Effects of magnesium source and monensin on nutrient digestibility and mineral balance in lactating dairy cows

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ABSTRACT

The interaction of monensin and 2 supplemental Mg sources (MgO and MgSO₄) on total-tract digestion of minerals and organic nutrients and milk production was evaluated in lactating dairy cattle. Eighteen multiparous Holstein cows (139 ± 35 DIM) were used in a split-plot experiment with 0 or 14 mg/kg diet DM of monensin as the whole-plot treatments and Mg source as split-plot treatments. During the entire experiment (42 d), cows remained on the same monensin treatment but received a different Mg source in each period (21 d) of the Latin square. Diets were formulated to contain 0.35% Mg with about 40% of that provided by MgO or MgSO₄. Diets were formulated to have similar concentrations of major nutrients and K concentrations were elevated (2.1% of DM) with K₂CO₃ to create antagonism to Mg absorption. Apparent digestibility was measured by total collection of urine and feces. Supplemental MgSO₄ decreased DMI (26.9 vs. 25.7 kg/d) and tended to decrease milk yield (40.2 vs. 39.3 kg/d), but increased the digestibility of OM (68.3 vs. 69.2%) and starch (91.9 vs. 94.4%) compared with MgO. Feeding MgSO₄ with monensin decreased NDF digestibility compared with other treatments (46.7 vs. 50.2%). That diet also had decreased apparent absorption of Mg compared with diets without monensin (15.6 vs. 19.2%), whereas MgO with monensin had greater apparent absorption of Mg (23.0%) than other treatments. Cows consuming MgSO₄ had increased apparent Ca absorption (32.2 vs. 28.1%) and balance. A diet with MgSO₄ without monensin increased the concentration of long-chain fatty acids in milk, suggesting increased mobilization of body fat or decreased *de novo* fatty acid synthesis in the mammary gland. Overall, when dietary Mg was similar, MgO was the superior Mg source for lactating dairy cattle, but inclusion of monensin in diets should be considered when evaluating Mg sources.

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INTRODUCTION

Relative to daily requirements, lactating dairy cows have small reservoirs of labile Mg (Blaxter and McGill, 1956); therefore, rations must consistently provide adequate absorbable Mg. Absorption of Mg does not appear to be under homeostatic regulation (Schweigel and Martens, 2000), which means that apparent absorption reflects availability of dietary Mg, and apparent absorption varies widely (−4 to 30% of intake; Weiss, 2004). Major factors affecting availability of Mg are dietary K concentration and source of Mg. Apparent absorption of Mg from MgSO₄ averaged about twice as great as Mg from MgO when fed to sheep (Van Ravenswaay et al., 1989). This likely reflects greater ruminal solubility of Mg from sulfate than from MgO. Ruminal microbes require ionized Mg for fiber degradation (Morales and Dehority, 2014), and increased Mg supply may improve total-tract NDF digestibility in lactating cows (Erdman et al., 1982).

Monensin is a feed additive used to improve feed efficiency by depleting cells and select microbes of intracellular K and altering rumen ecology (Bergen and Bates, 1984). Monensin may also improve digestibility, absorption and retention of nutrients in cattle (Spears, 1990). Feeding monensin has improved Mg apparent absorption 15 to 25% in ruminants by decreasing the antagonistic effect of K on Mg absorption (Greene et al., 1986), but the effects may be dependent on the source of Mg. In gestating beef cattle, monensin with MgSO₄ increased plasma glucose and decreased blood urea and ruminal butyrate concentrations in early lactation compared with diets with MgSO₄ but without monensin or MgO with monensin (Grings and Males, 1988), suggesting altered energy metabolism and digestibility.

Supplementing diets with a more soluble Mg source and monensin may be beneficial to the high producing dairy cow, especially when subject to K antagonism. The objective of this research was to investigate the

interaction of supplementing 2 different Mg sources and monensin on the digestion and balance of nutrients in lactating dairy cattle. We hypothesized a more soluble source of Mg (i.e., MgSO_4) combined with monensin would increase Mg apparent absorption and improve the digestibility of other nutrients in lactating cattle compared with diets without monensin or with MgO .

MATERIALS AND METHODS

Cows and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee. Eighteen multiparous Holstein cows (139 ± 35 DIM) were used in a split-plot experiment with subplot treatments in a Latin square design. Because our facility only has 6 stalls designed for total collection of urine and feces, cows were placed into 3 groups of 6 based on 10-d average milk yield (range in average group yields: 42.6 to 46.8 kg/d). Whole plot treatments were 0 or 14 mg/kg of DM of monensin (Rumensin 90, Elanco Animal Health, Greenfield, IN). The split-plot design allowed monensin treatments to be fed for the entire 42 d of the experiment without possibilities of carryover effects. Cows were also not fed monensin for at least 3 mo before the experiment. Split-plot treatments (arranged as a 2×2 Latin square) were Mg either from MgO (Animag Prilled 30/100, Martin Marietta Magnesia Specialties LLC, Baltimore, MD) or MgSO_4 (Magriculture, Giles Chemical, Waynesville, NC). Each Latin square consisted of two 21-d periods with total collection of urine and feces on d 16 to 20 of each period. Diets were formulated to contain about 40% Mg from supplemental Mg sources and have similar concentrations of total dietary Mg (target: 0.35% of DM; Table 1). All diets were formulated to contain similar concentrations of nutrients and have elevated K concentrations (basal diet was 1.3% K plus 0.8% K from K_2CO_3) to create antagonism on Mg absorption.

Cows were housed in individual tie stalls and fed once daily at a refusal rate of 3 to 5%. Individual feed delivery and refusal amounts were weighed and recorded daily. Cows were milked twice daily at 0200 and 1400 h, and milk yields were measured electronically (Afirmilk, Kibbutz Afikim, Israel). Cows were weighed on consecutive mornings (values were averaged within a cow) at the beginning and end of each period. Silages were sampled weekly, analyzed for DM (100°C for 48 h), and used to adjust diets. One refusal sample was taken on d 10 of each period, analyzed for DM (100°C for 48 h), and used to calculate DMI when in the tiestalls.

Digestion Collection Samples

For the measurement of apparent nutrient digestibility and balance, cows were moved from tiestalls to digestion stalls, and total output of urine and feces was measured for 4 d (Weiss et al., 2009). Urine (acidified with 50% sulfuric acid and nonacidified), feces, milk, refusals, and feeds were sampled daily (refrigerated between days) and composited by wet weight for each cow. Water intake was measured and recorded daily by water meters connected to each individual water bowl. One water sample from the inlet feeding the water bowl was taken during each digestion period and analyzed for minerals.

Milk, acidified urine, and wet feces composite samples were analyzed for N by the Kjeldahl method (AOAC International, 2000; 984.13.4.09) immediately following each digestion period. Subsamples of feed, refusal and fecal composites were analyzed for DM (100°C for 48 h). The remaining silage, fecal, and refusal composite samples were frozen, lyophilized, and ground through a 1-mm screen (Wiley mill, Arthur A. Thomas Co., Philadelphia, PA). Concentrates and dried, ground samples of silages, feces, and refusals were assayed for DM (100°C for 24 h), ash (muffle oven at 600°C

Table 1. Ingredient composition of the diets

Ingredient, % of DM	Diet ¹	
	MgO	MgSO ₄
Corn silage	34.2	34.2
Alfalfa silage	22.0	22.0
Ground corn	18.4	17.5
Soybean meal, 48% CP	12.9	13.0
Soy hulls	8.7	8.0
Animal/vegetable fat	0.49	0.48
Potassium carbonate ²	1.51	1.49
Dicalcium phosphate	0.35	0.35
Magnesium oxide ³	0.19	—
Magnesium sulfate ⁴	—	1.65
Trace-mineralized salt ⁵	0.70	0.70
Mineral-vitamin premix ⁶	0.62	0.62

¹198 g/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN) added to the diet of each Mg source at 14 mg/kg of the DM by replacing ground corn.

