



Short communication

## Magnesium butyrate is a readily available magnesium source in dairy cow nutrition

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## ABSTRACT

The aim of the present study was to measure the apparent absorption of magnesium (Mg) originating from Mg-butyrate. Six mid-lactation Holstein Friesian dairy cows were used with dietary treatments arranged in a cross-over design. Two different diets were fed during the experiment, consisting of a low Mg diet without Mg-butyrate (L-Mg, 3.1 g Mg/kg dry matter) or a high Mg diet with Mg-butyrate (H-Mg, 3.9 g Mg/kg dry matter). Cows offered the L-Mg diet ingested 54.7 g Mg/day while the cows fed the H-Mg diets ingested 66.3 g Mg/day ( $P < 0.001$ ). The fecal excretion of Mg, however, was similar between the two experimental diets ( $P = 0.174$ ). Consequently, apparent Mg absorption was found to be 7.9 % units greater ( $P = 0.038$ ) when the cows were fed the diet supplemented with Mg-butyrate. The greater Mg absorption after feeding the H-Mg diet was, however, not reflected by a greater urinary Mg concentration ( $P = 0.228$ ). The fractional Mg absorption from Mg-butyrate was calculated to be 71.6 %, which indicates that Mg from Mg-butyrate is readily available for absorption. In conclusion, Mg-butyrate is an attractive alternative to supplement dairy rations with Mg.

### 1. Introduction

Magnesium (Mg) is an essential nutrient for cows. This means that dairy rations need to supply a sufficient amount of absorbable Mg to safeguard the cow's health. Currently, there is no experimental proof for any specific Mg regulating hormone (Martens and Schweigel, 2000). This implies that Mg intake as such does not downregulate the efficiency of Mg absorption. Consequently, the amount of absorbed Mg in excess of requirement is excreted in the urine in order to maintain Mg balance. The efficiency of Mg absorption is considered the critical determinant in the Mg supply of dairy cows (Schonewille, 2013). The amount of Mg that is available for metabolism depends not only on the amount of Mg ingested by the animals but also on the solubility of the Mg source because only ionized Mg can be absorbed (Leonhard et al., 1990). Moreover, the amount of absorbable Mg also depends on the content of dietary potassium (K). It is well known that K inhibits Mg absorption in the rumen of the cow (Schonewille et al., 1999). This increases the risk of hypomagnesemia (Schonewille et al., 2000), and subsequently grass tetany or milk fever (Lean et al., 2006).

In many countries, grass and its co-products are usually rich in K. Consequently, it is common practice in such countries to supplement dairy rations with Mg. Magnesium-oxide (MgO) is currently widely used as a source of supplemental Mg. The effectiveness of

*Abbreviations:* DM, dry matter; K, potassium; Mg, magnesium; MgO, magnesium oxide; SE, standard error; SCFA, short chain fatty acids; Ti, titanium.

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MgO in terms of supplying Mg, however, is limited (Jittakhot et al., 2004) and can vary greatly between different MgO sources (Jesse et al., 1981; Schonewille et al., 1992; Xin et al., 1989). As a consequence alternative Mg sources, such as Mg-butyrate, might be of interest.

Currently, supplemental butyrate has gained interest for ruminants in both practice and research. This interest is related to the evidence that butyrate is a main stimulator of papillae growth and epithelial cell proliferation in the rumen (Mentschel et al., 2001; Malhi et al., 2013). By increasing the size of rumen papillae, and thus the rumen surface area, absorption of short chain fatty acids is enhanced. From this perspective, Mg supplementation in the form of Mg-butyrate can be considered opportune to use in the nutrition of dry cows due to the decrease in rumen surface area during the dry period (Dieho et al., 2016). Thus, the use of Mg-butyrate instead of MgO seems an attractive alternative to supplement dairy rations with Mg. However, the availability of Mg from Mg-butyrate is not known. The aim of the current experiment was, therefore, to measure the apparent Mg absorption when Mg-butyrate is used as a supplemental Mg source.

