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Effect of *in vitro* manipulation of pH on magnesium solubility in ruminal and caecal digesta in sheep

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SUMMARY

Rumen and caecal digesta were collected, under anaesthetic, from eight sheep offered either hay, pelleted concentrate or pasture at the Johnston Memorial Laboratory, Lincoln University during 1991. Subsamples of digesta were incubated at 39 °C for 1 h after adjustment of pH within the range 0·5–12 by the addition of H_2SO_4 or NaOH. The samples were centrifuged at 30000 ***g*** for 30 min and magnesium (Mg) concentration measured in the 30000 ***g*** supernatant fraction and in total digesta to assess Mg solubility. In rumen digesta, Mg solubility declined from 0·86 at pH 5 to 0·30 at pH 7 and differences in response between diets were small. Magnesium solubility in caecal digesta was generally higher than in ruminal digesta, and particularly at pH values > 6. At pH 7 the difference was twofold. Moreover, differences were observed between diets in the rate of decline in solubility in caecal digesta with increasing pH. At pH 5, 0·90 of Mg from hay and concentrate diets was soluble compared with only 0·8 for pasture. At pH 7, Mg solubility in caecal digesta from hay and concentrate-fed animals was almost double that from pasture-fed animals (0·64 and 0·62 *v.* 0·36, respectively). The implications of the findings for Mg homoeostasis in ruminants are discussed.

INTRODUCTION

The concentration of magnesium in the liquid phase at the site of Mg absorption is an important determinant of the proportion of dietary Mg that will be absorbed (Field 1983). Smith & Horn (1976) observed, during *in vitro* studies, and Johnson & Aubrey-Jones (1989), in *in vivo* studies, the sensitivity to pH of Mg distribution between liquid and solid phases of rumen digesta. Moreover, Horn & Smith (1978) observed reduction in the efficiency of absorption of Mg between the mouth and duodenum with increasing pH in the range 6·5–7·0.

Although the rumen has been recognised as the primary site of Mg absorption (Martens 1983; Gabel & Martens 1985) absorption from the large intestine has been observed (Meyer & Busse 1975; Field & Munro 1977; Reynolds *et al.* 1984; Bown *et al.* 1989; Dalley & Sykes 1989). Simulation modelling of Mg homoeostasis based on physiological studies in the literature (Robson *et al.* 1997) strongly suggests the existence of a significant post-ruminal site for Mg absorption. Moreover, very recently, Dalley *et al.* (1997) have provided evidence to suggest that the hindgut may be the major site of Mg absorption in K-induced depression of Mg absorption. The mechanism and conditions which influence Mg absorption from the hindgut are not, however, well understood, although Dalley & Sykes (1989) speculated that a passive transport mechanism is involved. Digesta pH in this region of the tract is generally high – pH 7·0– 8·0 (Williams 1965; Ben-Ghedalia *et al.* 1975). If the relationship between pH and Mg solubility in hindgut digesta is similar to that observed in the rumen then, at these pH values, $< 20\%$ of total Mg would be in the liquid phase, having implications for Mg absorption.

The present experiment was designed to generate Mg solubility–pH response curves for rumen and caecal digesta for three typical feed types, suitable for use in simulation model development.

MATERIALS AND METHODS

Eight female Coopworth sheep were allocated to one of three diets – chaffed lucerne hay (*Medicago sativa*), pelleted concentrate (g/kg; 697 barley grain, 220 barley malt culms, 60 barley straw, 10 NaCl, 10 $NaHCO_3$, 3 premix) or spring-grown ryegrass/white

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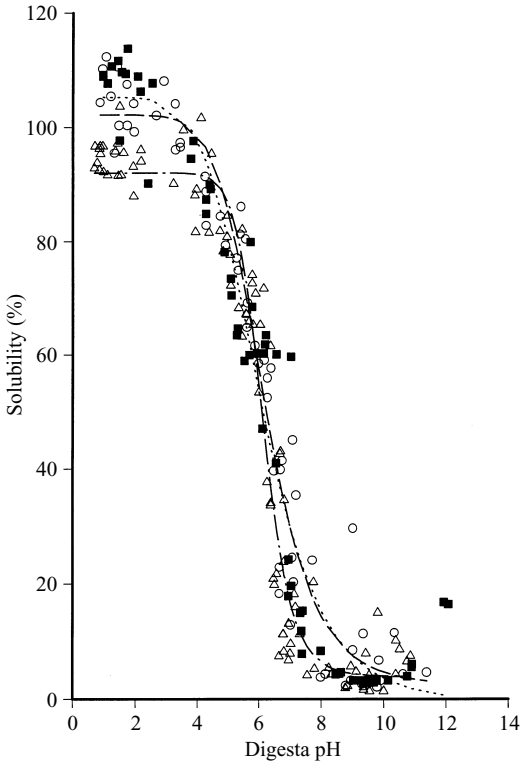

