Effects of potassium on potential difference across the rumen wall and magnesium metabolism in sheep

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ABSTRACT

Several *in vitro* studies have indicated that the potential difference (p.d.) across the rumen wall is important for magnesium (Mg) absorption and that increases in p.d. as a result of high rumen potassium (K) concentration may reduce Mg transport. The aim of this work was to provide quantitative data on the relationship between p.d. and Mg absorption in the live animal and on diurnal variation in p.d.

Twelve rumen cannulated ewes, weighing 45±2.5 kg, were used and randomly allotted according to a thrice-replicated 4x4 Latin square change-over design. Animals were offered 750 gDM/d (80% concentrate and 20% chaffed lucerne hay). Four levels of KCl were infused intraruminally to raise total K intake to 15, 25, 35 and 45 g/d, spanning the range of dietary concentration and intake normally experienced on spring herbage in New Zealand. A 14-d balance study followed by a 48-h continuous p.d. measurement was used.

Diurnal variation in p.d. was relatively small, less than 5 mV, but p.d. increased from 45.8±1.21 to 52.6±0.82 mV as K intake increased from 15 to 25 g/d and showed little change with higher levels. The relationship between p.d. and rumen K concentration was curvilinear and was described as $y = 18.89 + 0.66(\pm 0.175)\ [K] - 0.003(\pm 0.001)\ [K^2]$.

Apparent Mg absorption, as judged by urinary Mg excretion, decreased from 0.43±0.02 to 0.34±0.02 g/d, most of the depression occurring as K intake increased from 15 to 25 g/d. On the other hand, plasma Mg concentration decreased ($p<0.05$) linearly throughout the whole range of K intake from 1.08±0.03 to 0.90±0.01 mmol/l.

The results suggest at least two mechanisms of effect of K on Mg homeostasis and these are discussed.

Keywords: Magnesium, potassium, potential difference, absorption, sheep

INTRODUCTION

Economic losses from hypomagnesaemia in the dairy industry in New Zealand are considered to be $NZ28 million annually (Towers, 1984). Although the role of high herbage K during spring in depressing Mg absorption has been well documented, the precise mechanisms involved are poorly understood. Tomas and Potter (1976) and Martens and Blume (1986) demonstrated reduced Mg absorption with increase in p.d. following increases in rumen K concentration. Martens *et al.* (1988) demonstrated, *in vitro*, that p.d., and not K *per se*, was responsible for reducing net Mg absorption across the rumen epithelium.

Work in our laboratory has begun to model Mg metabolism to enable better prediction of risk of hypomagnesaemia. This work has highlighted the lack of reliable quantitative data on p.d. for the range of K intakes encountered by grazing animals. The aim of this work was to provide this. Previous studies have provided spot measurement of p.d.. This study allowed estimation of any diurnal variation in p.d.

MATERIALS AND METHODS

Experimental procedures

Twelve, 1-2 year-old, Coopworth ewes, averaging, 41.2 ± 2.2 kg liveweight, equipped with a rigid rumen cannula (3 cm, ID) were used. Animals were randomly assigned to a thrice-replicated 4x4 Latin square change-over design or Round Robin experiment. Treatments were allocated by using a single balanced square (Petersen, 1985). This experimental design aimed to observe any carry-over effects from period-to-period.

Animals were offered a diet consisting of chaffed lucerne hay and low Na pelleted concentrate (153 and 699 gDM/d, respectively). They were offered 50 gDM/kg$W^{0.75}$/d which was calculated to provide maintenance energy and protein intakes (ARC, 1980). The diet was given at 2-h intervals using over head automatic feeders.

