20, 29, 39, 49, and 59 of the experiment. Mid-stream urine was collected by massage of the perineal area, and the pH of urine was measured within 1 h of the collection using a portable pH meter (Orion Star A121, Thermo Fisher Scientific Inc.). A daily composited sample of concentrate urine was collected for each cow and stored in a 50-mL tube at -20°C for Ca and Mg analysis. In another 50-mL tube, containing 40 mL of 1 N H₂SO₄, a subsample of urine from each daily timepoint was transferred to the tube with acid and stored at -20°C for creatinine analysis.

Feces were collected directly from the rectum of the animals at the same timepoints as the urine collections described previously. Within 1 h of collection, the material was homogenized and a subsample of 150 g was collected and combined into a 2-d pool (d 18/19; 28/29; 38/39; 48/49; and 58/59) and then stored at -20°C . Samples of TMR and orts were collected for 4 d to better estimate Ca and Mg intake: the 2 d before the feces/urine collection and the 2 d of feces/urine collection (d 16 to 19, 26 to 29, 36 to 39, and 56 to 59). Immediately after collection, samples were weighed and placed in an oven for 72 h at 60°C and stored in paper bags.

Ruminal fluid from the selected cows was collected 4 h after the morning feeding using an orogastric tube and a vacuum pump, on d 20, 27, 37, 47, and 57 of the experiment. The first 100 mL of ruminal fluid was discarded to avoid saliva contamination. From the second amount of ruminal fluid collected, a sample was filtered through 4 layers of cheesecloth, and pH was measured. An aliquot of 10 mL was collected and stored at -20°C for Ca and Mg analysis.

Blood was collected, also from the 20 selected cows, 4 h after morning feeding on d 20, 26, 36, 46, and 56 of the experiment, from the caudal vein in BD Vacutainer tubes with lithium heparin; blood was centrifuged at $2,500 \times g$ for 15 min at 21°C . Plasma was transferred to microcentrifuge tubes and stored at -20°C for measurement of Ca and Mg concentration. Similarly, jugular venous blood was collected into 10-mL BD Vacutainer tubes with lithium heparin. Samples of whole blood were analyzed within 2 min of collection for pH, the concentration of HCO₃⁻, base excess, partial pressure of CO₂ (pCO₂), total dissolved CO₂ (tCO₂), partial pressure of O₂, O₂ saturation, concentrations of Na, K, and ionized Ca, hemoglobin, hematocrit, and concentration of glucose using a handheld biochemical analyzer (VetScan i-STAT, Abaxis).

Chemical Analyses

The concentrations of Ca and Mg were determined in milk, urine, and serum samples. Samples were thawed at room temperature and composited across days.

These analyses were performed at SDK Laboratories (Hutchinson, KS) by atomic absorption spectroscopy (iCE 3000 series, Thermo Scientific), according to official method 956.01 (AOAC International, 2000). Daily urinary excretion was estimated based on the creatinine concentration in the urine, according to Chizzotti et al. (2008), considering a daily excretion of 0.212 mmol of creatinine per kg of BW. Creatinine was analyzed using DetectX Urinary Creatinine Detection Kit (K002-H5, Arbor Assay), and the intra- and interassay coefficients of variation (CV) were 2.8 and 2.3%, respectively.

Analysis of Ca and Mg in the ruminal fluid was carried out according to Jittakhot et al. (2004b). Briefly, ruminal samples were centrifuged at 18°C at $2,700 \times g$ for 15 min; then the supernatant was harvested and transferred to another tube and centrifuged at $30,000 \times g$ and 20°C for 30 min in an ultra-speed centrifuge (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments). The supernatant was harvested and stored at -20°C. The concentration of soluble Ca and Mg was determined using the same procedure previously described.

Feces were thawed at 4°C and a sample of 25 g was mixed with 25 mL of H₂O, vortexed for 10 s, and filtered through 4 layers of cheesecloth before pH measurement. Thawed feces were then placed in a forced-air oven for 72 h at 60°C for drying. Then, dried feed, orts, and feces were ground in a Wiley mill (model no. 2; Arthur H. Thomas Co.) to pass through a 2-mm screen. Each ingredient/feces sample was homogenized, and one subsample was taken and ground to pass through a 1-mm screen to determine the chemical composition. Concentrations of Ca and Mg were determined by atomic absorption spectrometry, as previously described.

Indigestible NDF (iNDF) was used as an internal marker for fecal output (Lee and Hristov, 2013). Briefly, 2 sets of 1-g samples ground at 1 mm were weighed and placed in an F57 Ankom bag (Ankom Technology). Bags were heat-sealed, and 1 bag from each sample was placed in the rumen of 2 cannulated lactating dairy cows; bags were incubated for 240 h. After incubation, the bags were washed until recovery of clear water and dried at 60°C for 72 h. Analysis of NDF was carried out following the procedure described by Mertens (2002) with the addition of thermostable α -amylase and sodium sulfite in an Ankom200 Fiber Analyzer (Ankom Technology).

The digestibility of DM was calculated using the following equation:

$$\text{DM digestibility (\%)} = 100 - \left[100 \times \left(\frac{\% \text{ iNDF}_{\text{intake}}}{\% \text{ iNDF}_{\text{feces}}} \right) \right].$$

Fecal output on a DM basis was calculated using the following equation:

$$\text{Fecal output (kg of DM)} = \frac{\text{DMI} \times (100 - \text{DM digestibility})}{100}.$$

Statistical Analysis

The experiment consisted of a randomized complete block design in which cow within block was considered the experimental unit. Cows were blocked according to parity (primiparous and multiparous) and DIM of the current lactation. Then, cows were randomly assigned within their respective blocks to 1 of the 4 treatments. Therefore, 7 blocks each containing 4 primiparous cows and 8 blocks each containing 4 multiparous cows were used in the experiment.