Fig. 1. Data and fitted curves for magnesium solubility in ruminal digesta as a function of pH for hay (––○––), herbage (- - - ■ - - -) and concentrate (– · △– ·) diets.

clover pasture (*Lolium perenne*/*Trifolium repens*) at the Johnston Memorial Laboratory, Lincoln University, during 1991. Three animals were allocated, indoors, to each of a concentrate or hay diet and two remained outdoors and grazed pasture. The animals were offered their respective diets *ad libitum* for at least 1 week prior to the beginning of experimentation and were brought indoors for sample collection off full feed. Animals had access to fresh water at all times.

Individual animals were anaesthetized with 12–16 ml of pentobarbitone sodium, 60 mg/ml, given to effect by slow intravenous injection. Once anaesthetized, the animal was positioned on its left side and a 20 cm incision made into the lower right abdominal region *c*. 10 cm anterior to the udder and 10 cm dorsal to the midline. The caecum was located and exteriorized. A 3 cm incision was made into the blind sac of the caecum. The entire contents of the caecum and proximal colon were removed taking care to avoid contamination with blood. Sodium pentobarbitone (8 ml, 500 mg/ml) was then used to effect euthanasia. The abdominal incision was extended, the rumen located, a 5 cm incision made into the rumen and the entire contents removed.

The rumen and caecal digesta were transferred to the laboratory, in sealed plastic containers, within 10 min of collection. The pH was determined and the digesta gassed (10% carbon dioxide: 90% nitrogen) for 1 min, sealed and placed in a water bath at 39 °C. A stream of the same gas was periodically run through the samples to maintain anaerobic conditions.

Subsamples of unstrained rumen digesta (50 g) were added to a series of plastic containers and 2·5 ml of one of the reagents from the following range added: sulphuric acid (H_2SO_4) 4 M, 3 M, 2 M, 1·5 M, 1 M, 0·5 M, 0·25 M, 0·1 M, water, sodium hydroxide (NaOH) 0·5 M, 0·75 M, 1 M, 1·25 M, 1·5 M, 1·75 M, 2 M, 3 M and 4 M.

Samples were thoroughly mixed and placed in a 7 cm deep plastic tray to which water, at 39 °C, was added to a depth of *c*. 1·5 cm. The tray was placed in a plastic bag, the air displaced by gassing with the CO_2:N_2 mixture and the bag sealed. Samples were incubated in an oven at 39 °C for 1 h.

Following incubation, the pH was determined and the samples centrifuged at 30000 ***g*** for 30 min. Supernatant was collected and stored at −20 °C until analysed for Mg. The technique was repeated using a duplicate set of digesta samples.

Caecal digesta was treated in a similar manner. However, only 25 g of sample was used and correspondingly only 1·25 ml of the appropriate acid, water or alkali added.

Dry matter content of whole rumen and caecal digesta was determined. These samples were subsequently used for total Mg analysis following wet-ashing by the method of Thompson & Blanchflower (1971) and reconstitution in 20 ml 0·6 M HCl. The samples were then diluted in 0·1 M HCl containing 2000 μg/g strontium chloride and the Mg concentration determined by atomic absorption spectrophotometry. The proportion of total Mg present in the 30000 ***g*** supernatant fraction was calculated and assumed to represent soluble Mg for the purpose of this experiment.

Statistical analysis

The maximum likelihood program (MLP: Ross 1980) was used to generate the line of best fit to the Mg solubility–pH data for each diet at both digesta collection sites using the following four-parameter equation from Thornley (1976):

$$EY = A/((1+(X/D)^{**}B))+C$$

where EY = estimated Mg solubility, X = pH, A = upper Y asymptote, B = 'steepness' parameter, C = lower Y asymptote and D = midpoint of pH between the lower and upper asymptotes.

