Magnesium Solubility in the caecum in response to pH changes

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ABSTRACT

Five two-tooth Coopworth ewes were used to monitor the effects of changing caecal pH on magnesium (Mg) solubility and absorption pH change was induced by infusion of volatile fatty acid (VFA) (0, 220, 440, 660, 880 mmol; ratio 0.80:0.15:0.05 acetic:propionic:butyric in 2016 ml of deionised water) into the terminal ileum for 24 h. Treatments were randomly applied to each animal using a latin square design. During the infusion samples of blood and urine were taken at 4 h intervals and proximal colonic digesta at 4, 16 and 24 h. Apparent Mg absorption from the large intestine was estimated from changes in plasma Mg concentration and urinary Mg excretion. Sixteen hours after the commencement of infusion digesta pH decreased and Mg solubility increased. For example, with the 880 mmol infusion digesta pH had decreased by 2.1 ± 0.48 units and Mg solubility increased from 21 ± 2.6 to 52 ± 1.2 %. Increasing VFA infusion tended to decrease plasma Mg concentration. Urinary Mg excretion (mg/h) increased during the first 4 h of infusion on all treatments but this increase was not sustained so that by 24 h urinary excretion was at or below levels prior to infusion.

Results indicate that Mg solubility in caecal and proximal colonic digesta is responsive to changes in pH. The lack of response of Mg absorption was surprising and possible reasons are discussed.

Keywords Absorption, large intestine, magnesium, solubility, pH, volatile fatty acids, sheep.

INTRODUCTION

Within the ruminant digestive tract the reticulo-rumen appears to be the major site of Mg absorption (Tomas & Potter, 1976; Field & Munro, 1977; Gabel & Martens, 1985). However, the production of volatile fatty acids (VFA's) in the caecum as a result of carbohydrate fermentation (Orskov, *et al.*, 1971; Weston & Hogan, 1971), and the large scale absorption of water from the caecum and colon (Hyden, 1961), result in conditions within the large intestine that are conducive to net Mg absorption. Absorption from the large intestine does occur and appears to rely on passive transfer of Mg ions across the gut mucosa (Dalley & Sykes, 1989) but an active transport system cannot be discounted. Volatile fatty acid (VFA) production could potentially decrease the pH of caecal digesta and increase Mg solubility and absorption, provided that Mg solubility in caecal digesta responds to pH in a similar manner to that in rumen digesta (Smith & Horn, 1976). VFA's are also a readily available source of energy for an active transport system.

Because the amount of undigested carbohydrate arriving at the caecum differs between diets (MacRae & Armstrong, 1969; Orskov *et al.*, 1971) it is possible that this is a mechanism by which diet plays a role in Mg absorption.

In this experiment the caecal pH was manipulated by the infusion of increasing concentrations of a VFA solution into the terminal ileum. The effect of this pH change on Mg solubility and absorption from the large intestine was studied using urinary Mg excretion and plasma Mg concentrations as indicators of net Mg absorption.

MATERIALS & METHODS

Animals

Five two-tooth Coopworth ewes with a mean liveweight of 40kg, fitted with terminal ileal and proximal colonic cannulae were used. Animals were housed indoors in metabolism crates, designed for separation and collection of urine and faeces, and offered a pelleted concentrate diet at a rate of 800 g/d which was supplemented with 100 g chaffed lucerne hay to aid rumination. The daily ration was delivered at 2 h intervals from continuous feeders. Animals had free access to fresh tap water.

Infusion

Following a 2 week period for animals to adjust to the metabolism crates and feeders they were randomly allocated within a latin square design to one of 5 VFA infusion treatments. The VFA treatments were chosen based on results of studies by Faichney (1969) who determined, in sheep, the caecal VFA production rates and the ratio of individual acids.

The 5 treatments; - water, 220, 440, 660 and 880 mmol of VFA/d in the ratio of 0.8:0.15:0.05 for acetic, propionic and butyric acids respectively, were diluted in 2016 ml of distilled water and continuously infused during a 24 h period into the terminal ileum using a multichannel peristaltic pump.