The normality of residuals and homogeneity of variance were examined for each continuous dependent variable using the Shapiro-Wilk test. Means for DMI, milk yield and composition, pH, blood gas, electrolytes, and acid-base were used as repeated measures in a model. The most appropriate covariance structure was selected for each model based on the smallest corrected Akaike's information criterion. Statistical analyses were performed using the MIXED procedure of SAS 9.4 (SAS Institute Inc.) using the model

$$Y_{ijklm} = \mu + C_i + T_j + P_k + T_j \times P_k + B_m + C(B)_{lm} + e_{ijklm},$$

where Y_{ijklm} is the observation $ijklm$; μ is the overall mean; C_i is the true covariate; T_j is the fixed effect of treatment ($j = 1$ to 4); P_k is the fixed effect of period ($k = 1$ to 4); $T_j \times P_k$ is the interaction between T_j and P_k ; B_m is the random effect of block ($m = 1$ to 15); $C(B)_{lm}$ is the random effect of cow ($l = 1$ to 4) nested within block ($m = 1$ to 15); and e_{ijklm} is the random residual. The concentrations of Ca and Mg were analyzed similarly except that the interaction between treatment \times experimental period was not included in the model as samples were pooled across experimental periods. The model was

$$Y_{ijlm} = \mu + C_i + T_j + B_m + C(B)_{lm} + e_{ijlm},$$

where Y_{ijlm} represents the observation $ijlm$; μ represents the overall mean; C_i is the true covariate; T_j is the fixed effect of treatment ($j = 1$ to 4); B_m is the random effect of block ($m = 1$ to 15); $C(B)_{lm}$ is the random effect of cow ($l = 1$ to 4) nested within block ($m = 1$ to 15); and e_{ijlm} is the random residual. The main effects (Mg

Table 2. Effects of Mg source and buffer on DMI and production parameters in mid-lactation dairy cows (n = 60) fed either MgO or CaMg(OH)₂ with or without buffer

Variable	Treatment ¹				SEM	P-value ²		
	MgO	MgO ⁺	CaMgOH	CaMgOH ⁺		Source (S)	Buffer (B)	S × B
BW gain, kg	20.7	34.4	19.4	19.0	5.44	0.12	0.22	0.19
DMI, kg/d	22.0	23.1	23.0	21.2	0.53	0.39	0.51	0.01
Milk yield, kg/d	39.8	38.2	38.5	36.9	1.13	0.22	0.14	1.00
ECM, kg/d	35.9	36.1	34.6	34.3	1.06	0.15	0.94	0.83
FE, ³ kg of milk/kg of DMI	1.84 ^a	1.64 ^b	1.71 ^{ab}	1.74 ^{ab}	0.05	0.79	0.10	0.02
FE, ECM/kg DMI	1.65	1.55	1.54	1.62	0.04	0.57	0.77	0.04

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$) using the Tukey test.

¹MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

²Values from orthogonal contrast are significantly different if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

³FE = feed efficiency.

source and ruminal buffer) and their interaction were also analyzed through orthogonal contrasts in SAS.

RESULTS

Production Variables

Production variables, such as feed intake, milk yield, and milk composition are presented in Tables 2 and 3. Body weight gain, milk yield, and ECM were not affected by Mg source, ruminal buffer addition, or their interaction. There was an interaction between Mg source and ruminal buffer for DMI ($P = 0.01$) and feed efficiency using total milk production ($P = 0.02$) and ECM ($P = 0.04$). Cows fed CaMg(OH)₂ had the same feed efficiency (kg of milk/kg of DMI) regardless of ruminal buffer use; however, when cows were fed MgO, the inclusion of buffer reduced feed efficiency.

Lactose concentration and yield were not affected by mineral source, ruminal buffer, or their interaction. Somatic cell score ($P = 0.06$), MUN ($P = 0.06$), and protein yield ($P = 0.09$) tended to be reduced for cows fed CaMg(OH)₂ compared with cows fed MgO. Also, buffer addition tended to increase the concentration of fat ($P = 0.07$), protein ($P = 0.06$), and SNF ($P = 0.10$), without affecting the yields of these milk components.

Physiological Effects

The pH-related variables are presented in Table 4. The pH of body fluids was not affected by mineral source or buffer addition, and no interaction between these 2 factors was observed. Ruminal pH was not affected by source or buffer addition, but there was a trend ($P = 0.07$) for the interaction. Interaction or

Table 3. Effects of Mg source and buffer on milk yield and composition in mid-lactation dairy cows (n = 60) fed either MgO or CaMg(OH)₂ with or without buffer

Variable	Treatment ¹				SEM	P-value ²		
	MgO	MgO ⁺	CaMgOH	CaMgOH ⁺		Source (S)	Buffer (B)	S × B
SCS ³	4.02	3.90	3.52	3.50	0.24	0.06	0.77	0.84
MUN, mg/dL	12.0	12.4	11.5	11.5	0.38	0.06	0.71	0.55
Fat, %	2.97	3.19	2.93	3.16	0.13	0.78	0.07	0.98
Fat, kg/d	1.14	1.18	1.09	1.12	0.05	0.19	0.19	0.96
Lactose, %	5.00	5.00	4.97	4.98	0.02	0.22	0.83	0.80
Lactose, kg/d	1.93	1.86	1.86	1.79	0.06	0.19	0.19	0.96
Protein, %	3.14	3.24	3.14	3.18	0.04	0.42	0.06	0.40
Protein, kg/d	1.21	1.18	1.16	1.13	0.03	0.09	0.31	0.88
SNF, %	9.09	9.18	9.05	9.10	0.04	0.13	0.10	0.59
SNF, kg/d	3.51	3.40	3.38	3.26	0.10	0.14	0.20	0.94

¹MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

²Values from orthogonal contrast are significantly different if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

³SCS = $\text{Log}_2(\text{SCC}/100) + 3$.