²56% K (DCAD Plus, Church & Dwight Co. Inc., Piscataway, NJ).

³58% Mg, mean particle size: $923 \pm 20.3 \mu\text{m}$ (Animag Prilled 30/100, Martin Marietta Magnesia Specialties LLC, Baltimore, MD).

⁴9.8% Mg (Magriculture, Giles Chemical, Waynesville, NC).

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

⁶Premix contained 60 mg/kg of Se (sodium selenate), 111 mg/kg of biotin, 3.8 g/kg of Zn (Zimpro 120, Zimpro Corp., Eden Prairie, MN), 800 mg/kg of Cu (copper sulfate), 500 kIU/kg of vitamin A, 200 kIU/kg of vitamin D, and 3,600 IU/kg of vitamin E.

overnight), CP ($N \times 6.25$; method 984.13.4.09; AOAC International, 2000), long-chain fatty acids (**LCFA**; Weiss and Wyatt, 2003), and NDF (Ankom200 Fiber Analyzer, Ankom Technology Corp., Fairport, NY) with sodium sulfite and amylase (Sigma A3306, Sigma Diagnostics, St. Louis, MO). Starch was analyzed according to Weiss and Wyatt (2000) except that 0.1 M sodium acetate buffer (pH = 5.0) rather than molecular grade water was used to gelatinize starch before enzymatic digestion with amylase as suggested by Hall (2009). Residual organic matter (**ROM**) was calculated as

$$\begin{aligned}\text{ROM, \% of DM} &= 100 - \text{CP} - \text{LCFA} \\ &\quad - \text{ash} - \text{starch} - \text{NDF},\end{aligned}$$

where CP, LCFA, ash, starch, and NDF are % of the DM. The NDF residues were ashed (**NDI-ash**; muffle oven at 600°C overnight) or analyzed for CP (**NDICP**; $N \times 6.25$; method 984.13.4.09; AOAC International, 2000). The NDI-ash and NDICP values were then used to calculate NDF as ash-free, CP-free, or both (Tebbe et al., 2017). Nonacidified urine, water, milk, and dried ground feed, refusal, and fecal samples were analyzed for minerals (OARDC STAR Laboratory, Wooster, OH) by inductively coupled plasma emission spectroscopy (Isaac and Johnson, 1985) after microwave digestion in nitric acid (Jones et al., 1991). Chloride in feed samples was analyzed by potentiometric titration with silver nitrate using a chloride ion electrode after nitric acid extraction (LaCroix et al., 1970).

Milk and Blood Samples

Weekly composite (a.m. and p.m.) milk samples were collected and analyzed for milk fat, true protein, and lactose (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), and MUN (Skalar SAN Plus segmented flow analyzer, Skalar Inc., Norcross, GA) by DHI cooperative Inc. (Columbus, OH). Additional p.m. milk samples were collected on d 21 of each period and analyzed for milk fatty acid profile. Milk fat layer was removed and its fatty acid profile was determined using a 2-step procedure for methylation (Jenkins, 2000) with separation by gas-liquid chromatography using a CP-SIL88 capillary column (100 m $\times$ 0.25 mm $\times$ 0.2 μ m film thickness; Varian Inc., Palo Alto, CA). Blood was collected from the tail vein into sodium heparin Vacutainers (BD Vacutainer, Franklin Lakes, NJ) on d 21 of each period. Plasma was separated by centrifugation (2,500 $\times g$ at 4°C) and assayed for Mg using a colorimetric assay (Magnesium Liquicolor No. 0130, Stanbio Laboratory, Boerne, TX).

Calculations and Statistical Analyses

Two cows on the MgO with monensin treatment unknowingly received diets devoid of trace-mineralized salt (apparent by urine Na excretion <1 g/d and total dietary Na concentration <0.02% of DM) and subsequently had decreases in DMI and milk; all data from these 2 cow-periods were removed. Production measures were averaged for the entire period, and apparent digestibility and balance of nutrients were calculated for each cow-period. Digestible energy (Mcal/d) was estimated by multiplying measured apparently digested CP, starch, NDF, LCFA, and ROM by the following enthalpies, 5.65, 4.23, 4.2, 9.4, and 4.0 Mcal/kg, respectively. Data were analyzed using PROC MIXED (v9.4, SAS Institute Inc., Cary, NC) using a model that included the fixed effects of monensin (1 df), Mg source (1 df), their interaction (1 df), and the random effects of group (2 df), period within group (1 df), cow within group $\times$ monensin (12 df), and residual error. All model denominator degrees of freedom were adjusted using the Kenward-Rogers option. Because of the missing data, the highest standard error of the mean was reported for all measures.

RESULTS AND DISCUSSION

Diet Mg content was similar for the MgSO₄ and MgO diets (Table 2), but S content doubled from 0.21 to 0.42% of DM and DCAD decreased 40% (356 to 214 mEq/kg) when adding MgSO₄. Because of the lesser concentration of Mg in MgSO₄ compared with MgO (9.8 vs. 58% Mg), supplementing MgSO₄ slightly lowered OM, NDF, LCFA, and starch content compared with MgO because it replaced corn and soyhulls. Mineral concentrations in the drinking water were generally low (Table 3), and average increases in mineral intake from water was greatest for Ca (5.7% or 9.4 g/d) and Na (4.8% or 3.2 g/d) followed by Mg (3.8% or 3.4 g/d) and S (2.7% or 2.1 g/d). Intakes of minerals provided by drinking water were included when calculating apparent absorption and balance; however, treatment inferences on macro-mineral metabolism were the same with or without inclusion of water minerals (data not shown).

Production

Monensin did not affect any production measures ($P \geq 0.15$; Table 4). Feeding MgSO₄ decreased DMI [kg/d ($P < 0.01$)] and as a percent of BW ($P < 0.03$) compared with MgO diets, likely because of excess S as sulfate. Previous studies feeding high S concentrations by

Table 2. Nutrient composition of diets and forages¹

Nutrient ²	Control		Monensin		Corn silage	Alfalfa silage
	MgO	MgSO ₄	MgO	MgSO ₄		
DM, %	59.0	58.6	59.2	58.6	35.0	38.4
OM, %	92.0	91.6	92.2	91.9	96.1	90.7
CP, %	17.0	16.7	16.6	16.8	8.72	19.1
NDF, %	31.6	30.8	31.8	30.9	39.6	38.6
NDI-ash, %	0.66	0.56	0.59	0.53	0.59	0.77
NDICP, %	1.00	0.89	0.97	0.98	0.54	1.19
NDF _{om+cp} , %	29.9	29.4	30.2	29.4	38.5	36.6
Starch, %	25.4	25.2	25.4	25.2	33.6	3.05
LCFA, %	3.46	3.40	3.49	3.40	3.22	2.85
ROM, %	14.6	15.5	14.9	15.5	11.0	27.2
ROM _{om+cp} , %	16.2	16.9	16.4	17.0	12.1	29.1
Ca, %	0.66	0.69	0.65	0.69	0.21	1.25
P, %	0.34	0.35	0.34	0.35	0.21	0.28
Mg, %	0.33	0.38	0.34	0.37	0.16	0.29
K, %	2.12	2.09	2.12	2.11	1.20	2.56
Cl, %	0.59	0.66	0.43	0.59	0.32	0.24
S, %	0.21	0.43	0.21	0.42	0.13	0.32
Na, %	0.26	0.29	0.24	0.27	0.003	0.02
Cu, mg/kg	13	14	14	16	5	9
Mn, mg/kg	49	51	51	51	36	41
Mo, mg/kg	2.6	2.7	2.6	2.7	0.5	1.4
Zn, mg/kg	74	83	79	86	33	28
DCAD, mEq/kg	356	202	357	226	137	392

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄. Treatments contained different concentrate but similar forages.