Four levels of potassium chloride (KCl) were infused intraruminally to achieve total K intake of 15, 25, 35 and 45 g/d for treatments 15K, 25K, 35K and 45K, respectively, representing dietary concentrations of 20, 30 40 and 50 g K/kgDM. The total amounts of K used were designed to span the range of dietary concentration commonly found in New Zealand pastures during spring, the major period of high incidence of hypomagnesaemia (Smith and Middleton, 1978). Infusates were delivered to the rumen via polyvinyl tubing (2 mm ID) inserted through the cap of the rumen cannula using a multichannel peristaltic pump at a measured rate of approximately 500 ml/d. Infusates were subsampled before and after infusion periods and bulked daily during the collection period for subsequent analysis.

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Balance study period

Each balance study period consisted of a 5-d transition period, a 7-d preliminary period and a 6-d collection period followed by a 2-d measurement period. Before commencing the experiment, animals were adjusted to metabolism crates and feeding regimen for 2 weeks. Following the 7-d preliminary period the collection period commenced, feeds were sampled from 2 d prior to until 2 d before the end of the collection period. Faeces and urine were separated using fibreglass separators located under the metabolism crates. Faeces were weighed daily at 0930 h, bulked and then stored at 4 °C in plastic bags. Urine was collected daily in polythene containers located under the metabolism crates to which 100 ml of 10.5 M glacial acetic acid had been added to adjust urine pH to 3-4. An aliquot (10 % by volume) of daily output was subsampled, bulked and then stored at -20 °C. The bulked urine collections were subsampled in duplicate for analysis at the end of each period. Voluntary water intake was measured daily throughout the collection period. At the end of each period, DM contents of the bulked feeds and faeces were determined by oven-drying at 105 °C for 24 h and then ground to pass through a 1 mm screen prior to chemical analysis.

Potential difference measurement period

Potential difference was measured using a modified technique according to the procedure of Dobson and Phillipson (1958) as described by Wachirapakorn (1995). Twelve readings were made and then averaged for that minute using dataloggers (CR10WP, Campbell Scientific Inc., USA). The p.d. was monitored continuously and recorded for 48 h during the measurement periods. Saturated KCl-agar bridges were connected to animals and silver/silver chloride ($Ag/AgCl_2$) half cells. These bridges were made by filling vinyl tubing with a hot solution of saturated KCl and 3% agar. One tip (1 mm ID) was inserted into the jugular vein via an indwelling jugular catheter, and the other tip (2 mm ID) inserted via a cannula in the rumen contents. The tip of the rumen bridge in the rumen was renewed regularly by cutting obliquely to ensure stability of the reading.

During the p.d. measurement period, 10 ml blood was taken by jugular venipuncture into lithium heparin vacutainer tubes at 0700, 1500 and 2300 h during 2 consecutive days. Blood was immediately centrifuged at 1000 *g* for 15 min. Plasma was removed and stored at -20 °C until analysed. Fluid samples from the ventral sac of the rumen were taken shortly after blood sampling by suction via the rumen cannula through a stainless steel perforated tube. Approximately 30 ml of fluid was taken on each occasion and the pH measured immediately using a portable pH meter. The rumen samples were centrifuged at 30 000 *g* for 30 min and the supernatant removed and stored at -20 °C until analysed.

Chemical analyses

Dried feed and faeces were wet-ashed as described by Thompson and Blanchflower (1971) prior to mineral analyses. Magnesium in all samples was determined using atomic absorption spectrophotometry. Potassium and Na were measured by flame emission spectrophotometry.

Statistical analyses

Data were subjected to analysis of variance using the general linear model procedure (Proc GLM) (SAS, 1989). Treatment means were compared using predicted difference (PDIFF) option of least-squares means. Regression analyses were performed using the regression procedure (Proc REG) (SAS, 1989).

RESULTS

Dry matter, water and mineral intakes

The mean DM intake averaged 813±6.1 g/d. Intakes of Mg and Na were not different between treatments, averaging 1.66±0.05 and 1.23±0.07 g/d, respectively. Total K intakes were 15.8±0.10, 25.0±0.23, 35.2±0.60 and 44.9±0.69 g/d for the 15K, 25K, 35K and 45K treatments, respectively. Increasing K by infusion did not affect ($p>0.05$) feed DM or OM digestibilities. Water intake was similar on all treatments.