Table 4. Effects of Mg source and buffer on ruminal, blood, urinary, and fecal pH in mid-lactation dairy cows (n = 20) fed either MgO or CaMg(OH)₂ with or without buffer

Variable	Treatment ¹				SEM	P-value ²		
	MgO	MgO+	CaMgOH	CaMgOH ⁺		Source (S)	Buffer (B)	S × B
Ruminal fluid pH	6.43	6.26	6.28	6.48	0.10	0.80	0.91	0.07
Blood pH	7.49	7.47	7.49	7.49	0.01	0.35	0.52	0.69
Urine pH	7.99	8.17	8.06	8.19	0.03	0.18	<0.01	0.36
Feces pH	6.56	6.50	6.47	6.46	0.07	0.36	0.61	0.70

¹MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

²Values from orthogonal contrast are significantly different if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

effects of mineral source were not observed for urinary pH; nevertheless, we observed an increase in the urinary pH for animals supplemented with buffer ($P < 0.01$).

Blood gases, electrolytes, and acid-base balance variables are presented in Table 5. No effects due to buffer addition or interaction of mineral and buffer were observed in the analyzed blood gas parameters. Blood oxygenation (partial pressure of O₂ and O₂ saturation) was not affected by the mineral source. Compared with MgO, cows fed CaMg(OH)₂ had increased ($P < 0.01$) concentrations of pCO₂ (33.9 to 35.8 mmHg), HCO₃⁻ (25.3 to 27.4 mmol/L), tCO₂ (26.3 to 28.35 mmol/L), and base excess (1.71 to 4.04 mmol/L).

In contrast, blood Na concentration tended to decrease ($P = 0.06$) in cows fed CaMg(OH)₂ compared with those fed MgO (137.9 vs. 138.7 mmol/L, respectively); no other electrolyte, glucose, hematocrit proportion, or hemoglobin concentration differed across treatments.

Ca and Mg Balance

Balances of Ca and Mg are presented in Table 6. Interactions were observed for Ca ($P = 0.05$) and Mg ($P = 0.06$) intake. The concentration of soluble Ca and Mg in the ruminal fluid and Ca concentration in plasma were not affected by Mg source, ruminal buffer, or their interaction; nevertheless, plasma Mg concentration was greater ($P = 0.03$) in cows fed MgO source. Excretion of Ca and Mg in milk and urine is reported in Table 6. No differences were observed for Ca and Mg excretion in milk, except for an interaction ($P = 0.02$) between source and buffer use in the relative excretion of Ca. Similarly, no effects were observed for Ca and Mg excretion in urine, except for an increase in urine yield ($P = 0.01$) and a trend to reduce Ca concentration ($P = 0.06$) in urine of cows supplemented with buffer.

The fecal excretion of Ca and Mg and manure waste (the sum of feces and urine) of these nutrients are also

Table 5. Effects of source of Mg and buffer on blood gases, electrolytes, and acid-base measures in mid-lactation dairy cows (n = 20) fed either MgO or CaMg(OH)₂ with or without buffer

Variable ¹	Treatment ²				SEM	P-value ³		
	MgO	MgO ⁺	CaMgOH	CaMgOH ⁺		Source (S)	Buffer (B)	S × B
Blood gases								
pCO ₂ , mmHg	34.0	33.7	36.2	35.4	0.71	<0.01	0.43	0.75
pO ₂ , mmHg	33.6	37.5	35.5	40.7	2.87	0.37	0.12	0.81
Base excess, mmol/L	2.32	1.11	4.47	3.60	0.80	<0.01	0.14	0.82
HCO ₃ , mmol/L	25.7	24.8	27.7	27.0	0.67	<0.01	0.15	0.85
tCO ₂ , mmol/L	26.8	25.7	28.7	28.0	0.70	<0.01	0.13	0.72
sO ₂ , %	69.2	73.7	71.6	71.9	2.10	0.86	0.25	0.33
Chemistry and electrolytes								
Na, mmol/L	138.8	138.6	137.9	137.9	0.39	0.06	0.78	0.85
K, mmol/L	3.73	3.73	3.78	3.86	0.08	0.22	0.53	0.60
iCa, mmol/L	1.22	1.25	1.22	1.23	0.02	0.81	0.31	0.54
Glucose, mg/dL	63.8	64.3	65.6	68.5	1.75	0.11	0.36	0.50
Hematology								
Hematocrit, %	26.2	26.5	25.5	25.5	0.61	0.16	0.78	0.79
Hemoglobin, g/dL	8.91	9.02	8.65	8.67	0.21	0.16	0.77	0.83

¹pCO₂ = partial pressure of CO₂; pO₂ = partial pressure of O₂; HCO₃ = concentration of HCO₃⁻; tCO₂ = total dissolved CO₂; sO₂ = oxygen saturation; iCa = ionized Ca.

²MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

³Values from orthogonal contrast are significantly different if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

Table 6. Effects of Mg source and buffer on the balance of Ca and Mg in mid-lactation dairy cows ($n = 20$) fed either MgO or CaMg(OH)₂ with or without buffer

Variable	Treatment ¹				SEM	P-value ²		
	MgO	MgO ⁺	CaMgOH	CaMgOH ⁺		Source (S)	Buffer (B)	S × B
Intake								
Ca, g/d	171	182	186	166	7.3	0.91	0.54	0.05
Mg, g/d	62.5	66.4	67.8	60.6	2.6	0.93	0.55	0.06
Rumen fluid								
Ca, mmol/L	1.66	1.96	1.69	2.10	5.70	0.69	0.11	0.76
Mg, mmol/L	2.43	2.62	2.77	2.12	0.49	0.86	0.63	0.45
Plasma								
Ca, mmol/L	2.39	2.39	2.37	2.38	0.07	0.81	0.91	0.97
Mg, mmol/L	1.18	1.15	1.12	1.10	0.03	0.03	0.30	0.82
Milk								
Ca, mmol/L	18.1	18.0	16.3	17.2	0.87	0.17	0.69	0.52
Ca, g/d	28.4	25.4	23.8	25.0	1.78	0.17	0.63	0.27
Ca, % of Ca intake	16.8	14.0	13.2	15.2	0.97	0.24	0.69	0.02
Mg, mmol/L	3.54	3.67	3.90	3.82	0.23	0.30	0.92	0.64
Mg, g/d	3.39	3.25	3.41	3.28	0.25	0.92	0.58	1.00
Mg, % of Mg intake	5.3	4.9	5.2	5.5	0.30	0.38	0.89	0.22
Urine								
Yield, L/d	22.4	35.3	24.1	30.5	3.35	0.61	0.01	0.30
Ca, mmol/L	0.63	0.15	0.29	0.20	0.13	0.30	0.06	0.17
Ca, mg/d	516	167	276	239	127	0.52	0.17	0.26
Ca, % of Ca intake	0.29	0.10	0.14	0.14	0.1	0.42	0.23	0.21
Mg, mmol/L	17.3	14.9	15.0	13.7	1.84	0.34	0.37	0.75
Mg, g/d	9.53	9.35	9.01	9.89	0.85	1.00	0.71	0.51
Mg, % of Mg intake	15.0	14.9	13.7	16.1	1.5	0.96	0.51	0.41
Feces								
Output, kg of DM/d	6.5	6.6	7.2	6.1	0.42	0.85	0.29	0.16
Ca, g/d	104	107	115	98	8.3	0.89	0.40	0.22
Ca, % of Ca intake	61.5	59.5	63.2	55.9	3.5	0.76	0.17	0.37
Mg, g/d	38.9	38.3	37.2	30.2	2.6	0.08	0.16	0.23
Mg, % of Mg intake	61.7	59.3	55.4	48.2	4.8	<0.01	0.07	0.30
Manure ³								
Ca manure, g/d	104	107	116	98	8.4	0.90	0.40	0.24
Ca manure, % of Ca intake	61.7	59.6	63.3	56.1	3.5	0.75	0.17	0.40
Mg manure, g/d	48.3	47.8	46.2	40.1	2.9	0.08	0.27	0.31
Mg manure, % of Mg intake	77.8	73.1	69.9	63.3	3.4	0.01	0.10	0.73

¹MgO = magnesium oxide; MgO⁺ = magnesium oxide and sodium sesquicarbonate; CaMgOH = calcium magnesium hydroxide; CaMgOH⁺ = calcium magnesium hydroxide and sodium sesquicarbonate.

²Values from orthogonal contrasts are significantly different if $P \leq 0.05$ and tendency if $0.05 < P \leq 0.10$.

³Manure is the sum of urine and fecal excretion of a given nutrient.

reported in Table 6. No effects on fecal excretion or total waste were observed for Ca; however, CaMg(OH)₂ tended to reduce the total (g/d) fecal excretion of Mg ($P = 0.08$) and Mg waste ($P = 0.08$). Also, a reduction of the Mg excretion in feces ($P < 0.01$) and manure ($P = 0.01$) relative to intake was observed in cows fed CaMg(OH)₂ compared with MgO.

DISCUSSION

Production Variables

In dairy cow diets, DMI is highly affected by dietary changes and the addition of alkalinizing products (Bach et al., 2018). For instance, compared with control diets (no supplementation of Mg or ruminal buffer), the addition of Mg sources with alkalinizing potential

has shown divergent results in the literature, showing that cattle fed Mg sources have greater (Bach et al., 2018), similar (Erdman et al., 1982; Rauch et al., 2012; Colombo et al., 2022), or lower (Thomas et al., 1984) DMI. These highly variable results could be related to the intrinsic characteristics of the different sources of Mg (Beede, 2017) or the acidotic ruminal environment (Schaefer et al., 1982).

Some studies have also evaluated whether a similar effect on DMI is observed for animals fed ruminal buffer or Mg sources (Rauch et al., 2012; Bach et al., 2018), and other studies evaluated the interactions of these 2 dietary additives (Erdman et al., 1980, 1982). However, to our knowledge, studies evaluating more than one Mg source and the interaction with ruminal buffer on performance parameters of dairy cows are scarce. Our results show an interaction on DMI and feed efficiency,

which indicates that cows had a lower feed efficiency when fed MgO together with ruminal buffer; however, cows fed CaMg(OH)₂ were able to maintain similar feed efficiency regardless of buffer supplementation.