For one week prior to infusion urine samples were collected daily and acidified to pH 2-3 with concentrated acetic acid. Blood samples were collected daily into heparinised tubes by jugular venepuncture. The tubes were centrifuged at 1200 g for 20 min and the plasma transferred into storage containers. A digesta sample was taken from the proximal colon on the day prior to the infusion and pH determined immediately. All samples were stored at -20°C for analysis.

On the morning of the infusion, urine and blood samples were collected and urinary catheters inserted to enable regular urine collection. During the infusion blood and urine samples were collected at 4 h intervals. Colonic digesta samples were collected at 4, 16 and 24 h and the pH determined. Blood and urine samples were collected following the removal of the infusion lines at the end of the infusion period and subsequently, at 6 h

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intervals for 12 h and then 12 h intervals for 24 h. A colonic digesta sample was collected 4 h post infusion and then 3 days later. A 7 day period was allowed before the animals were reallocated to their treatment and the procedure repeated.

Laboratory analysis

Feeds, urine, plasma and colonic digesta were analysed chemically for Mg using atomic absorption spectrophotometry, following wet digestion (Thompson & Blanchflower, 1971) and dilution with 1% strontium chloride in 0.1 M HCl. Digesta (10 g) was weighed into a high speed centrifuge tube to which was added 10 ml of a phosphate buffer which had the same pH as the fresh digesta sample. The sample and buffer mixture were mixed for 30 sec and the pH determined. Following 30 min centrifugation at 30,000 *g* the supernatant was collected and subsequently analysed for Mg by atomic absorption spectrophotometry. The soluble Mg being defined as the amount of Mg present in the 30,000 *g* supernatant expressed as a percentage of the total Mg in the digesta.

Statistical Analysis

Treatment means and standard errors for urinary Mg excretion, Mg solubility, digesta pH and plasma Mg concentration were generated using the general linear model procedure (GLM) in SAS. Minitab was used to determine the correlation coefficients.

RESULTS

Feed Intake

The daily maintenance diet of concentrate (800 g) and chaffed lucerne hay (100 g) supplied 1.87 g Mg/d. All animals showed some degree of intake depression on the day of the VFA infusion and for 2-10 days thereafter. The magnitude of depression and the period required for recovery increased as the VFA concentration of the infusate increased (Table 1).

TABLE 1 Mean (± sem) percentage decline in feed intake following VFA infusion and days for consumption to return to pre-infusion levels.

Treatment mmol VFA/d	Mean decrease in feed intake (%)		Time to recover (days)	
water	1	(1.4)	1.2	(0.97)
220	22	(14.4)	2.3	(1.14)
440	41	(5.0)	2.2	(0.49)
660	78	(1.9)	5.0	(1.52)
880	87	(4.7)	6.4	(1.03)

Infusions

The mean infusion volume for all five animals across all VFA treatments was 1936 ± 11.4 ml delivering 96 % of the calculated VFA treatment. Actual amounts of each VFA treatment infused were thus 209 ± 2.2, 424 ± 5.5, 634 ± 11.0 and 844 ± 12.0 mmol for treatments 220, 440, 660 and 880, respectively.

Digesta pH

The pre-treatment colonic pH was 7.32 ± 0.098 and a non significant increase in colonic pH of 0.11 ± 0.22 pH units was observed following 16 h of water infusion. There was a significant negative correlation (r= -0.99) between amount of VFA infused (mmol/d) and the subsequent change in colonic digesta pH (Table 2). Four hours after infusion proximal colonic pH values were within 0.5 pH units of the pre-infusion value for all treatments.

TABLE 2 Mean (± sem) change in colonic digesta pH, Mg solubility and plasma Mg concentration after 16 hours of volatile fatty acid infusion into the terminal ileum.