²LCFA = long-chain fatty acids; residual organic matter, ROM (% DM) = 100 - %CP - %LCFA - %Ash - %Starch - % NDF.

³NDF_{om+cp} = NDF - NDI-ash (ashed NDF residues) - NDICP (NDF residues analyzed for CP); ROM_{om+cp} (% of DM) = 100 - %CP - %LCFA - %Ash - %Starch - % NDF_{om+cp}.

⁴DCAD (mEq/kg) = Na + K - Cl - S.

adding sulfate salts linearly decreased DMI in lactating cattle (Bouchard and Conrad, 1973; Ivancic and Weiss, 2001). The observed 1.1 kg/d decrease in DMI when dietary S increased by 0.2% units of DM is similar to the change in DMI by steers when dietary S increases by 0.25 units (Drewnoski et al., 2014). Potential modes of action for the negative effect of sulfate on DMI include altered microbial metabolism in the rumen (Kandylis, 1984), excessive ruminal H₂S production (Uwituze et al., 2011b), or decreased smooth muscle contraction

and rumen motility (Delfiol et al., 2013). Lower DMI resulting in decreased weight from gut fill is likely the primary reason why BW was lower for cows consuming MgSO₄. Cows fed MgSO₄ also tended to gain less ($P < 0.06$) BW compared with MgO, which is plausible given the lower ($P < 0.01$) intake of digestible energy (72.0 vs. 76.3 Mcal/d; Table 5) at similar milk energy outputs (29.6 vs. 30.2 Mcal/d; $P > 0.31$).

Milk fat concentration tended to increase ($P < 0.09$; Table 4) for cows consuming MgSO₄ compared with MgO likely from dilution because milk fat yield was unaffected ($P \geq 0.88$). The absence of treatment effects on milk fat yield and higher fat concentration for MgSO₄ was unexpected considering this diet was 142 mEq/kg lower in DCAD than MgO diets (Table 1). Dietary DCAD is positively correlated with milk fat concentration and yield (Iwaniuk and Erdman, 2015), but most studies evaluating DCAD in lactating cows increase DCAD by supplementing cations, whereas we reduced DCAD by supplementing anions (S).

Yields of protein and lactose were lower ($P < 0.04$) and milk yield tended to be lower ($P < 0.09$) for cows on MgSO₄ likely because of decreased DMI. Without monensin, MgSO₄ decreased protein concentration

Table 3. Average mineral analysis of water during digestion trials¹

Mineral	Average (SD)
Ca, mg/L	84.1 (1.25)
P, mg/L	<0.01
Mg, mg/L	30.1 (0.63)
K, mg/L	3.4 (1.25)
S, mg/L	18.9 (0.59)
Na, mg/L	28.6 (1.81)
Cu, µg/L	7.7 (7.23)
Mn, µg/L	4.0 (2.12)
Mo, µg/L	2.0 (0.01)
Zn, µg/L	24.4 (30.2)

¹One water sample taken during each digestion trial from the inlet to water drinking bowl (n = 6).

compared with MgO, but with monensin, MgSO₄ concentration was similar to cows fed MgO because of numerically higher protein yield and dilution (monensin × Mg interaction; $P < 0.05$). The yield of milk energy was not affected by monensin or Mg source ($P \geq 0.31$). When accounting for maintenance and BW change, NE_L use was less for cows consuming MgSO₄ compared with MgO ($P < 0.03$) but not the efficiency of NE_L use per kg of DMI ($P > 0.18$). Milk MUN was not affected by monensin or Mg source ($P \geq 0.25$). Statistical inferences made on production should be interpreted carefully because this experiment was designed to investigate digestibility and has low cow numbers and short period lengths for production.

Nutrient Intake and Apparent Digestibility

Supplementing MgSO₄ compared with MgO decreased DMI and milk yield during the 4-d total collection period (Table 5), which is similar to production measures from the entire period. The intake of dietary and free water followed DMI and was lower for cows consuming MgSO₄ compared with those fed MgO ($P < 0.04$); total water intake was reduced about 8.5% or 11.4 kg/d. The intakes and outputs of all nutrients were greater ($P \leq 0.06$) for cows consuming MgO with the exception of CP and ROM. Without monensin, MgSO₄ decreased CP intake compared with MgO, but with monensin, the CP intake was similar for cows

fed MgO and MgSO₄ (Mg × monensin interaction; $P < 0.04$). The interaction on CP intake was caused by the numerically higher DMI and slightly increased CP content of the MgO diet with monensin compared with MgO without monensin (17.0 vs. 16.6%). Intake of ROM was similar across treatment ($P \geq 0.84$) because of the higher ROM concentration in the MgSO₄ diets.

Apparent DM, CP, and LCFA digestibilities were not affected by treatments ($P \geq 0.11$). When cows consumed MgSO₄ diets, apparent OM digestibility tended to be higher ($P < 0.08$) and total-tract starch digestibility was higher ($P < 0.01$) compared with MgO. In one study, linearly increasing dietary S (0.15 to 0.25%) with ammonium sulfate did not affect total-tract digestion of OM or starch in steers (Zinn et al., 1997). However in another study, OM digestibility was greater when steers were fed a diet with 0.65% S compared with a diet with 0.42% S (Uwituze et al., 2011a). The authors proposed that increased digestibility was caused by a reduction in gastrointestinal tract motility from excessive H₂S production, which would subsequently create slower passage rates and more complete ruminal digestion. Slower passage for MgSO₄ diets in this study is possible given the lower DMI. The observed difference in OM and starch digestibility between cows fed MgO and MgSO₄ was 3 to 6 times greater than expected differences based solely on changes in DMI (Huhtanen et al., 2009; Ferraretto et al., 2013), suggesting a beneficial effect of supplementing MgSO₄ independent of intake.