Davenport et al. (1990) evaluated DMI in steers fed MgO, Mg(OH)₂, and a control diet (no Mg supplementation), and reported that animals supplemented with Mg had an increased DMI, regardless of Mg source. In contrast, Thomas et al. (1984), in a study evaluating the effects of MgO sources with different particle sizes, Mg(OH)₂, NaHCO₃, and control (no buffer or Mg supplementation) in lactating dairy cows, reported that animals fed control diets had greater DMI than Mg-supplemented cows. A smaller decrease in intake was observed in animals fed MgO with a larger particle size (>1.7 mm) or NaHCO₃, and a greater decrease in intake was observed in animals fed MgO with a smaller particle size (<1.7 mm) and Mg(OH)₂. However, neither of these studies evaluated the interaction of Mg sources and ruminal buffer supplementation on production parameters.

Thomas et al. (1984) reported a reduction in milk yield for cows fed small-particle (<0.450 mm) MgO or Mg(OH)₂ sources and an increase in the milk production for cows fed ruminal buffer compared with cows fed the control diet (no supplement). Interestingly, our results corroborate in part the findings of Thomas et al. (1984) in that cows produced a similar amount of milk when fed small-particle MgO or Mg(OH)₂; however, no effect of buffer was observed on milk yield.

Also, Thomas et al. (1984) reported that supplementation of ruminal buffer improved milk fat concentration, and a further increase in milk fat was observed for cows fed MgO or Mg(OH)₂. Our results are consistent with the finding of Thomas et al. (1984) because the inclusion of buffer improved milk fat concentration and no effects of Mg source were observed on milk fat concentration. However, we observed the opposite of Thomas et al. (1984), as their results show a greater effect on milk fat from the Mg source than ruminal buffer. An increase in milk fat by ruminal buffer supplementation is well documented and was an expected result in our study (Solorzano et al., 1989; Iwaniuk and Erdman, 2015).

Physiological Effects

Some sources of Mg can have alkalinizing potential, which has implications for production parameters and the acid-base system of the body (Erdman et al., 1980). The alkalinizing potential of oxide and hydroxide sources of Mg is due to the production of OH⁻ in the dissolution of the mineral in water. First, MgO is hydrated, generating Mg hydroxide [MgO + H₂O → Mg(OH)₂],

which dissociates aqueous solution into Mg ion (Mg²⁺) and hydroxides (OH⁻) (Fruhirth et al., 1985). Similarly, Na sesquicarbonate, which is a double salt of sodium bicarbonate (NaHCO₃) and sodium carbonate (Na₂CO₃), will dissociate into both salts when added to an aqueous solution. These 2 salts further dissociate on bicarbonate (HCO₃⁻), hydroxide ions (OH⁻), and sodium ions (Na⁺) in the rumen. Further reaction of OH⁻, from Mg sources and ruminal buffers, with available H⁺ forms water and increases the pH of the solution (Fruhirth et al., 1985).

Our data show that Na sesquicarbonate effectively increased urinary pH; however, Mg source did not affect urinary, fecal, blood, or ruminal pH. The increase in urinary pH can be explained by the alkalinizing potential of sodium sesquicarbonate. There is a consensus that ingested Na⁺ ions are fully absorbed in the gastrointestinal tract of dairy cows (NASEM, 2021). The renal response to greater Na absorption is typically an increase in cation and water excretion and a reduction in the renal reabsorption of HCO₃⁻ (Lindinger et al., 2000; NASEM, 2021), which explains the increase in urinary pH and urine output in animals fed Na sesquicarbonate.

In one of the early studies on the alkalinizing potential of sodium bicarbonate and MgO, Erdman et al. (1980) showed that NaHCO₃ and MgO can increase the urinary pH of early lactation dairy cows by about 0.39 pH units; however, no further benefits of the use of both alkalizers together were observed. Also, those authors reported an increase of 0.8 pH units in the fecal pH of animals fed MgO, indicating a possible low digestive tract action of the Mg source. In a more recent study, Bach et al. (2018) examined the effects of NaHCO₃ and a blend of MgO and CaO in mid-lactation Holstein cows when cows were challenged with increasing amounts of barley grain (3 kg/d of additional barley). They observed that animals fed NaHCO₃ had a similar ruminal pH to animals fed a control diet (without supplementation of NaHCO₃ or the blend of MgO/CaO), and both treatments had lower ruminal pH compared with animals fed a magnesium-based product.

Smith and Correa (2004) studied the effects of Mg hydroxide sources as an antiacid treatment for acidosis and laxative inducer on ruminal and blood physiological parameters in dairy cows. They found that treatment with 450 g/d of powdered Mg hydroxide was able to increase ruminal pH by about 2 pH units 72 h after the first administration; interestingly, no changes in blood pH were observed. However, the authors pointed out that possible changes in pH could have occurred and been missed during the 24-h interval between blood samplings.