## 2. Materials and methods

The animal study protocol was approved by the Institutional Review Board of the Faculty of Veterinary Medicine, Utrecht University (approval number 10803-2020-05).

### 2.1. Experimental design

Six, multiparous, mid-lactation cows (approx. 120 days in milk) producing around 30 kg of milk per day were used in the experiment. The experiment was designed as a cross-over with 2 experimental periods of 14 days each, preceded by a 14 day pre-experimental period to allow the cows to become adapted to the experimental rations. The cows were randomly assigned to the order of the two experimental treatments (N = 6 per dietary treatment). The cows were individually fed in a stanchion barn with unrestricted access to fresh water.

### 2.2. Experimental rations

During the pre-experimental period all six cows were fed a basal ration (Table 1) supplemented with 2.7 kg dry matter (DM) commercial compound feed and 1.8 kg DM low sugar beet pulp without any additives. During the experimental periods, the beet pulp was replaced by experimental beet pulp, i.e., beet pulp with or without supplemented Mg-butyrate (molar ratio of Mg: butyric acid= 1: 2), which was termed either high or low Mg beet pulp, respectively (Table 1). The supplemental Mg-butyrate preparation used, RumensReady® (Palital Feed Additives, Veldriel, The Netherlands), was in the form of a micro pellet with a diameter of ~ 1.2 mm and consists of (as fed) 70 % Mg-butyrate encapsulated in a fat matrix. Both types of experimental beet pulp contained titanium oxide (TiO<sub>2</sub>), which was used as an inert marker for determination of fecal output.

### 2.3. Feeding procedure

At 9:00 h the daily individual feed refusals, if any, were collected. These were then weighed and stored at – 18 °C. The rations were

**Table 1**  
Ingredients and calculated chemical composition of the experimental rations.

Items	Treatment	
	Low Mg	High Mg
Ingredient composition (kg DM):		
Basal ration <sup>a</sup>	13.6	13.6
Compound feed	2.7	2.7
Experimental beet pulp		
Low Mg <sup>b</sup>	1.8	-
High Mg <sup>c</sup>	-	1.8
Total (kg DM)	18.1	18.1
Calculated composition of the whole experimental ration		
NEL, MJ/kg DM <sup>d</sup>	6.91	6.91
Mg, g/kg DM	3.1	3.9
K, g/kg DM	23.7	23.7
Ca, g/kg DM	6.0	6.0
P, g/kg DM	3.3	3.3

<sup>a</sup> Basal ration consisted of (% DM): grass silage, 66.9; corn silage, 26.5; bypass soybean meal, 5.9; mineral premix, 0.7.

<sup>b</sup> Low Mg beet pulp; pellet containing 99.2 % low sugar beet pulp and 0.8 % TiO<sub>2</sub>.

<sup>c</sup> High Mg beet pulp; pellet containing 91.7 % low sugar beet pulp, 0.8 % TiO<sub>2</sub> and 7.5 % RumensReady®.

<sup>d</sup> NEL = Net Energy Lactation.

offered twice daily in two equal portions starting at ~ 9:15 h and ~ 16:30 h. At each feeding time, cows were first offered their allocated amounts of compound feed and beet pulp and then ~ 30 min later, half of the daily portion of the basal ration was offered (Table 1).

#### 2.4. Sampling procedure and chemical analysis

During the last 4 days of each 14 day experimental period, all spontaneously voided feces and urine were collected between 9:00 and 17:00. At the end of each collection day, the individual feces collections were stored at  $-18^{\circ}\text{C}$  whereas the individual urine collections were stored at  $5^{\circ}\text{C}$ . At the end of each experimental period, the individual feces collections were thawed. Then, all individual feed refusals, feces and urine collections were pooled per cow and mixed thoroughly. Thereafter, the pooled fecal and urine samples were stored at  $-18^{\circ}\text{C}$  pending chemical analysis. All pooled samples were air dried at  $60^{\circ}\text{C}$  for 24 h, and the dry matter (DM) content of the air-dried samples was determined with the use of a forced-air oven ( $105^{\circ}\text{C}$ , 24 h) according to ISO (1999). The Mg content of feces, urine, feed and feed refusals was measured by means of inductively coupled plasma mass spectrometry. The titanium concentration of the experimental beet pulp and feces was analyzed using a spectrophotometer (Beckman Coulter Inc., Brea, USA) equipped with sipper module for 408 nm instead of 410 nm as reported by Myers et al. (2006).