Mean values for the fitted coefficients are given with one standard error unit. The estimated solubility

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value from the prediction equation was overplotted on the Mg solubility–pH data. A *t*-test was used to test for differences in pH between diets.

RESULTS

Digesta pH

Initial pH values of rumen and caecal digesta did not differ between diets and were 6·62, 6·61 and 6·68 (S.E. 0·136) for ruminal and 6·84, 6·77 and 6·81 (S.E. 0·170) for caecal contents from the herbage, concentrate and hay diets, respectively. Caecal digesta tended to have a higher pH than rumen digesta ($P < 0{\cdot}04$).

Manipulation of ruminal and caecal digesta *in vitro*, using H_2SO_4 and NaOH, resulted in pH values ranging from 0·5 following treatment with 4 M H_2SO_4 to pH 12 after treatment with 4 M NaOH.

Relationship between magnesium solubility and pH in ruminal digesta

Magnesium solubility in the 30000 ***g*** supernatant fraction was a function of pH (Fig. 1), and differed between diets as demonstrated by the coefficients of the fitted curves (Table 1).

The main difference between the diets was a lower maximum solubility value between pH 1 and 4 for digesta from the concentrate diet and an initially slower but ultimately more rapid decline in solubility than with digesta from hay and herbage diets which was shown in the different values for the upper asymptote (*A*) and the steepness factor (*B*) in the equations in Table 1.

Table 1. *Coefficients* (*with S.E. in parentheses*) *and D.F. from equations used to fit curves to the magnesium solubility–pH relationship in ruminal and caecal digesta generated by* in vitro *manipulation of pH in digesta collected from sheep offered a range of diets*

Diet	Coefficient (S.E. of coefficient) A	B	C	D	D.F.
Ruminal digesta					
Herbage	1·06 (0·048)	6·11 (0·753)	−0·01 (0·035)	6·04 (0·121)	58
Concentrate	0·88 (0·025)	13·29 (1·468)	0·03 (0·018)	6·13 (0·053)	88
Hay	1·00 (0·041)	7·55 (0·964)	0·02 (0·031)	6·15 (0·053)	57
Caecal digesta					
Herbage	0·77 (0·049)	8·99 (1·490)	0·01 (0·039)	6·58 (0·114)	39
Concentrate	1·26 (0·145)	4·32 (0·446)	−0·28 (0·139)	8·63 (0·465)	58
Hay	0·72 (0·055)	5·34 (0·611)	0·28 (0·050)	7·07 (0·214)	62

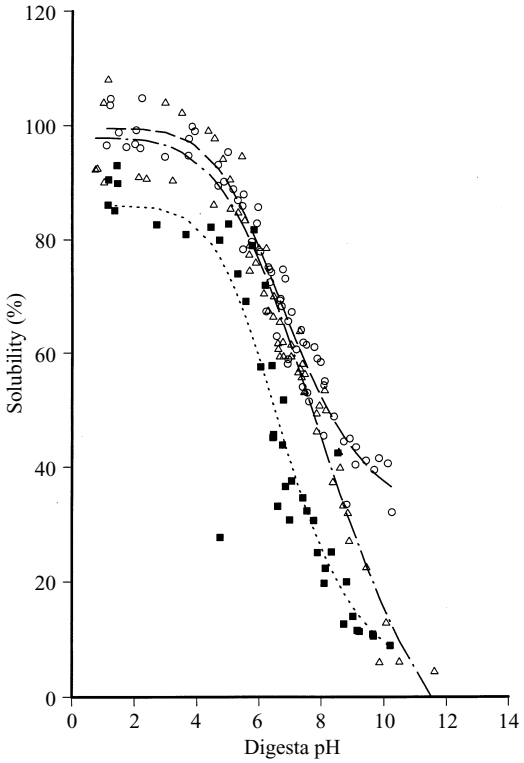

Fig. 2. Data and fitted curves for magnesium solubility in caecal digesta as a function of pH for hay (––○––), herbage (--- ■ ---) and concentrate (– · △ – ·) diets.

Relationship between magnesium solubility and pH in caecal digesta

As with ruminal digesta, Mg solubility in caecal digesta was a function of pH (Fig. 2) and differed between diets as demonstrated by the coefficients in Table 1. There was a greater difference between diets in both the maximum Mg solubility achieved and the response to pH than was the case with rumen digesta. These show that solubility of Mg in herbage was lower at low pH and tended to fall more rapidly with increasing pH than in the hay or concentrate diets.