Arce-Cordero et al. (2020) observed no difference in pH-related variables in ruminal fluid in an in vitro dual-flow continuous culture system when comparing diets supplemented with oxide and hydroxide sources of Mg. Both treatments showed a decrease in duration of pH <6 compared with carbonate sources of Mg. In another experiment, Arce-Cordero et al. (2021) evaluated the interaction of 2 sources of Mg (oxide and a blend of hydroxide and carbonate) and buffer use (Na sesquicarbonate) in an in vitro dual-flow continuous culture system. The authors reported no interactions of the 2 factors evaluated and no effects of Mg sources on the pH-related variables evaluated; however, an increase in ruminal pH was detected from fermentation systems in which buffer was included. Similar results were observed by Agostinho et al. (2022), using oxide and carbonate sources of Mg and NaHCO₃ as a buffer source.

In addition to the lack of pH changes in ruminal fluid and blood with buffer supplementation, we are confident that urinary pH changes support the hypothesis that buffer affects the acid-base balance of dairy cows. Also, based on the blood gas analysis, we did not observe an interaction or buffer effect; nevertheless, an increase in HCO₃ concentration and base excess was observed in animals fed CaMg(OH)₂.

In addition, the cows in our study were maintained on a common dairy diet for the eastern United States (44% corn silage), and more pronounced effects on the acid-base balance might have been observed if a more challenging diet (i.e., high grain) was used, due to an increase in acid production in the rumen, as demonstrated by Schaefer et al. (1982). A recent study evaluating ruminal challenge diets reported that average pH decreased with the inclusion of barley for cows fed control (no additive supplementation) and sodium bicarbonate diets; however, Mg supplementation was able to prevent the pH decline (Bach et al., 2018).

Despite the increase in blood parameters that indicate an increase in the alkalinity of the blood, the average blood gas results were within the normal range for lactating dairy cows: pH varying from 7.35 to 7.50; pCO₂ varying from 35 to 44 mmHg; HCO₃ varying from 20 to 30 mmol/L; and tCO₂ varying from 22 to 34 mmol/L (DiBartola, 2011).

Ca and Mg Balance

In addition to the importance of acid-base balance in the body, macromineral balance is a critical parameter to evaluate when testing different sources of Mg. Davenport et al. (1990) evaluated Mg balance in steers fed MgO, Mg(OH)₂, and a control diet (no Mg supplementation), and reported an increase in plasma

Mg concentration and Mg excretion in feces and urine, regardless of Mg source. Their results indicate that an increase in dietary Mg input can increase Mg availability in the gastrointestinal tract and, consequently, a greater concentration in the plasma and urine was observed.

The ruminal solubility of Ca and Mg of the minerals tested in our study was also evaluated, and no differences were observed. This corroborates with the findings of Thomas et al. (1984) and Arce-Cordero et al. (2020), in which no differences in Mg solubility in ruminal fluid were observed between MgO and Mg(OH)₂. Although Ca solubility was not evaluated in either of these 2 studies, Arce-Cordero et al. (2021) reported a reduction in the Mg solubility of Mg hydroxide compared with an oxide source used in combination with a carbonate source.

The solubility of Mg in the ruminal fluid is important because the rumen is a major site for Mg absorption in adult dairy cows, and Mg concentration in the rumen is correlated with absorption (NASEM, 2021). However, Jittakhot et al. (2004a) suggested that this system of absorption is saturable, meaning that an increase in soluble Mg in the rumen does not directly implicate a linear increase in serum Mg concentration. Interestingly, our results indicate that, despite no difference observed in the Mg concentration in the rumen fluid, a reduction in the plasma concentration of Mg in animals fed CaMg(OH)₂ was observed compared with the plasma of animals fed MgO.

Despite a lower plasma Mg concentration, both mineral sources had plasma Mg concentrations above the recommended threshold (1 mmol/L) for dairy cows described in NASEM (2021). The values of both treatments were similar to the maximum plasma Mg concentration observed by Jittakhot et al. (2004a), which ranged from 1.07 to 1.28 mmol/L. These results indicate that Mg hydroxide is a suitable source of Mg and could replace MgO in the diet of mid-lactating Holstein dairy cows with no risk of a possible plasma Mg deficiency. However, further research is needed to test the suitability of this source when cows are supplemented CaMg(OH)₂ for extended periods, such as the entire lactation, or when supplementation is provided in the transition period or early lactation.

The urinary excretion of Mg is a major mechanism that regulates its balance in dairy cow. According to Tebbe and Weiss (2018) and Tebbe et al. (2018), urinary excretion of Mg can vary from 7.3 to 13.9% of Mg intake, which is similar to the observed values from our results (average of 14.9% of Mg intake). The urinary excretion of Mg is a suitable method by which to evaluate the relative bioavailability of Mg (van Ravenswaay et al., 1992; Tebbe and Weiss, 2018), which is highly

variable and can differ among Mg sources (Jesse et al., 1981; Thomas et al., 1984).

Thomas et al. (1984) reported that animals fed supplemental Mg had a greater excretion of Mg in feces compared with animals fed control diets (no Mg supplementation). Also, the authors reported that animals fed large MgO particles (>0.425 mm) were less reactive, and consequently, greater excretion was observed compared with small MgO particles (<0.425 mm) and Mg(OH)₂ sources. Differently from Thomas et al. (1984), our findings indicate that CaMg(OH)₂ sources have a lower excretion than MgO. These results could be explained by the observed particle size: CaMg(OH)₂ had a smaller and more uniform particle size. These results indicate that CaMg(OH)₂ may be more reactive and consequently less excretion was observed. Also, when comparing the Mg excretion in the manure, lower values were observed for animals fed CaMg(OH)₂.