Table 4. Effects of supplemental monensin and Mg source on production during the entire period

Item	Treatment ¹				SEM	P-value		
	Control		Monensin			Monensin	Mg	Monensin × Mg
	MgO	MgSO ₄	MgO	MgSO ₄				
DMI, kg/d	27.6	25.9	26.1	25.4	0.61	0.15	0.01	0.22
BW, kg	736	720	707	702	22.0	0.45	0.03	0.26
BW change, kg/d	0.51	−0.25	0.42	0.06	0.36	0.69	0.06	0.51
DMI/BW, %	3.78	3.62	3.74	3.64	0.14	0.97	0.03	0.65
Yield								
Milk, kg/d	40.6	39.3	39.7	39.2	2.29	0.86	0.09	0.52
Fat, kg/d	1.63	1.64	1.61	1.62	0.12	0.85	0.88	0.99
Protein, kg/d	1.29	1.20	1.25	1.23	0.06	0.97	0.04	0.18
Lactose, kg/d	2.05	1.90	1.97	1.94	0.12	0.88	0.02	0.15
Energy, ² Mcal	30.5	29.5	29.8	29.6	1.59	0.88	0.31	0.56
NE _L use, ³ Mcal/d	44.7	39.4	43.0	41.1	2.04	0.98	0.03	0.27
NE _L use, Mcal/kg of DM	1.61	1.52	1.68	1.62	0.08	0.23	0.18	0.82
Composition								
Fat, %	3.91	4.09	3.94	4.14	0.18	0.83	0.09	0.97
Protein, %	3.14	3.07	3.10	3.13	0.12	0.97	0.37	0.05
Lactose, %	4.86	4.81	4.87	4.89	0.07	0.53	0.61	0.38
Energy, Mcal/kg of milk	0.73	0.74	0.73	0.75	0.02	0.75	0.09	0.64
MUN, mg/dL	12.8	12.9	12.7	13.5	1.20	0.67	0.25	0.39

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄.

²Energy, Mcal = 9.29 × fat (kg) + 5.63 × protein (kg) + 3.95 × lactose (kg).

³NE_L use, Mcal = 0.08 × BW^{0.75} + milk energy + BW change, where BW change, Mcal = 4.7 × BW loss or 5.3 × BW gain (NRC, 2001).

Monensin decreased ($P < 0.05$) NDF digestibility, and digestibility of NDF on an ash-free basis was essentially the same as NDF (Supplemental Table S1; <https://doi.org/10.3168/jds.2017-13782>). Digestibility of NDF was not different between Mg sources for cows not fed monensin, but with monensin, MgSO_4 lowered NDF digestibility 6% compared with MgO (interaction $P < 0.10$). Because dietary S concentration and Mg source are completely confounded (by design), the interaction may actually be between S and monensin. Increased sulfate has decreased cellulose degradation in vitro (Hubbert et al., 1958) and ADF digestion in steers (Zinn et al., 1997), whereas monensin has not affected (Benchaa et al., 2006; Martineau et al., 2007) or increased (Plaizier et al., 2000) fiber digestibility in lactating dairy cows. Dietary sulfate (van Zijderveld et al., 2010) and monensin (Schären et al., 2017) can alter microbial populations. The toxic effect of feeding MgSO_4 on ruminal bacteria has previously been shown in sheep (van Zijderveld et al., 2010). A synergistic effect of monensin and MgSO_4 may have inhibited a

broadier population of cellulolytic bacteria or commensal microbes to cellulolytic bacteria and reduced NDF digestibility.

Apparent digestibility of ROM was about 3 percentage units greater when feeding monensin or MgSO_4 , but there was no interaction ($P = 0.76$). Removing NDF-ash and NDICP contamination consistently decreased apparent digestibility of ROM across all diets, but did not affect treatment inferences (Supplemental Table S1; <https://doi.org/10.3168/jds.2017-13782>). Because ROM has a metabolic fecal fraction that increases with DMI (Tebbe et al., 2017), the effects of monensin and MgSO_4 on ROM digestibility may have been caused by reduced endogenous fecal ROM.

Cows fed diets with MgO consumed and digested more ($P < 0.01$) N than cows on MgSO_4 diets, which tended to increase ($P < 0.06$) urinary N for these cows (Table 6). Fecal N followed N intake; without monensin, MgSO_4 had lower fecal N compared with MgO, but with monensin, MgSO_4 was similar to cows fed MgO (monensin $\times$ Mg interaction; $P < 0.03$). The efficiency

Table 5. Effects of supplemental monensin and Mg source on nutrient intake and digestibility and water intake

Item	Treatment ¹						SEM	P-value	
	Control		Monensin		Mg	Monensin × Mg			
	MgO	MgSO ₄	MgO	MgSO ₄					
Water intake, kg/d									
Dietary	29.3	28.4	28.6	27.8	2.14	0.32	0.04	0.81	
Free	116.3	104.2	117.6	109.0	3.53	0.45	0.01	0.49	
Total	145.6	132.6	146.6	136.8	4.08	0.55	0.01	0.61	
Output, kg/d (as is)									
Milk	40.6	38.9	41.1	39.6	2.36	0.85	0.01	0.84	
Feces	61.7	55.6	60.7	58.9	2.50	0.57	0.04	0.25	
Fecal water	53.2	48.1	52.7	51.2	2.26	0.48	0.06	0.32	
Urine	35.8	30.2	36.1	31.9	2.51	0.70	0.01	0.68	
Total manure	97.7	85.6	95.6	91.0	3.76	0.65	0.01	0.10	
Intake, ² kg/d									
DM	26.3	24.4	25.8	24.5	0.59	0.72	0.01	0.32	
OM	24.1	22.4	23.7	22.5	0.54	0.78	0.01	0.31	
CP	4.47	4.01	4.28	4.09	0.13	0.66	0.01	0.04	
NDF	8.29	7.68	8.13	7.62	0.22	0.58	0.01	0.59	
Starch	6.77	6.18	6.74	6.25	0.17	0.91	0.01	0.59	
LCFA	0.92	0.83	0.91	0.84	0.02	0.99	0.01	0.60	
ROM	3.71	3.72	3.73	3.73	0.14	0.84	0.93	0.93	
Energy digested, ³ Mcal/d	76.0	72.1	76.5	71.8	1.77	0.98	0.01	0.63	
Apparent digestibility, ² %									
DM	67.8	69.1	68.4	68.5	0.62	0.98	0.11	0.17	
OM	67.7	69.4	68.9	69.0	0.79	0.66	0.08	0.17	
CP	64.3	64.9	66.8	65.2	1.19	0.24	0.51	0.20	
NDF	50.2	50.7	49.6	46.7	1.21	0.05	0.23	0.10	
Starch	91.1	94.2	92.6	94.6	0.87	0.34	0.01	0.31	
LCFA	73.9	75.1	73.0	72.4	2.32	0.43	0.84	0.49	
ROM	65.4	68.9	68.3	72.8	2.65	0.06	0.02	0.76	

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

²LCFA = long-chain fatty acids; ROM = residual organic matter.

³Energy digested, Mcal/d = CP digested, kg/d $\times$ 5.65 + NDF digested, kg/d $\times$ 4.2 + starch digested, kg/d $\times$ 4.23 + LCFA digested, kg/d $\times$ 9.4 + ROM digested, kg/d $\times$ 4.0.

Table 6. Effects of supplemental monensin and Mg source on nitrogen metabolism

Item	Treatment ¹						P-value			
	Control			Monensin						
	MgO	MgSO ₄		MgO	MgSO ₄		SEM	Monensin	Mg	Monensin × Mg
Intake, g/d	715	641		686	654		20.7	0.66	0.01	0.04
Digested (ND), g/d	459	417		461	426		17.3	0.76	0.01	0.68
Milk, g/d	197	189		205	199		8.81	0.43	0.03	0.71
Urinary, g/d	231	205		214	210		12.1	0.63	0.06	0.18
Fecal, g/d	255	225		228	227		9.28	0.18	0.01	0.03
Retention, g/d	30.3	24.3		43.2	17.9		17.9	0.88	0.13	0.37
Milk, % of intake	27.7	29.6		29.7	30.5		1.30	0.41	0.01	0.10
Urinary, % of intake	32.6	31.9		30.3	32.5		1.79	0.56	0.52	0.22
Retention, % of intake	4.13	3.28		6.20	2.34		2.70	0.79	0.16	0.41
Milk, % of ND	43.3	45.6		44.4	46.9		2.27	0.67	0.01	0.94
Urinary, % of ND	50.6	49.3		45.5	49.9		2.77	0.35	0.43	0.15
Retention, % of ND	6.24	4.98		9.36	3.30		4.22	0.83	0.15	0.39

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄.