Magnesium metabolism

Magnesium intake, excretion in faeces and urine, apparent absorption, retention and availability are shown in Table 1. The apparent absorption of Mg declined ($p<0.01$) as K intake increased from 15 g/d to 25 g/d, but only slightly decreased ($p>0.05$) as K intake increased from 25 to 45 g/d.

Urinary Mg excretion showed the same trend as apparent Mg absorption with increasing infusion of K. A positive correlation ($r=0.76$, $p<0.01$) between urinary Mg excretion and apparent Mg absorption was observed. The regression equation obtained was $y = 0.19 + 0.46 (\pm 0.06)$ x, where y = urinary Mg excretion (g/d), x = apparent Mg absorption (g/d). Increasing dietary K did not affect Mg retention ($p>0.05$).

TABLE 1: Magnesium metabolism in sheep receiving a diet with differing levels of infused K (mean±sem, n=12).

	15K[1]	25K	35K	45K
Magnesium metabolism, g/d				
Intake	1.66±0.05	1.66±0.05	1.66±0.05	1.66±0.05
Faecal output	1.16±0.04	1.28±0.04	1.30±0.03	1.31±0.05
Urinary excretion	0.43±0.02	0.37±0.03	0.36±0.02	0.34±0.02
Apparent absorption	0.50±0.02	0.38±0.04	0.35±0.03	0.35±0.04
Retention	0.07±0.02	0.01±0.02	-0.00±0.02	0.01±0.03
Availability*	30.2±1.24	22.8±1.93	21.1±1.38	20.8±2.08
Availability**	26.8±1.05	21.8±1.10	21.5±0.95	20.7±1.33

[1]15K = 15g K/d, 25K = 25 g K/d, 35K = 35 g K/d, 45K = 45 g K/d

* Calculated from faecal output as % of intake

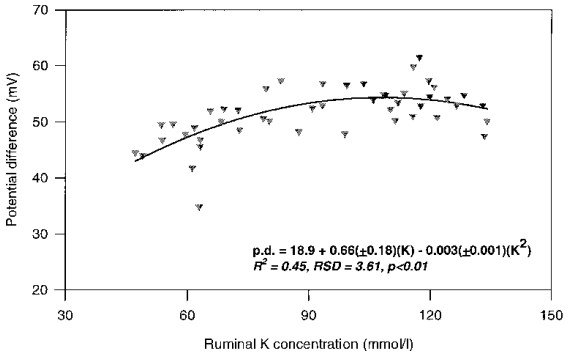
** Calculated from feed intake - urinary excretion as % of intake

sem = standard error of mean

Potential difference across the rumen wall

The p.d. within animals showed low consistent diurnal variation, varying by only 3-4 mv. Fluctuations were observed around feeding, but overall the coefficient of variation within an animal was about 15%. The mean p.d. in treatment 15K (45.8±1.21 mV) was lower ($p<0.05$) than in treatments 25K, 35K and 45K (52.6±0.82, 53.9±1.02, and 53.8±0.94 mV). Highly significant relationships between the p.d. and the concentration of K in rumen fluid were found. A curvilinear relationship gave a better statistical fit than a linear one and is described in Figure 1.

FIGURE 1: Relationships between the p.d. (y, mV) and K concentration (mmol/l) in rumen fluid.



Mineral concentration in plasma and rumen fluid

Mean plasma Mg concentration decreased significantly ($p<0.01$) and progressively as K intake increased, but plasma K concentrations were not affected (Table 2). The concentration of Mg in the $31,000_g$ supernatant fraction of rumen fluid tended to be increased by K intake, though not significantly ($p>0.05$). Increasing K intake resulted in an increase ($p<0.01$) in the concentration of K and a decrease ($p<0.01$) of Na in the supernatant fraction. The correlation between K intake (g/d) and the concentrations (mmol/l) of K and Na in the rumen fluid were 0.87 and -0.88, respectively ($p<0.001$ in both cases). Rumen digesta pH was not altered ($p>0.05$) by intraruminal infusion of KCl, though the concentration of Mg in the supernatant fraction was negatively correlated ($r = -0.57$, $p<0.01$) with rumen digesta pH.