Estimates of Mg solubility in ruminal and caecal digesta at pH 5, 6, 7 and 8, derived from the equations are given in Table 2. These show that Mg solubility in caecal digesta was always higher than in ruminal digesta at the same pH and that this difference was greatest at the higher pH values and was particularly apparent on the hay and concentrate diets. Solubility of Mg in both ruminal and caecal digesta from the herbage diet was always lower than in corresponding samples from hay or concentrate-fed sheep, but

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Table 2. *Proportion of total magnesium present in the 30000* ***g*** *supernatant fraction of ruminal and caecal digesta as estimated from the maximum likelihood prediction equation at pH 5, 6, 7 and 8 for sheep offered a range of diets*

	Magnesium solubility (proportion of total Mg)							
	pH 5		pH 6		pH 7		pH 8	
Diet	Ruminal	Caecal	Ruminal	Caecal	Ruminal	Caecal	Ruminal	Caecal
Herbage	0·80	0·80	0·52	0·63	0·30	0·36	0·15	0·22
Concentrate	0·86	0·88	0·53	0·76	0·27	0·62	0·08	0·45
Hay	0·86	0·90	0·56	0·78	0·29	0·64	0·15	0·52

solubility in caecal digesta from the herbage diet was particularly low at pH values > 6.

DISCUSSION

The finding of a change in solubility with change in pH in rumen digesta is not surprising, since such associations have been well demonstrated by Smith & Horn (1976) and Johnson & Aubrey-Jones (1989). The present findings confirm these in a general sense, although the decline in solubility with increasing pH occurred around pH 5·5–6·0 in the present work compared to 6·5 in the work of Smith & Horn (1976). The differences may be the result of differences in technique which need further investigation, but the generally lower solubility observed in the present work at normal rumen pH (6·0–7·0) suggests that the rumen may be less important as a site of absorption of Mg than hitherto assumed. Interestingly, the wide range of diets used had no effect on rumen pH or on the relationship between pH and solubility of Mg.

On the other hand, there was very significant variation between diets in the relationship between pH and the solubility of Mg in caecal digesta (Table 2, Fig. 2). The precise significance of this cannot be established at this stage because of our lack of knowledge of mechanisms regulating hindgut transport of Mg. However, if the electrical potential difference between the lumen of the hindgut and plasma is as low as that in the duodenum (viz. 13·3 ($\pm$1·55) mV; McLean *et al*. 1984) and much lower than that across the rumen wall (viz. 37·6 ($\pm$1·11) mV), diffusional transport and therefore Mg solubility could be very important. The pH observed *in vivo*, 6·8, was similar on the three diets and to values observed in other studies (Ben-Ghedalia *et al*. 1975). The major differences in solubility of Mg observed in caecal digesta between diets at pH values *c*. 6·8 are therefore likely to be of physiological significance. Dalley *et al*. (1997) have demonstrated that under conditions of high potassium intake, such as occurs during rapid herbage growth, the site of Mg absorption may shift substantially from the rumen to the hindgut. Under these circumstances, the finding of low solubility of Mg from the fresh ryegrass/white clover diet could be of particular importance in the context of early spring post-calving hypomagnesaemia. It is interesting in this context that anecdotal evidence from practical farmer experience would argue the positive value of hay feeding in dairy cattle nutrition, though there could be other reasons for such a view. We have, as yet, no explanation for this low solubility, but there is documented evidence for variation with diet in caecal fermentation (Karr *et al*. 1966; MacRae & Armstrong 1969; Ørskov *et al*. 1971). The present work suggests the need to search for the presence of compounds or characteristics of digesta which bind significant amounts of Mg in this region of the tract.

REFERENCES

Ben-Ghedalia, D., Tagari, H., Zamwel, S. & Bondi, A. (1975). Solubility and net exchange of calcium, magnesium and phosphorus in digesta flowing along the gut of the sheep. *British Journal of Nutrition* **33**, 87–94.

Bown, M. D., Poppi, D. P. & Sykes, A. R. (1989). The effects of concurrent infection of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* on calcium, phosphorus and magnesium transactions along the digestive tract of lambs. *Journal of Comparative Pathology* **101**, 11–20.