Table 7. Effect of supplemental monensin and Mg source on macro-mineral metabolism

Item	Treatment ¹						P-value		
	Control		Monensin		SEM	Monensin			Mg
	MgO	MgSO ₄	MgO	MgSO ₄					
Dietary intake, g/d									
Ca	168	163	166	163	7.3	0.80	0.15	0.77	
P	90	83	89	85	3.0	0.89	0.01	0.11	
K	551	502	536	513	19.1	0.89	0.01	0.14	
Mg	85	88	88	88	4.0	0.75	0.45	0.54	
S	56	99	56	98	3.4	0.50	0.01	0.76	
Na	70	69	62	62	5.9	0.23	0.66	0.72	
Total intake, ² g/d									
Ca	178	172	176	172	7.3	0.83	0.10	0.72	
P	90	83	89	85	3.0	0.89	0.01	0.11	
K	551	502	536	513	19.1	0.89	0.01	0.14	
Mg	89	91	91	91	4.1	0.74	0.57	0.58	
S	58	101	55	100	3.4	0.52	0.01	0.74	
Na	73	70	66	65	6.0	0.24	0.59	0.70	
Apparent absorption, ³ %									
Ca	28.4	33.8	27.8	30.5	3.58	0.48	0.03	0.47	
P	38.8	40.6	41.5	41.7	1.74	0.29	0.41	0.54	
K	82.8	84.1	85.1	83.1	2.41	0.77	0.78	0.29	
Mg	18.1	20.3	23.0	15.6	3.06	0.98	0.16	0.03	
S	52.7	69.9	53.9	68.6	1.54	0.97	0.01	0.27	
Na	79.7	83.1	76.8	82.0	4.61	0.61	0.22	0.81	
Apparent balance, ³ g/d									
Ca	9.2	17.0	6.9	11.1	8.32	0.47	0.09	0.63	
P	2.5	3.0	2.4	3.3	2.42	0.92	0.58	0.87	
K	48.7	56.0	55.6	36.4	18.3	0.69	0.65	0.37	
Mg	4.8	5.8	8.9	4.0	2.84	0.65	0.26	0.15	
S	3.6	11.9	4.1	6.7	2.64	0.32	0.01	0.14	
Na	1.6	6.7	0.6	1.2	5.13	0.44	0.36	0.53	
Blood									
Mg, mM	1.06	1.02	1.06	1.02	0.05	0.72	0.27	0.72	

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄.

²Total intake, g/d = dietary minerals + water minerals.

³Calculated using total intake.

of N (as a % of intake and % of N digested) secreted in milk was 5% lower ($P < 0.01$) for MgO diets compared with MgSO₄ because of higher milk protein production.

Macro-Mineral Metabolism

Absorption and balance of Na, P and K were unaffected by treatment ($P \geq 0.22$; Table 7). Intake of Ca was similar ($P \geq 0.10$) because the slightly higher Ca content in MgSO₄ diets compared with MgO compensated for lower DMI. Apparent absorption increased 14.6% or about 5.1 g/d when cows were fed MgSO₄. Milk Ca concentration was unaffected by treatment (average: 1.04 Ca g/kg of milk) but was 17% lower than the NRC (2001) estimate of 1.22 Ca g/kg of milk (Table 8). In theory, increased absorption of Ca with similar output in milk (Table 8) should have increased urinary Ca excretion; however, urinary excretion was

similar ($P > 0.39$) and MgSO₄ increased apparent Ca balance about 6 g/d. Although statistically all treatments had a Ca balance equal to zero ($P > 0.10$), the greater apparent absorption without increased urinary excretion suggests greater Ca balance with MgSO₄. An alternative explanation for increased apparent absorption and balance is lower endogenous fecal losses of Ca. In ruminants, DMI has a positive correlation with endogenous fecal Ca (Chrisp et al., 1989). The greater DMI for cows fed MgO could have increased endogenous fecal losses, resulting in reduced apparent absorption and balance of Ca. However, the 5 g/d increase in the amount apparently absorbed was much larger than the 1 to 1.5 g/d increase in endogenous calcium predicted by Chrisp et al. (1989). Adding DMI as a covariate to the model analyzing apparently absorbed Ca also did not affect ($P \geq 0.30$) absorption suggesting a true benefit of MgSO₄ on Ca metabolism. In dry cows, MgSO₄

Table 8. Effect of supplemental monensin and Mg source on macro-mineral excretion

Item	Treatment ¹					
	Control		Monensin		<i>P</i> -value	
	MgO	MgSO ₄	MgO	MgSO ₄	Monensin	Monensin × Mg
Milk concentration, g/kg						
Ca	1.03	1.06	1.02	1.03	0.65	0.26
P	0.81	0.80	0.82	0.80	0.84	0.13
K	1.29	1.30	1.31	1.29	0.77	0.85
Mg	0.099	0.104	0.097	0.099	0.41	0.03
S	0.32	0.32	0.31	0.32	0.92	0.20
Na	0.40	0.38	0.35	0.36	0.52	0.81
Milk output, % of total intake						
Ca	23.5	23.8	23.8	24.0	0.89	0.62
P	35.7	36.8	38.1	37.3	2.20	0.87
K	9.6	10.2	10.2	10.0	0.76	0.28
Mg	4.6	4.5	4.4	4.3	0.35	0.36
S	21.6	12.0	23.2	12.7	0.96	0.01
Na	21.2	21.0	23.8	22.1	2.74	0.50
Fecal output, g/d						
Ca	127	113	126	119	4.78	0.61
P	55.1	49.2	52.3	49.6	2.45	0.01
K	94.7	78.5	79.9	86.9	12.4	0.79
Mg	71.6	72.5	71.2	76.0	2.93	0.60
S	27.3	30.3	25.9	31.5	1.63	0.95
Na	14.5	12.0	12.2	12.2	2.46	0.60
Urine output, g/d						
Ca	0.71	1.12	0.78	0.82	0.30	0.68
P	0.62	0.37	0.35	0.55	0.17	0.81
K	354	317	350	338	20.5	0.67
Mg	7.98	8.77	8.06	6.69	0.91	0.33
S	14.5	47.2	13.1	50.0	1.49	0.86
Na	41.3	38.2	40.0	37.2	4.44	0.76
Urine output, % of total intake						
Ca	0.4	0.6	0.4	0.5	0.18	0.76
P	0.7	0.4	0.4	0.7	0.18	0.88
K	64.7	62.9	63.8	66.4	2.96	0.65
Mg	9.2	9.7	8.8	7.3	1.11	0.18
S	24.9	46.9	23.4	49.4	1.72	0.79
Na	56.2	55.6	60.1	57.3	5.25	0.71

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄.

reduced the incidence of hypocalcemia and increased blood Ca after parturition compared with MgO (Stockdale, 2004). However, in dry cows, supplemental anions are fed at rates to induce metabolic acidosis (i.e., decreased urinary pH) and increase urinary Ca losses (Schonewille et al., 1994). In our study, dietary DCAD was positive for all diets and urinary pH was not affected by treatments (data not shown). Grazing cows supplemented with MgSO₄ prepartum had increased plasma Ca postpartum compared with cows fed equal milliequivalents of MgCl₂, and treatments did not differ in the urinary Ca to creatinine ratio (Roche et al., 2002). These results, in concert with the greater Ca absorption without concomitant increases in urinary Ca excretion and decreases in urine pH found in our study, suggest a biological role of MgSO₄ independent of acid-base balance and deserves more investigation.