TABLE 2: Concentrations of Mg and K in plasma, concentrations of Mg, K and Na in supernatant fraction and rumen digesta pH. The mean values for each during a p.d. measurement period were used. Each value is the mean of 12 periods

	15K[1]	25K	35K	45K
Plasma Mg concentration, mmol/l	1.08±0.03	1.01±0.02	0.93±0.02	0.90±0.01
Plasma K concentration, mmol/l	3.93±0.07	4.13±0.15	4.08±0.06	3.99±0.05
Rumen Mg concentration, mmol/l	5.20±0.22	5.70±0.23	5.73±0.24	5.62±0.24
Rumen K concentration, mmol/l	58.7±1.21	80.6±2.17	115.3±2.30	117.2±2.33
Rumen Na concentration, mmol/l	76.2±1.40	62.0±1.48	40.2±1.07	33.9±1.06
Rumen digesta pH	5.92±0.04	5.82±0.04	5.96±0.03	5.90±0.04

[1]15K = 15g K/d, 25K = 25 g K/d, 35K = 35 g K/d, 45K = 45 g K/d
sem = standard error of mean

DISCUSSION

The present work confirms the general relationship established in the literature of increasing p.d. with increasing dietary K intake in association with a decrease in Na:K ratio in rumen contents (Sellers and Dobson; 1960, Scott, 1966). Potential difference across the rumen wall showed only small diurnal variation. This work supports previous work (Martens and Blume, 1986) in showing that while the change in p.d. is essentially directly related to the increase in rumen K concentration major changes in Mg absorption occur only at dietary K concentrations between 20 and 30 g/kgDM corresponding to rumen K concentrations of 60 and 80 mmol/l respectively. Perhaps surprisingly, in this context, there was a progressive reduction in plasma Mg concentration with increase in K intake to levels greater than these at which K intake had ceased to affect p.d. or apparent absorption of Mg. This suggests an effect of K intake on plasma Mg concentration which was independent of its effect on p.d. between rumen and the body.

The continuous recording showed the p.d. to vary little within individual animals. Periodic cyclical variations of around 3-4 mV at approximately two-hourly intervals occurred and were probably caused by the feed intake *per se* or by an associated influx of saliva into the rumen. Variation between animals and of a diurnal nature were very small compared to the effect of KCl infusion. The majority of intake in grazing animals occurs during discrete periods both in the morning and before sunset (Cruickshank, 1986), and diurnal variation in the field may well be greater than recorded here. The present technology by enabling continuous recording will allow variation under grazing conditions to be measured.

The curvilinear relationship between p.d. across the rumen wall and concentration of K - in rumen fluid - Table 3 - contrasts with other work (Scott, 1966; Martens and Blume, 1986) which has suggested a linear relationship. Moreover, there is considerable variation between reports in the absolute values of p.d. recorded (Table 3).

The present values are in better agreement with the data of Scott (1966) than those of Martens and Blume (1986). This variation between data in this work and others may reflect differences in measurement methods, but may also be caused by nutritional factors apart from K itself, for example, sulphate (Martens and Blume, 1986), phosphate (Beardsworth *et al.*, 1989), rumen digesta pH (Gäbel *et al.*, 1987) or hypertonicity (Stacy and Warner, 1972). While, these factors were not determined in the present work, the variation does indicate a real problem in defining predictive relationships which integrate the literature on this important dietary factor with major influence on Mg absorption.

TABLE 3: Predicted p.d. values (mV) over a wide range of rumen fluid (mmol/l) using the equations reported by Scott (1966), Martens and Blume (1986) and the present work.