CONCLUSIONS

We observed an interaction between Mg source and buffer, in which cows fed CaMg(OH)₂ had similar feed efficiency, regardless of ruminal buffer supplementation; however, when MgO was provided together with ruminal buffer, a reduction in the feed efficiency was observed. Also, ruminal buffer had a positive effect on milk fat concentration. We found no evidence of a beneficial interaction between buffer supplementation and Mg sources with alkalinizing potential on mid-lactating dairy cows on the overall physiological response and Ca and Mg balance. There was evidence that MgO could be replaced by CaMg(OH)₂ without affecting the physiological response of mid-lactating Holstein dairy cows. There was no strong evidence that the balance of Ca and Mg was affected by the replacement of MgO with CaMg(OH)₂, aside from a small reduction in Mg fecal excretion.

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REFERENCES

Agustinho, B. C., A. Ravelo, J. R. Vinyard, R. R. Lobo, J. A. Arce-Cordero, H. F. Monteiro, E. Sarmikasoglou, S. Bennett, M. L.

- Johnson, E. R. Q. Vieira, C. Stoffel, S. E. Stocks, and A. P. Faciola. 2022. Effects of replacing magnesium oxide with calcium-magnesium carbonate with or without sodium bicarbonate on ruminal fermentation and nutrient flow in vitro. *J. Dairy Sci.* 105:3090–3101. <https://doi.org/10.3168/jds.2021-20995>.
- AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International.
- Arce-Cordero, J. A., H. F. Monteiro, V. L. N. Brandao, X. Dai, S. L. Bennett, and A. P. Faciola. 2020. Effects of calcium–magnesium carbonate and calcium–magnesium hydroxide as supplemental sources of magnesium on microbial fermentation in a dual-flow continuous culture. *Transl. Anim. Sci.* 5:txaa229. <https://doi.org/10.1093/tas/txaa229>.
- Arce-Cordero, J. A., A. Ravelo, J. R. Vinyard, H. F. Monteiro, B. C. Agustinho, E. Sarmikasoglou, S. L. Bennet, and A. P. Faciola. 2021. Effects of supplemental source of magnesium and inclusion of buffer on ruminal microbial fermentation in continuous culture. *J. Dairy Sci.* 104:7820–7829. <https://doi.org/10.3168/jds.2020-20020>.
- Bach, A., I. Guasch, G. Elcoso, J. Duclos, and H. Khelil-Arfa. 2018. Modulation of rumen pH by sodium bicarbonate and a blend of different sources of magnesium oxide in lactating dairy cows submitted to a concentrate challenge. *J. Dairy Sci.* 101:9777–9788. <https://doi.org/10.3168/jds.2017-14353>.
- Beede, D. K. 2017. Can we differentiate magnesium sources nutritionally? Pages 99–107 in *Tri-State Dairy Nutrition Conference*. M. L. Eastridge, ed. The Ohio State University.
- Chizzotti, M. L., S. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livest. Sci.* 113:218–225. <https://doi.org/10.1016/j.livsci.2007.03.013>.
- Colombo, E. A., R. F. Cooke, A. C. R. Araújo, K. M. Harvey, K. G. Pohler, and A. P. Brandão. 2022. Supplementing a blend of magnesium oxide to feedlot cattle: Effects on ruminal, physiological, and productive responses. *J. Anim. Sci.* 100:skab375. <https://doi.org/10.1093/jas/skab375>.
- Davenport, G. M., J. A. Boling, and N. Gay. 1990. Bioavailability of magnesium in beef cattle fed magnesium oxide or magnesium hydroxide. *J. Anim. Sci.* 68:3765–3772. <https://doi.org/10.2527/1990.68113765x>.
- DiBartola, S. P., ed. 2011. *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice*. 4th ed. Elsevier Health Sciences.
- Erdman, R. A., R. L. Botts, R. W. Hemken, and L. S. Bull. 1980. Effect of dietary sodium bicarbonate and magnesium oxide on production and physiology in early lactation. *J. Dairy Sci.* 63:923–930. [https://doi.org/10.3168/jds.S0022-0302\(80\)83027-X](https://doi.org/10.3168/jds.S0022-0302(80)83027-X).
- Erdman, R. A., R. W. Hemken, and L. S. Bull. 1982. Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: Effects of production, acid-based metabolism, and digestion. *J. Dairy Sci.* 65:712–731. [https://doi.org/10.3168/jds.S0022-0302\(82\)82259-5](https://doi.org/10.3168/jds.S0022-0302(82)82259-5).
- Fruhwith, O., G. W. Herzog, I. Hollerer, and A. Rachetti. 1985. Dissolution and hydration kinetics of MgO. *Surf. Technol.* 24:301–317. [https://doi.org/10.1016/0376-4583\(85\)90080-9](https://doi.org/10.1016/0376-4583(85)90080-9).
- Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *J. Dairy Sci.* 101:2763–2813. <https://doi.org/10.3168/jds.2017-13112>.
- Gordon, A. M., E. Homsher, and M. Regnier. 2000. Regulation of contraction in striated muscle. *Physiol. Rev.* 80:853–924. <https://doi.org/10.1152/physrev.2000.80.2.853>.
- Iwaniuk, M. E., and R. A. Erdman. 2015. Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. *J. Dairy Sci.* 98:8973–8985. <https://doi.org/10.3168/jds.2015-9949>.
- Jesse, B. W., J. W. Thomas, and R. S. Emery. 1981. Availability of magnesium from magnesium oxide particles of differing sizes and surfaces. *J. Dairy Sci.* 64:197–205. [https://doi.org/10.3168/jds.S0022-0302\(81\)82554-4](https://doi.org/10.3168/jds.S0022-0302(81)82554-4).