Total Mg intake did not differ ($P \geq 0.57$) and averaged 90.3 g/d across treatments. Average apparent absorption (19.3%), urine excretion (7.9 g/d), and milk concentration (0.10 Mg g/kg of milk) were similar to lactating cattle fed 71 g/d of Mg and dietary K at 1.8% of DM (17.1%, 7.2 g/d, and 0.10 g/kg, respectively; Holtenius et al., 2008). Average plasma Mg was higher than in the previous study (1.04 vs. 0.85 mM) but was not affected by treatments ($P \geq 0.27$; Table 7).

Two proposed mechanisms of ruminal Mg absorption exist: (1) a K-sensitive transport mechanism dependent on transmural potential difference and (2) a saturable, carrier-mediated transport system (Martens et al., 1991). Feeding monensin is thought to improve Mg ap-

parent absorption through increased Na⁺/K⁺-ATPase activity (Greene et al., 1986) and create a greater driving force for the K-sensitive transport mechanism (Martens and Schweigel, 2000). We hypothesized feeding MgSO₄ would be additive to the effects of monensin because of its higher solubility compared with MgO (Ammerman et al., 1972) creating more Mg available for absorption. Increases in apparent absorption would then be reflected by increases in output of Mg in urine. In our study, differences in urinary Mg output reflected differences in apparent absorption of Mg (Tables 7 and 8).

As expected, feeding MgSO₄ without monensin increased Mg apparent absorption and urinary Mg excretion compared with MgO. The 10% increase in Mg absorption when supplementing MgSO₄ compared with MgO was substantially lower than the 20 to 40% increase predicted by the NRC (2001) and Van Ravenswaay et al. (1989). Apparent absorption of Mg from MgO was increased by monensin, similar to previous observations in steers (Starnes et al., 1984) and sheep (Greene et al., 1986), although average dietary Mg concentrations were much higher in our study (0.15 vs. 0.36% Mg). However, with monensin, MgSO₄ had decreased Mg absorption and urinary excretion compared with MgO (monensin $\times$ Mg interactions; $P < 0.04$). Greater endogenous fecal loss (Greene et al., 1988) or lower ruminal and intestinal absorption could have caused decreased apparent absorption when feeding both monensin and MgSO₄. An alternative reason for the decreased absorption could be from the higher amounts of undigested

Table 9. Fatty acid composition of diets and forages

Composition, g/100 g of total fatty acids	Treatment ¹				
	Control		Monensin		
	MgO	MgSO ₄	MgO	MgSO ₄	Alfalfa silage
8:0	1.10	1.12	1.08	1.10	1.81
10:0	0.76	0.76	0.76	0.76	0.60
12:0	0.57	0.57	0.57	0.57	0.25
14:0	0.40	0.40	0.40	0.40	0.27
14:1	0.14	0.14	0.15	0.14	0.05
16:0	13.9	14.0	13.9	14.0	13.1
16:1	0.47	0.48	0.47	0.48	0.29
18:0	3.06	3.05	3.02	3.05	2.19
<i>cis</i> -9, 18:1	15.5	15.7	15.5	15.6	14.9
Other 18:1	1.86	1.90	1.86	1.91	2.37
18:2	38.0	37.8	38.1	37.9	45.3
18:3	10.9	10.9	10.9	10.9	5.27
20:0	0.83	0.82	0.83	0.84	1.46
22:0	0.42	0.44	0.45	0.44	0.42
Other	12.1	11.9	11.9	11.9	11.7
Unsaturated	67.6	67.7	67.7	67.6	69.1
Saturated	21.5	21.5	21.6	21.6	20.4

¹Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO₄. Treatments contained different concentrate but similar silage.

NDF in this diet. High amounts of undigested fiber can bind cationic minerals and reduce absorption (Spears, 2003). A recent experiment within our laboratory also found 90% greater Mg apparent absorption (22.3 vs. 11.8% of intake) and 15% greater NDF digestibility (51 vs. 44%) for byproduct-based diets compared with the forage (Faulkner et al., 2017; Faulkner and Weiss, 2017). The authors accredited most of the increased absorption primarily to lower Mg apparent absorption from MgO compared with the Mg contained in the byproduct feeds, but differences in indigestible fiber could have contributed to the effects. Concentration of Mg in milk was greater ($P < 0.03$) for cows fed MgSO_4 , but the difference was small and did not affect output of Mg in milk ($P \geq 0.73$). The balance of Mg was also not affected by treatments ($P \geq 0.15$).

Supplementing MgSO_4 compared with MgO almost doubled S intake, and almost 90% of that difference was apparently absorbed. Metabolic fecal S may have been diluted by the greater S intake, causing the greater ($P < 0.01$) apparent absorption. The primary form of S in the MgO diets was probably organic. Absorption of S from inorganic S can be greater than organic forms (Bouchard and Conrad, 1973) and could also explain increased absorption. Increased S apparently absorbed from MgSO_4 increased urinary S excretion about 35 g/d accounting for about 89% of the difference in absorbable intake. Total amount of S in milk was not different ($P \geq 0.20$) and averaged 0.32 g/kg of milk. The remainder of absorbed S increased apparent balance 5.4 g/d in MgSO_4 diets ($P < 0.01$) and may partially reflect losses as eructated H_2S .