Rumen K concentration mmol/l	Scott (1966) y (mV) = 43 log[K] -29	Martens and Blume (1986) y (mV) = 37.1 log[K] -28.9	The present work y (mV) = 18.9 + 0.66 (K) - 0.003 (K^2)
20	26.9	19.4	30.8
40	39.9	30.5	40.3
60	47.5	37.1	47.4
80	52.8	41.7	52.1
100	57.0	45.3	54.4
120	60.4	48.2	54.3
Mean	47.4	37.0	46.6

The increase in dietary K and in rumen K concentration similarly had a non-linear effect on Mg absorption. Martens *et al.* (1988), using in vivo studies with the isolated rumen technique, showed similar relationships within the same range of rumen concentration viz. 20-120m mol/l. Martens *et al.* (1987) showed that p.d., but not K itself, influences net Mg absorption. The small change in the p.d. across the rumen wall from 53 to 54 mV as rumen K concentration increased from 80 to 120 mmol/l in the present work emphasises the need to base prediction of Mg absorption on p.d. rather than K intake or rumen K concentration *per se*.

The change in absorption of Mg with increasing dietary KCl intake in the present work agrees quantitatively with the findings of Greene *et al.* (1983b) and Dalley (1992) even though Greene *et al.* (1983b) increased the concentration of K in the diet by adding $KHCO_3$.

Previous recommendations have suggested 30 gK/kg feed DM is the upper limit for minimising risk of hypermagnesaemia (Green *et al.* 1983 a,b). The present work suggests that a ruminant diet with greater than 20 K g/KgDM may put animals at risk.

Reduction in Mg concentration in plasma in response to K infusion has been reported previously and has generally been attributed to reduction in Mg absorption (Tomas and Potter, 1976; Wylie *et al.*, 1985). The progressive decline in plasma Mg concentration with increase in dietary K above 25g/KgDM, despite lack of change in p.d. or decrease in apparent absorption of Mg, is a new finding and difficult to explain. There is evidence that high rates of K absorption may result in an increase in circulating insulin concentration which subsequently stimulates the uptake of K as well as Mg into the cells (Miller *et al.*, 1980; Yano *et al.*, 1988). We are unable to speculate further on the mechanism of this effect, but it suggests a secondary level of effect of K on Mg metabolism.

In conclusion, this work confirms the important of K induced elevation of the p.d. across the rumen wall for net Mg absorption but suggests that the effect operates above a relatively low threshold for dietary K, viz 20 g K/kgDM. Moreover K itself may alter Mg homeostasis by two mechanisms. Firstly by reducing Mg absorption, and secondly by inducing reduction in plasma Mg concentration independent of the effect on Mg absorption.

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REFERENCES

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, UK.

Beardsworth, L.J., Beardsworth, P.M., Care, A.D. 1989. Calcium fluxes across the wall of the ovine reticulo-rumen in vivo. *Research in Veterinary Science* **47:** 404-405.

Cruickshank, G.J. 1986. Nutritional Constraints to Lamb Growth at Pasture. PhD Thesis, Lincoln College, University of Canterbury, Canterbury, New Zealand.

Dalley, D.E. 1992. Studies of Magnesium Metabolism in Ruminants. PhD Thesis, Lincoln University, Canterbury, New Zealand.

Dobson, A., Phillipson, A.T. 1958. The absorption of chloride ions from the reticulo-rumen sac. *Journal of Physiology* **140:** 94-104.

Gäbel, G., Suendermann, M., Martens, H. 1987. The influence of osmotic pressure, lactic acid and pH on ion and fluid absorption from the washed and temporarily isolated reticulo-rumen of sheep. *Journal of Veterinary Medicine, A.* **34:** 220-226.

Godwin, I.R., Williams, V.J. 1986. Effects of intraruminal sodium chloride infusion on rumen and renal nitrogen and electrolyte dynamics in sheep. *British Journal of Nutrition* **56:** 379-394.