- Jittakhot, S., J. T. Schonewille, H. Wouterse, A. W. J. Uijtewaal, C. Yuangklang, and A. C. Beynen. 2004a. Increasing magnesium intakes in relation to magnesium absorption in dry cows. *J. Dairy Res.* 71:297–303. <https://doi.org/10.1017/S0022029904000275>.
- Jittakhot, S., J. T. Schonewille, H. Wouterse, C. Yuangklang, and A. C. Beynen. 2004b. Apparent magnesium absorption in dry cows fed at 3 levels of potassium and 2 levels of magnesium intake. *J. Dairy Sci.* 87:379–385. [https://doi.org/10.3168/jds.S0022-0302\(04\)73177-X](https://doi.org/10.3168/jds.S0022-0302(04)73177-X).
- Katz, B., and R. Miledi. 1967. A study of synaptic transmission in the absence of nerve impulses. *J. Physiol.* 192:407–436. <https://doi.org/10.1113/jphysiol.1967.sp008307>.
- Lee, C., and A. N. Hristov. 2013. Short communication: Evaluation of acid-insoluble ash and indigestible neutral detergent fiber as total-tract digestibility markers in dairy cows fed corn silage-based diets. *J. Dairy Sci.* 96:5295–5299. <https://doi.org/10.3168/jds.2012-6442>.
- Lindinger, M. I., T. W. Franklin, L. C. Lands, P. K. Pedersen, D. G. Welsh, and G. J. F. Heigenhauser. 2000. NaHCO_3 and KHCO_3 ingestion rapidly increases renal electrolyte excretion in humans. *J. Appl. Physiol.* 88:540–550. <https://doi.org/10.1152/jappl.2000.88.2.540>.
- Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* 85:1217–1240.
- NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. *Nutrient Requirements of Dairy Cattle*. 8th rev. ed. National Academies Press.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academies Press.
- Rauch, R. E., P. H. Robinson, and L. J. Erasmus. 2012. Effects of sodium bicarbonate and calcium magnesium carbonate supplementation on performance of high producing dairy cows. *Anim. Feed Sci. Technol.* 177:180–193. <https://doi.org/10.1016/j.anifeedsci.2012.08.016>.
- Rude, R. K., M. E. Kirchen, H. E. Gruber, M. H. Meyer, J. S. Luck, and D. L. Crawford. 1999. Magnesium deficiency-induced osteoporosis in the rat: Uncoupling of bone formation and bone resorption. *Magnes. Res.* 12:257–267.
- Schaefer, D. M., L. J. Wheeler, C. H. Noller, R. B. Keyser, and J. L. White. 1982. Neutralization of acid in the rumen by magnesium oxide and magnesium carbonate. *J. Dairy Sci.* 65:732–739. [https://doi.org/10.3168/jds.S0022-0302\(82\)82260-1](https://doi.org/10.3168/jds.S0022-0302(82)82260-1).
- Smith, G. W., and M. T. Correa. 2004. The effects of oral magnesium hydroxide administration on rumen fluid in cattle. *J. Vet. Intern. Med.* 18:109–112. <https://doi.org/10.1111/j.1939-1676.2004.tb00143.x>.
- Solorzano, L. C., L. E. Armentano, R. R. Grummer, and M. R. Dentine. 1989. Effects of sodium bicarbonate or sodium sesquicarbonate on lactating Holsteins fed a high grain diet. *J. Dairy Sci.* 72:453–461. [https://doi.org/10.3168/jds.S0022-0302\(89\)79127-X](https://doi.org/10.3168/jds.S0022-0302(89)79127-X).
- Storry, J. E., and J. A. F. Rook. 1963. Magnesium metabolism in the dairy cow V. Experimental observations with a purified diet low in magnesium. *J. Agric. Sci.* 61:167–171. <https://doi.org/10.1017/S002185960000589X>.
- Tebbe, A. W., and W. P. Weiss. 2018. Evaluation of creatinine as a urine marker and factors affecting urinary excretion of magnesium by dairy cows. *J. Dairy Sci.* 101:5020–5032. <https://doi.org/10.3168/jds.2017-14098>.
- Tebbe, A. W., D. J. Wyatt, and W. P. Weiss. 2018. Effects of magnesium source and monensin on nutrient digestibility and mineral balance in lactating dairy cows. *J. Dairy Sci.* 101:1152–1163. <https://doi.org/10.3168/jds.2017-13782>.
- Thomas, J. W., R. S. Emery, J. K. Breaux, and J. S. Liesman. 1984. Response of milking cows fed a high concentrate, low roughage diet plus sodium bicarbonate, magnesium oxide, or magnesium hydroxide. *J. Dairy Sci.* 67:2532–2545. [https://doi.org/10.3168/jds.S0022-0302\(84\)81610-0](https://doi.org/10.3168/jds.S0022-0302(84)81610-0).
- van Ravenswaay, R. O., P. R. Henry, C. B. Ammerman, and R. C. Littell. 1992. Relative bioavailability of magnesium sources for ruminants as measured by urinary magnesium excretion. *Anim. Feed Sci. Technol.* 39:13–26. [https://doi.org/10.1016/0377-8401\(92\)90028-5](https://doi.org/10.1016/0377-8401(92)90028-5).