#### 2.5. Statistical analysis

All data were subjected to analysis of variance with the general linear model procedure in SPSS, using the model:  $Y_{ij} = \mu + \text{PERIOD}_i + \text{TREATMENT}_j + e_{ij}$ .

where  $Y_{ij}$  = a response variable (e.g., Mg intake, fecal Mg excretion, etc.);  $\mu$  = overall mean;  $\text{PERIOD}_i$  = experimental period ( $i = 1$  or  $2$ );  $\text{TREATMENT}_j$  = level of dietary magnesium ( $j = \text{low or high}$ ); and  $e_{ij}$  = residual error. Both, the PERIOD and TREATMENT were set as fixed factors in the statistical model. Throughout, the level of statistical significance was pre-set at  $P \leq 0.05$ .

### 3. Results

#### 3.1. Feed intake and milk yield

The amount of DM offered was not fully consumed by the cows and the mean amounts of DM refused were 1.1 kg DM and 0.7 kg DM for the cows fed the high- and low Mg rations, respectively. Consequently, DM intakes were found to be 16.9 kg (SE  $\pm$  0.51) and 17.4 kg (SE  $\pm$  0.43) for high- and low Mg treatment groups ( $P = 0.543$ ), respectively. The mean milk production was similar ( $P = 0.836$ ) between the two treatments, i.e. 26.5 kg/day (SE  $\pm$  2.39) and 27.2 kg/day (SE  $\pm$  2.04) for the high- and low Mg treatments, respectively.

#### 3.2. Mg absorption and urinary Mg concentration

The intake of Mg increased ( $P < 0.001$ ) from 54.7 g/day to 66.3 g/day when the cows were fed the high- instead of the low Mg ration (Table 2). However, fecal Mg excretion was found to be similar ( $P = 0.174$ ) between the two experimental rations. Consequently, Mg absorption, either expressed as g/day or as a % of intake, was greater ( $P \leq 0.038$ ) when the high Mg ration was fed. Numerically, the urinary Mg concentration increased by 31 % (Table 2) when the animals were fed the high Mg ration, however, the difference was not statistically significant ( $P = 0.228$ ).

### 4. Discussion

In the current study, the amount of absorbed Mg was found to be 14.3 g/day when the low Mg ration, containing 23.7 g K/kg DM, was fed. The value on Mg absorption observed in the current study is 13.4 % greater than predicted based on the regression formula reported by Schonewille et al. (2008), i.e.,  $\text{Mg absorption (g/day)} = 3.6 + 0.2 \times \text{Mg intake (g/day)} - 0.08 \times \text{Dietary K (g/kg DM)}$ . Thus, when the low Mg ration was fed, absolute Mg absorption (g/day) was more or less in line with expectation. When the cows were fed the ration supplemented with Mg-butyrate, predicted Mg absorption (Schonewille et al., 2008) was calculated to be 15.0 g/day but

**Table 2**

Mg intake, fecal Mg excretion, Mg absorption and urinary Mg concentration in cows fed the experimental rations with or without Mg-butyrate supplementation (i.e., high Mg and low Mg, respectively).