Dalley, D. E. & Sykes, A. R. (1989). Magnesium absorption from the large intestine of sheep. *Proceedings of the New Zealand Society of Animal Production* **49**, 229–232.

Dalley, D. E., Isherwood, P., Sykes, A. R. & Robson, A. B. (1997). Effect of intraruminal infusion of potassium on the site of magnesium absorption within the digestive tract in sheep. *Journal of Agricultural Science, Cambridge* **129**, 99–105.

Field, A. C. (1983). Dietary factors affecting magnesium utilisation. In *Proceedings of John Lee Pratt International Symposium on the Role of Magnesium in Animal Nutrition* (Eds J. P. Fontenot, G. E. Bunce, V. G. Allen & K. E. Webb, Jr), pp. 159–171. Blacksburg, Virginia: Virginia Polytechnic Institute and State University.

Field, A. C. & Munro, C. S. (1977). The effect of site and quantity on the extent of absorption of Mg infused into

the gastro-intestinal tract of sheep. *Journal of Agricultural Science*, *Cambridge* **89**, 365–371.

Gabel, G. & Martens, H. (1985). Magnesium absorption from the rumen of heifers. *Zentralblatt fur Veterinärmedizin Reihe A* **32**, 636–639.

Horn, J. P. & Smith, R. H. (1978). Absorption of magnesium by the young steer. *British Journal of Nutrition* **40**, 473–484.

Johnson, C. L. & Aubrey Jones, D. A. (1989). Effect of change of diet on the mineral composition of rumen fluid, on magnesium metabolism and on water balance in sheep. *British Journal of Nutrition* **61**, 583–594.

Karr, M. R., Little, C. O. & Mitchell, G. E., Jr (1966). Starch disappearance from different segments of the digestive tract of steers. *Journal of Animal Science* **25**, 652–654.

MacRae, J. C. & Armstrong, D. G. (1969). Studies on intestinal digestion in the sheep. 2. Digestion of some carbohydrate constituents in hay, cereal and hay–cereal rations. *British Journal of Nutrition* **23**, 377–387.

Martens, H. (1983). Saturation kinetics of magnesium efflux across the rumen wall in heifers. *British Journal of Nutrition* **49**, 153–158.

McLean, A. F., Buchan, W. & Scott, D. (1984). Magnesium absorption in mature ewes infused intraruminally with magnesium chloride. *British Journal of Nutrition* **52**, 523–527.

Meyer, H. & Busse, F. W. (1975). Zur rektalen absorption von magnesium bein wiederkauer. *Deutsche Tierarztliche Wochenschrift* **82**, 140–141.

Ørskov, E. R., Fraser, C. & McDonald, I. (1971). Digestion of concentrates in sheep. 3. Effects of rumen fermentation of barley and maize diets on protein digestion. *British Journal of Nutrition* **26**, 477–486.

Reynolds, C. K., Bell, M. C. & Sims, M. H. (1984). Changes in plasma, red blood cell and cerebrospinal fluid mineral concentrations in calves during magnesium depletion followed by repletion with rectally infused magnesium chloride. *Journal of Nutrition* **114**, 1334–1341.

Robson, A. B., Field, A. C., Sykes, A. R. & McKinnon, A. E. (1997). A model of magnesium metabolism in young sheep. Magnesium absorption and excretion. *British Journal of Nutrition*.

Ross G. J. S. (1980). *MLP*, *Maximum Likelihood Program*. Rothamsted Experimental Station: Lawes Agricultural Trust.

Smith, R. H. & Horn, J. P. (1976). Absorption of magnesium, labelled with magnesium-28, from the stomach of the young steer. *International Symposium on Nuclear Techniques in Animal Production and Health*, pp. 253–260. Vienna: International Atomic Energy Agency.

Thompson, T. H. & Blanchflower, W. J. (1971). Wet ashing apparatus to prepare biological materials for atomic absorption spectrophotometry. *Laboratory Practice* **20**, 859–861.

Thornley, J. M. H. (1976). *Mathematical Models in Plant Physiology: a Quantitative Approach to Problems in Plant and Crop Physiology*. London: Academic Press.

Williams, V. J. (1965). Microbial metabolism in the forestomachs and the large intestine of sheep. *Australian Journal of Agricultural Research* **16**, 77–91.