Table 10. Effects of monensin and Mg source on milk fatty acids

Fatty acid, ¹ g/kg	Control			Treatment ²			<i>P</i> -value		
				Monensin					
	MgO	MgSO ₄		MgO	MgSO ₄	SEM	Monensin	Mg	Monensin × Mg
4:0	26.9	28.0		26.5	28.2	0.84	0.94	0.05	0.67
6:0	21.7	21.0		21.3	22.7	0.62	0.23	0.21	0.01
8:0	12.1	11.3		12.3	12.9	0.49	0.04	0.82	0.01
10:0	29.7	26.8		31.0	31.9	1.49	0.03	0.13	0.02
12:0	34.4	30.6		36.6	37.3	1.90	0.03	0.06	0.03
<i>iso</i> 13:0	0.42	0.37		0.46	0.58	0.07	0.08	0.56	0.22
<i>anteiso</i> 13:0	0.20	0.13		0.12	0.43	0.09	0.20	0.19	0.04
13:0	1.05	0.88		1.22	1.07	0.07	0.03	0.01	0.71
<i>iso</i> 14:0	1.08	1.10		1.02	1.08	0.11	0.79	0.48	0.74
14:0	123.2	115.1		124.8	125.5	3.68	0.15	0.06	0.04
14:1	9.07	8.21		9.23	9.38	0.76	0.41	0.45	0.34
<i>iso</i> 15:0	2.64	2.53		2.45	2.42	0.12	0.32	0.16	0.49
<i>anteiso</i> 15:0	5.39	4.97		5.66	5.12	0.24	0.48	0.01	0.67
15:0	11.1	10.0		12.2	11.0	0.50	0.06	0.01	0.88
<i>iso</i> 16:0	2.69	2.84		2.45	2.94	0.32	0.82	0.04	0.30
16:0	325	321		334	338	12.1	0.23	0.99	0.46
<i>iso</i> 17:0	4.17	4.20		4.28	3.67	0.29	0.43	0.27	0.25
<i>cis</i> -9 16:1 + <i>anteiso</i> 17:0	7.13	4.21		3.24	6.71	3.23	0.83	0.92	0.23
17:0	6.81	6.91		7.30	6.98	0.13	0.03	0.35	0.07
17:1	1.81	2.22		1.96	1.86	0.18	0.60	0.22	0.08
18:0	112.6	120.1		105.5	101.5	7.12	0.11	0.73	0.28
<i>trans</i> -6 + <i>trans</i> -8 18:1	3.82	3.63		3.69	3.43	0.18	0.29	0.02	0.70
<i>trans</i> -9 18:1	5.06	5.19		6.02	5.49	0.54	0.35	0.51	0.28
<i>trans</i> -10 18:1	12.2	11.7		12.4	11.3	0.60	0.86	0.11	0.52
<i>trans</i> -11 18:1	2.58	2.37		2.70	2.42	0.15	0.53	0.07	0.81
<i>trans</i> -12 18:1	4.82	4.62		4.86	4.93	0.29	0.40	0.73	0.50
<i>cis</i> -9 18:1	189.8	209.4		185.1	178.1	7.44	0.02	0.20	0.02
<i>cis</i> -11 18:1	5.30	6.00		5.53	5.38	0.39	0.57	0.39	0.23
18:2	25.1	24.5		26.3	25.4	2.22	0.63	0.57	0.90
18:3	5.47	5.22		5.51	5.14	0.47	0.93	0.10	0.76
20:0	0.34	0.34		0.33	0.37	0.03	0.61	0.33	0.34
20:1	0.21	0.28		0.24	0.29	0.05	0.60	0.08	0.86
<i>cis</i> -9, <i>trans</i> -11 18:2	5.28	4.92		5.20	5.16	0.39	0.86	0.47	0.57
<i>trans</i> -10, <i>cis</i> -12 18:2	0.06	0.04		0.05	0.04	0.03	0.80	0.48	0.91
Other CLA	0.12	0.12		0.16	0.16	0.03	0.33	0.89	0.90
Short- and medium-chain fatty acids	279	261		285	290	8.32	0.05	0.07	0.01
Long-chain fatty acids	386	411		375	362	11.7	0.02	0.43	0.03

¹Number of carbons:number of double bonds.

²Diets with 0 or 14 mg/kg of monensin and with Mg from MgO or MgSO_4 .

Fatty Acid Metabolism and Milk Fatty Acid Profile

The fatty acid concentrations of the diets were similar (Table 9). Apparent digestibility of total LCFA was not affected ($P \geq 0.43$) by dietary treatment (Table 5). In milk fat, monensin tended to increase ($P < 0.06$) C15:0 and increased ($P < 0.03$) C13:0 and 17:0 (Table 10). Increased proportions of linear odd-chain fatty acids are associated with increases in ruminal fatty acids (Vlaeminck et al., 2006) and is commonly observed when supplementing monensin (Ipharraguerre and Clark, 2003; Duffield et al., 2008). When feeding MgSO_4 without monensin, the concentrations of short- and medium-chain fatty acids were lower and LCFA was greater in milk fat compared with the other diets (monensin $\times$ Mg source interaction; $P < 0.03$). In particular, C6, 8, 10, 12, and 14 decreased, and *cis*-9:18:1 increased, suggesting these cows were mobilizing fat (Jorjong et al., 2014). The higher concentration of LCFA in milk could also explain the numerical BW loss of cows consuming MgSO_4 without monensin (Table 4).

CONCLUSIONS

Our data suggest that the NRC (2001) model substantially overestimates the absorption of Mg from MgSO_4 relative to that from MgO. The interaction between Mg source and monensin indicates that monensin should be considered when determining Mg supplementation rates. Besides decreasing Mg apparent absorption, feeding monensin with MgSO_4 decreased NDF digestibility. Although supplementing MgSO_4 did improve Ca metabolism and starch digestibility, at equal dietary Mg concentrations MgO was still the superior source for milk production, whereas monensin had minor effects on production and digestibility.

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REFERENCES

- Ammerman, C., C. Chicco, P. Loggins, and L. Arrington. 1972. Availability of different inorganic salts of magnesium to sheep. *J. Anim. Sci.* 34:122–126.

- AOAC International. 2000. Official methods of analysis of the AOAC International. Vol. 1 and 2. AOAC International, Gaithersburg, MD.
- Benchear, C., H. Petit, R. Berthiaume, T. Whyte, and P. Chouinard. 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. *J. Dairy Sci.* 89:4352–4364.
- Bergen, W. G., and D. B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. *J. Anim. Sci.* 58:1465–1483.
- Blaxter, K., and R. F. McGill. 1956. Magnesium metabolism in cattle. *Vet. Rev.* 2:35–55.
- Bouchard, R., and H. Conrad. 1973. Sulfur requirement of lactating dairy cows. I. Sulfur balance and dietary supplementation. *J. Dairy Sci.* 56:1276–1282.
- Chrip, J., A. R. Sykes, and N. Grace. 1989. Faecal endogenous loss of calcium in young sheep. *Br. J. Nutr.* 61:59–65.
- Delfiol, D. J., D. Q. Cagnini, P. H. Cunha, N. Crosignani, A. T. Wouters, F. Wouters, D. Driemeier, and A. S. Borges. 2013. Clinical and laboratory aspects of sheep supplemented with high levels of sulfur in diet to induce polioencephalomalacia. *Pesqui. Vet. Bras.* 33:435–442.
- Drewnoski, M., D. Pogge, and S. Hansen. 2014. High-sulfur in beef cattle diets: A review. *J. Anim. Sci.* 92:3763–3780.
- Duffield, T., A. Rabiee, and I. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. *J. Dairy Sci.* 91:1334–1346.
- Erdman, R., R. Henken, and L. 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.
- Faulkner, M., N. St-Pierre, and W. Weiss. 2017. Effect of source of trace minerals in either forage or by-product-based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. *J. Dairy Sci.* 100:5368–5377.
- Faulkner, M., and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product-based diets fed to dairy cows: 1. Production and macronutrient digestibility. *J. Dairy Sci.* 100:5358–5367.
- Ferraretto, L., P. Crump, and R. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. *J. Dairy Sci.* 96:533–550.
- Greene, L., B. May, G. Schelling, and F. Byers. 1988. Site and extent of apparent magnesium and calcium absorption in steers fed monensin. *J. Anim. Sci.* 66:2987–2991.
- Greene, L., G. Schelling, and F. Byers. 1986. Effects of dietary monensin and potassium on apparent absorption of magnesium and other macroelements in sheep. *J. Anim. Sci.* 63:1960–1967.
- Grings, E., and J. Males. 1988. Performance, blood and ruminal characteristics of cows receiving monensin and a magnesium supplement. *J. Anim. Sci.* 66:566–573.
- Hall, M. B. 2009. Determination of starch, including maltoligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. *J. AOAC Int.* 92:42–49.
- Holtenius, K., C. Krongvist, E. Briland, and R. Spörndly. 2008. Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *J. Dairy Sci.* 91:743–748.
- Hubbert, F., E. Cheng, and W. Burroughs. 1958. Mineral requirement of rumen microorganisms for cellulose digestion. *J. Anim. Sci.* 17:559–568.
- Hultanen, P., M. Rinne, and J. Nousiainen. 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. *J. Dairy Sci.* 92:5031–5042.
- Ipharraguerre, I. R., and J. H. Clark. 2003. Usefulness of ionophores for lactating dairy cows: A review. *Anim. Feed Sci. Technol.* 106:39–57.
- Isaac, R., and W. Johnson. 1985. Elemental analysis of plant tissue by plasma emission spectroscopy: Collaborative study. *J. Assoc. Off. Anal. Chem.* 68:499.