Items	Low Mg	High Mg	SEM	P-value
Mg intake, g/day	54.7	66.3	1.40	<0.001
Fecal Mg excretion, g/day	40.4	43.7	1.58	0.174
Apparent Mg absorption				
g/day	14.3	22.6	1.66	0.006
% of intake	26.2	34.1	2.30	0.038
Urinary Mg concentration, mM	10.6	13.9	1.82	0.228

observed Mg absorption was found to be ~1.5 times greater than predicted (Schonewille et al., 2008). This indicates that Mg from Mg-butyrate is readily available for absorption. Indeed, the absolute difference in Mg absorption ( $\Delta$ Mg absorption) between the current low- and high Mg diet was 8.3 g/day while the absolute difference in Mg intake between the diets ( $\Delta$ Mg intake) was 11.6 g/day. Thus, the fractional Mg absorption from Mg-butyrate was calculated to be 71.6 % (i.e., calculated as  $\Delta$ Mg absorption /  $\Delta$ Mg intake  $\times$  100 %). The calculated fractional Mg absorption from Mg-butyrate is much greater compared to the likewise derived values on the fractional Mg absorption from MgO (Table 3). Thus, it appears that Mg-butyrate, relative to MgO, is superior in rendering Mg available for absorption.

For obvious reasons, the current study does not provide clues to explain the difference in Mg absorption between Mg-butyrate and MgO, but it is well known that only ionized Mg can be absorbed (Leonhard et al., 1990). In contrast to Mg-butyrate, the soluble part of MgO will react with water which results in  $Mg^{2+}$  and two hydroxide ions (OH<sup>-</sup>). The latter is a strong base and will act as a sink for H<sup>+</sup> ions which can be donated, amongst others, from short chain fatty acids (SCFA). This reasoning is in line with the observation that supplemental MgO increases the buffer capacity of rumen contents (Erdman, 1988). It can thus be speculated that the  $Mg^{2+}$  ions originating from solubilized MgO are absorbed in association with dissociated SCFA. Taking the aforementioned reasoning into account, it is suggested that the greater Mg absorption from Mg-butyrate versus MgO is related to a greater solubility of Mg-butyrate.

To the best of the authors' knowledge, there are currently no published data reporting on the rumen solubility of Mg-butyrate or the availability of Mg from Mg-butyrate. However, Ross and Gibson (1969) reported that supplemental Mg in the form of either calcined magnesite or Mg-acetate prevented hypomagnesemia in grazing cows. Unfortunately, Mg intakes were not kept constant between supplemental Mg sources and Mg absorption was not measured in the Ross and Gibson (1969) study, thereby hindering proper interpretation of the data. Interestingly, Giduck and Fontenot (1987) reported on the stimulatory effect of readily fermentable carbohydrates on Mg absorption in sheep. This observation in sheep fuels the idea that SCFA are instrumental in stimulating Mg absorption in ruminants. This notion is in line with the outcome of fundamental in-vitro research on Mg transport across the rumen epithelium.

The process of Mg uptake by rumen epithelial cells (Tomas and Potter, 1976) consists of two components, one sensitive and one insensitive to K (Leonhard-Marek and Martens, 1996). It was shown by Leonhard-Marek et al. (1998) that SCFA can stimulate Mg absorption in-vitro with butyrate, relative to acetate and propionate, being most effective. The authors suggested that SCFA absorption rate may play a role in this, as the ability of individual SCFA to stimulate  $Mg^{2+}$  absorption followed the same sequence as their absorption rate. The study results prompted the authors (Leonhard-Marek et al., 1998; Leonhard-Marek et al., 2010) to propose that a K-independent transporter was located at the apical membrane of the rumen epithelial cell, which exchanges one Mg ion for two hydrogen ions. It can, therefore, be speculated that Mg butyrate provided the protons to stimulate the K-independent transport across the apical membrane of the epithelial cells in the rumen. However, Schweigel and Martens (2003) were not able to demonstrate a  $Mg^{2+} / 2 H^{+}$  exchanger in isolated rumen epithelium cells and these authors suggested the involvement of H<sup>+</sup>-ATPase activity to explain the butyrate induced stimulation in Mg transport across rumen epithelial cells. The involvement of H<sup>+</sup>-ATPase activity implicates that SCFA-mediated Mg transport may also involve a voltage dependent pathway (Schweigel and Martens, 2003). Clearly, the mechanism underlying the stimulatory effect of SCFA/butyrate on Mg absorption is not settled yet (Martens et al., 2018).