	VFA Treatment				
	0	220	440	660	880
pH change after16 h	0.11 ±0.220	-0.24 ±0.107	-0.70 ±0.138	-1.57 ±0.272	-2.12 ±0.481
Mg solubility after 16 h (%)	29 ±4.3	34 ±4.8	41 ±4.3	43 ±4.3	52 ±1.2
plasma Mg change after 16 h (mg/100 ml)	-0.01 ±0.08	-0.05 ±0.05	-0.36 ±0.14	-0.28 ±0.17	-0.23 ±0.16

Magnesium Content and Solubility in Proximal Colonic Digesta

Although the Mg intake was variable during the study no changes in Mg concentration of the whole digesta with time or VFA treatment were observed during the infusions; the mean concentration being 463.5 ± 3.33 mg Mg/100 g DM. Prior to VFA treatment 21 ± 2.6 % of Mg was soluble. pH had declined significantly and Mg solubility increased after 16 h of infusion, the two parameters being negatively correlated (r = -0.968)(Table 2). Infusion of 880 mmol VFA increased Mg solubility by 31 percentage units. Despite there being no significant change in the pH of proximal colonic digesta following 16 h of water infusion there was an 8 percentage unit increase in Mg solubility (Table 2).

Urinary Magnesium Excretion

Urinary Mg excretion (mg Mg/h) increased from 16.3 ± 1.22 mg/h prior to infusion to 23.4 ± 5.84, 45.3 ± 6.60, 36.9 ± 4.26, 33.9 ± 5.21 and 24.5 ± 2.32 mg/h during the first 4 hours of water, 220, 440, 660 and 880 mmol infusions, respectively. This increase was not sustained such that after 24 h of infusion urinary excretion had declined to pre-infusion levels for the water, 220 and 440 mmol treatments and to below pre-infusion levels with 660 and 880 mmol infusions.

Plasma Magnesium.

Plasma Mg concentration did not fall below 2.0 mg/100 ml throughout the experiment. Water and 220 mmol infusions had no effect on plasma Mg while 440, 660 and 880 mmol treatments tended to decrease plasma Mg concentrations (Table 2). There were no long term effects of VFA treatment on plasma Mg concentration.

DISCUSSION

The response of caecal Mg solubility to pH was anticipated and was similar to that observed during *in vitro* studies with rumen fluid (Smith & Horn, 1976).

A model of Mg metabolism (Robson, 1989) has shown the importance of hindgut absorption in Mg homeostasis. These

results suggest that factors which influence pH of digesta in the hindgut, such as variation in the proportion of organic matter digested in the rumen and therefore caecal fermentation and VFA production (MacRae & Armstrong, 1969; Orskov, *et al.,* 1969; 1971) may be important. Morever variation in digesta pH in the large intestine associated with commonly used diets has been observed, though the practical importance of the present findings will require further investigation.

The lack of significant increase in apparent Mg absorption other than during the first four hours of VFA infusion was surprising. Previous studies (Dalley & Sykes, 1989) had suggested that Mg absorption from the large intestine may occur by passive transfer. The lack of response of Mg excretion despite a 2.5 fold increase in Mg solubility in the present work must cast doubt on this hypothesis.

The possibility that Mg absorption is energy dependent must be considered from the present data. The fact that absorption during the first fours hours was elevated suggests initial stimulation of the transport mechanism possibly reflecting increased availability of VFA's as an energy source (Bugart, 1987). The subsequent marked reduction in absorption may well reflect poisoning of epithelial tissue by VFA, particularly since the depression was related to infusion rate. Evidence for such physiological damage is shown by the marked depression in food intake which, at the infusion of 880 mmol was greater (87 %) than the 10 % increase as energy intake provided. It may be that caecal motility was disturbed causing inappetance (Ruckebusch, 1970; Svendsen, 1972) or that receptors exist in the caecal epithelium which are very sensitive to VFA and are capable of inducing changes in the appetite centre.

It seems that VFA infusion may influence two components of Mg transfer from the large intestine. Firstly having a positive influence on Mg solubility but ultimately a negative influence on the transport mechanism.

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