Greene, L.W., Fontenot, J.P., Webb, Jr., K.E. 1983a. Effect of dietary potassium on absorption of magnesium and other macroelements in sheep fed different levels of magnesium. *Journal of Animal Science* **56:** 1208-1213.

Greene, L.W., Webb, Jr., K.E., Fontenot, J.P. 1983b. Effect of potassium levels on site of absorption of magnesium and other macroelements in sheep. *Journal of Animal Science* **56:**1214-1221.

Leonhard, S., Martens, H, Gäbel, G. 1989. New aspects of magnesium transport in ruminants. *Acta Veterinaria Scandinavica* **86:** 146-151.

Martens, H, Blume, I. 1986. Effect of intraruminal sodium and potassium concentrations and of the trasmural potential difference on magnesium absorption from the temporarily isolated rumen of sheep. *Quarterly Journal of Experimental Physiology* **71:** 409-415.

Martens, H., Gäbel, G., Strozyk, H. 1987. The effect of potassium and the transmural potential difference on magnesium transport across an isolated preparation of sheep rumen epithelium. *Quarterly Journal of Experimental Physiology* **72:** 181-188.

Martens, H., Heggemann, G., Regier, K. 1988. Studies on the effect of K, Na, NH_4^+, VFA and CO_2 on the net absorption of magnesium from the temporarily isolated rumen of heifers. *Journal of Veterinary Medicine, A* **35:** 73-80.

Miller, J.K., Madsen, F.C., Lentz, D.E., Wong, Wei-o, Ramsey, N, Tysinger, C.E., Hansard, S.L. 1980. Blood plasma magnesium, potassium, glucose and immunoreactive insulin changes in cows moved abruptly from barn feeding to early spring pasture. *Journal of Dairy Science* **63:** 1073-1079.

Petersen, R.G. 1985. Design and Analysis of Experiments. Marcel Dekker, Inc., New York, USA.

SAS. 1989. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1and 2. Statistical Analysis Institute Inc., Cary, North Carolina, USA.

Scott, D. 1966. The effects of sodium depletion and potassium supplement upon electrical potentials in the rumen of the sheep. *Quarterly Journal of Experimental Physiology* **72:** 181-188.

Sellers, A.F., Dobson, A. 1960. Studies on the reticulo-rumen sodium and potassium concentrations and electrical potentials in sheep. *Research in Veterinary Science* **1:** 95-102.

Smith, G.S., Middleton, K.R. 1978. Sodium and potassium content of topdressed pastures in New Zealand in relation to plant and animal nutrition. *New Zealand Journal of Experimental Agriculture* **6:** 217-225.

Stacy, B.D., Warner, A.C.I. 1972. Acute effects of hypertonicity on the potential difference across the rumen wall in sheep. Comparative Biochemistry and Physiology 43A:637-641.

Thompson, R.H., and Blanchflower, W.J. 1971. Wet-ashing apparatus to prepare biological materials for atomic absorption spectrophotometry. *Laboratory Practice* **20:**859-861.

Tomas, F.M., Jones, G.B., Potter, B.J., Langsford, G.L. 1973. Influence of saline drinking water on mineral balances in sheep. *Australian Journal of Agricultural Research* **24:**377-386.

Tomas, F.M., Potter, B.J. 1976. The effect and site of action of potassium upon magnesium absorption in sheep. *Australian Journal of Agricultural Research* **27:**873-880.

Towers, N.R. 1984. Animal limitations to the efficient use of pasture. *New Zealand Agricultural Science* **18:**157-162.

Wachirapakorn, C. 1995. Magnesium Metabolism Studies. PhD Thesis, Lincoln University, Canterbury, New Zealand.

Wylie, M.J., Fontenot, J.P., Greene, L.W. 1985. Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *Journal of Animal Science* **61:** 1219-1229.

Yano, F., Horiuchi, K, Kawashima, R. 1988. Effect of potassium infusion into the rumen on magnesium absorption from the rumen wall of sheep. Memoirs of the College of Agriculture, Kyoto University, Japan.