- Ivanic, J., and W. Weiss. 2001. Effect of dietary sulfur and selenium concentrations on selenium balance of lactating Holstein cows. *J. Dairy Sci.* 84:225–232.
- Iwaniuk, M., and R. 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.
- Jenkins, T. 2000. Feeding oleamide to lactating jersey cows. Effects on lactation performance and milk fatty acid composition. *J. Dairy Sci.* 83:332–337.
- Jones, J., B. Wolf, and H. Mills. 1991. Microwave digestion using cem microwave digestion system. *Plant Analysis Handbook*. Micro-Macro Publishing. Athens, GA.
- Jorjong, S., A. Van Kneusel, J. Verwaeren, M. V. Lahoz, R. Bruckmaier, B. De Baets, B. Kemp, and V. Fievez. 2014. Milk fatty acids as possible biomarkers to early diagnose elevated concentrations of blood plasma nonesterified fatty acids in dairy cows. *J. Dairy Sci.* 97:7054–7064.
- Kandylis, K. 1984. Toxicology of sulfur in ruminants. *J. Dairy Sci.* 67:2179–2187. (Review)
- LaCroix, R., D. Keeney, and L. Walsh. 1970. Potentiometric titration of chloride in plant tissue extracts using the chloride ion electrode. *Commun. Soil Sci. Plant Anal.* 1:1–6.
- Martens, H., S. Leonhard, and G. Gäbel. 1991. Minerals and digestion: Exchanges in the digestive tract. Page 199–216 in *Rumen Microbial Metabolism and Ruminant Digestion*. J.-P. Jouany, ed. Institut National de la Recherche Agronomique, Clermont-Ferrand, France.
- Martens, H., and M. Schweigel. 2000. Pathophysiology of grass tetany and other hypomagnesemias: Implications for clinical management. *Vet. Clin. North Am. Food Anim. Pract.* 16:339–368.
- Martineau, R., C. Benchaar, H. Petit, H. Lapierre, D. Ouellet, D. Pelletier, and R. Berthiaume. 2007. Effects of lasalocid or monensin supplementation on digestion, ruminal fermentation, blood metabolites, and milk production of lactating dairy cows. *J. Dairy Sci.* 90:5714–5725.
- Morales, M., and B. Dehority. 2014. Magnesium requirement of some of the principal rumen cellulolytic bacteria. *Animal* 8:1427–1432.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Plaizier, J., A. Martin, T. Duffield, R. Bagg, P. Dick, and B. McBride. 2000. Effect of a prepartum administration of monensin in a controlled-release capsule on apparent digestibilities and nitrogen utilization in transition dairy cows. *J. Dairy Sci.* 83:2918–2925.
- Roche, J., J. Morton, and E. Kolver. 2002. Sulfur and chlorine play a non-acid base role in periparturient calcium homeostasis. *J. Dairy Sci.* 85:3444–3453.
- Schären, M., C. Droug, K. Kiri, S. Riede, M. Gardener, U. Meyer, J. Hummel, T. Urich, G. Breves, and S. Dänicke. 2017. Differential effects of monensin and a blend of essential oils on rumen microbiota composition of transition dairy cows. *J. Dairy Sci.* 100:2765–2783.
- Schnewille, J. T., A. T. Van't Klooster, A. Dirkwager, and A. Beynen. 1994. Stimulatory effect of an anion (chloride)-rich ration on apparent calcium absorption in dairy cows. *Livest. Prod. Sci.* 40:233–240.
- Schweigel, M., and H. Martens. 2000. Magnesium transport in the gastrointestinal tract. *Front. Biosci.* 5:666–677.
- Spears, J. W. 1990. Ionophores and nutrient digestion and absorption in ruminants. *J. Nutr.* 120:632–638.
- Spears, J. W. 2003. Trace mineral bioavailability in ruminants. *J. Nutr.* 133:1506S–1509S.
- Starnes, S., J. Spears, M. Froetschel, and W. J. Croom. 1984. Influence of monensin and lasalocid on mineral metabolism and ruminal urease activity in steers. *J. Nutr.* 114:518–525.
- Stockdale, C. 2004. Effects of feeding magnesium sulfate to dry pregnant dairy cows with different body condition scores on intake in late gestation, periparturient blood calcium concentrations and production in early lactation. *Aust. J. Exp. Agric.* 44:539–546.
- Tebbe, A., M. Faulkner, and W. Weiss. 2017. Effect of partitioning the nonfiber carbohydrate fraction and neutral detergent fiber method on digestibility of carbohydrates by dairy cows. *J. Dairy Sci.* 100:6218–6228.
- Uwituze, S., G. Parsons, K. Karges, M. Gibson, L. Hollis, J. Higgins, and J. Drouillard. 2011a. Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets. *J. Anim. Sci.* 89:2817–2828.
- Uwituze, S., G. Parsons, C. Schneider, K. Karges, M. Gibson, L. Hollis, J. Higgins, and J. Drouillard. 2011b. Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn. *J. Anim. Sci.* 89:2582–2591.
- Van Ravenswaay, R., P. Henry, C. Ammerman, and R. Littell. 1989. Comparison of methods to determine relative bioavailability of magnesium in magnesium oxides for ruminants. *J. Dairy Sci.* 72:2968–2980.
- van Zijderveld, S., W. Gerrits, J. Apajalahti, J. Newbold, J. Dijkstra, R. Leng, and H. Perdok. 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. *J. Dairy Sci.* 93:5856–5866.
- Vlaeminck, B., V. Fievez, A. Cabrita, A. Fonseca, and R. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Anim. Feed Sci. Technol.* 131:389–417.
- Weiss, W. 2004. Macromineral digestion by lactating dairy cows: Factors affecting digestibility of magnesium. *J. Dairy Sci.* 87:2167–2171.
- Weiss, W. P., N. St-Pierre, and L. Willett. 2009. Varying type of forage, concentration of metabolizable protein, and source of carbohydrate affects nutrient digestibility and production by dairy cows. *J. Dairy Sci.* 92:5595–5606.
- Weiss, W. P., and D. Wyatt. 2000. Effect of oil content and kernel processing of corn silage on digestibility and milk production by dairy cows. *J. Dairy Sci.* 83:351–358.
- Weiss, W. P., and D. Wyatt. 2003. Effect of dietary fat and vitamin E on α -tocopherol in milk from dairy cows. *J. Dairy Sci.* 86:3582–3591.
- Zinn, R., E. Alvarez, M. Mendez, M. Montano, E. Ramirez, and Y. Shen. 1997. Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle. *J. Anim. Sci.* 75:1723–1728.