Magnesium transport across the basolateral membrane of rumen epithelial cells depends on a carrier mediated process based on the exchange of a Mg ion for two Na ions (Schweigel et al., 2000; Leonhard-Marek et al., 2010). In principle, the transport of Mg across the rumen epithelium can become saturated (Martens, 1983). Jittakhot et al. (2004) reported that the apparent Mg absorption in dry cows became saturated when a ration was fed containing ~12 g Mg/kg dry matter. Therefore, it can be speculated that the process of Mg absorption was not saturated in the current feeding experiment due to the rations containing a much lower amount of Mg.

Despite the numerical increase in the urinary Mg concentration after the feeding of supplemental Mg-butyrate, the difference in the urinary Mg concentration did not reach statistical significance. This observation is not easy to explain because both DM intake and milk yield were found to be similar between the two experimental rations. It can be speculated that both Mg excretion with milk and the intake of electrolytes (i.e., Na and K), was similar between the two rations. In view of the latter, the volume of urine produced is likely to have been similar between the two treatments. It seems, therefore, fair to assume that the lack of response in urinary Mg excretion

**Table 3**

Mg intake, Mg absorption,  $\Delta$ Mg intake,  $\Delta$ Mg absorption in cows fed increasing amounts of Mg in the form of MgO (Jittakhot et al., 2004).

Ration <sub>i</sub>	Mg intake (g/day)	Mg absorption (g/day)	$\Delta$ Mg intake <sup>a</sup> (g/day)	$\Delta$ Mg absorption (g/day) <sup>b</sup>	(% of intake) <sup>c</sup>
1 <sup>d</sup>	27.1	3.4	-	-	-
2	44.6	7.0	17.5	3.6	20.6
3	64.6	11.9	20.0	4.9	24.5
4	83.5	17.1	18.9	5.2	27.5
5	100.4	20.2	16.9	3.1	18.3
6	124.3	22.0	23.9	1.8	7.5

<sup>a</sup>  $\Delta$ Mg intake is calculated as the difference in Mg intake between ration<sub>i+1</sub> and ration<sub>i</sub>. For example, the difference in Mg intake between ration 2 and ration 1 = 44.6 – 27.1 = 17.5 g Mg/day.

<sup>b</sup>  $\Delta$ Mg absorption (g/day) is calculated as the difference in Mg absorption between ration<sub>i+1</sub> and ration<sub>i</sub>. For example, the difference in Mg absorption between ration 2 and ration 1 = 7.0 – 3.4 = 3.6 g Mg/day

<sup>c</sup>  $\Delta$ Mg absorption expressed as % of intake is the percentage absorption of  $\Delta$ Mg intake, i.e. ( $\Delta$ Mg absorption /  $\Delta$ Mg intake)  $\times$  100 %.

<sup>d</sup> Ration<sub>1</sub> is the control ration, not supplemented with MgO.

cannot be explained by a difference in urine volume. Thus, the probability of type-1 error was most likely unfavorably affected by the high variation in the urinary Mg concentration.

## 5. Conclusion

Mg-butyrate is a readily available Mg source and can be considered an attractive alternative to MgO to supplement dairy rations with Mg.

## CRedit authorship contribution statement

Conceptualization, J.E. Edwards (J.E.E.), J.T. Schonewille (J.T.S.); Methodology, J.T.S.; Formal analysis, J.T.S.; Investigation, B.M. de Groot (B.M.G.); Resources, J.T.S.; Data curation, B.M.G.; Writing – original draft, J.T.S and B.M.G.; Writing – review & editing, J.T.S., B.M.G., J.E.E.; Supervision, J.T.S.; Project administration, J.T.S.; Funding acquisition, J.T.S.. All authors have read and agreed to the published version of the manuscript.

## Declaration of Competing Interest

The authors herewith confirm that there are no conflicts of interest.

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