

# Omasal sampling technique in estimation of the site and extent of mineral absorption in dairy cows fed rapeseed and soybean expellers

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The effects of rapeseed and soybean expeller on digestion of sodium, potassium, calcium, magnesium, phosphorus and sulphur in dairy cows were investigated in a study conducted as an incomplete Latin square. The experimental diets consisted of five concentrates fed at a rate of 9 kg d<sup>-1</sup>: a mixture of barley and oats (control), which was replaced either with rapeseed or soybean expeller both at two levels (130, 180 and 230 g crude protein per kg dry matter). A mixture of grass and red clover silage (1:1) was fed *ad libitum*. No mineral supplements except for NaCl and trace minerals were used. Ruminal digestion was estimated by omasal sampling technique and total digestion from total faecal collection. Intake of all minerals except sodium increased with the level of protein supplementation and it was generally higher when rapeseed compared with soybean expeller diets were offered. Reticulo-rumen was the major site of net absorption of magnesium, whereas calcium, phosphorus, sodium and potassium were absorbed postruminally. Net absorption of sulphur took place both in the rumen and postruminally. Omasal flow of sodium and phosphorus indicated substantial secretion of these minerals into the rumen via saliva. Compared with the published data based on duodenal sampling, the results indicated that omasum has an important role in the absorption of minerals, especially sodium and phosphorus. Omasal sampling technique is a useful tool in studying ruminal mineral metabolism.

*Key words:* calcium, magnesium, potassium, phosphorus, sodium, sulphur, digestibility

## Introduction

Nutrition and feeding recommendations of minerals have traditionally been set to ensure sufficient supply with safety margin in all dietary circumstances. However, this approach can nowadays not be justified due to economic reasons, and especially because the nutrient losses from agriculture have become the most important factor causing eutrophication in surface waters (Valpasvuo-Jaatinen et al. 1997, Ekholm et al. 1999, Granlund et al. 2005). Eutrophication has many negative effects like growth of algae and aquatic weeds and reduced water transparency. Shifts in algae species composition towards cyanobacteria or blue-green algal dominance can produce toxins, odour and taste problems for water treatment (Carpenter et al. 1998, Nash and Haygarth 2005). Owing to the need to reduce especially phosphorus leaching in agriculture, reduction of P overfeeding is of special importance in milk production.

The extent of mineral absorption has traditionally been investigated using the total faecal collection method. The site of mineral absorption has generally been investigated using animals cannulated at different segments of the digestive tract. Duodenal sampling is the most common method used to estimate the net absorption from or secretion into the rumen. However, duodenal sampling technique ignores absorption of minerals from the omasum. Absorption of some minerals from the omasum can be considerable (Engelhardt and Hauffe 1975, Edrize et al. 1986), especially for adult cattle which have more developed omasum than young cattle or sheep (Mäkelä 1956, Church 1988). Poutiainen (1968) investigated the flow of Na and K through the reticulo-omasal orifice by estimating liquid outflow using polyethylene glycol as a liquid phase marker and analysing the concentrations of sodium (Na) and potassium (K) in rumen fluid. This approach may be appropriate for minerals flowing mainly in the liquid phase (e.g. Na), but unrepresentative sample composition can result in biased flow estimates of minerals partly associated with digesta solid phase.

Omasal sampling technique as described by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000) provides another alternative for investigating ruminal mineral metabolism. The method has successfully been used in investigating ruminal metabolism of nitrogen and carbohydrates (Ahvenjärvi et al. 1999, Choi et al. 2002, Reynal et al. 2003), and also in studying biohydrogenation of dietary fatty acids in the rumen (Shingfield et al. 2003). Because of difficulties in obtaining a representative sample from the digesta flowing into the omasal canal, advanced marker techniques are required for reliable estimation of nutrient flow from the rumen (Ahvenjärvi et al. 2003).

The first objective of the present study was to investigate the potential of omasal sampling technique in estimating the ruminal metabolism of macro minerals [calcium (Ca), phosphorus (P), K, Na, magnesium (Mg) and sulphur (S)]. The second objective was to examine the effects of the level and type (rapeseed vs. soybean expeller) of protein supplementation on mineral metabolism of dairy cows fed a diet based on red clover-grass silage and cereal grains. The supply of most of the minerals varied greatly between the diets due to differences in mineral concentrations between rapeseed and soybean. The lowest supplies of phosphorus and calcium were below the requirements (MTT 2006), which is a prerequisite to truly determine the efficiency of mineral absorption. The effects of the diets on nitrogen and carbohydrate digestion will be published separately.

## Material and methods

### Animals and diets

The effects of increasing dietary crude protein (CP) concentration with rapeseed and soybean expeller (Mildola Ltd., Finland) on the site of mineral absorption were studied with five multiparous Finnish Ayrshire cows fitted with rumen cannulas (Bar Diamond, Inc., Parma, ID, USA). The cows weighed 654 kg (SD 63) and were on average 51

(SD 12.6) days in milk at the beginning of the experiment. Experimental design was an incomplete Latin square (five diets, five cows, and four 21 day periods). The cows were kept in tie stalls. They were fed twice daily at 0600 and 1800, and milked at 0700 and 1700.

The basal diet consisted of silage supplemented with a concentrate comprising of a mixture of barley and oats (1:1). The silage was prepared by mixing pure red clover (*Trifolium pratense*) silage and a timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) silage 1:1. Both silages were made from 1st cut. Silage was fed *ad libitum* during the first 14 days of each 21 d period allowing proportionally 0.05 to 0.10 for refusals. Daily allowances of silage and concentrates were divided to four equal portions and fed to cows at 0600, 0900, 1800 and 2000. During the sampling period from d 14 to 21 the intake was restricted to 0.95 of the *ad libitum* consumption to avoid refusals. The five dietary treatments were the basal concentrate (Control; 9 kg d<sup>-1</sup>), which was gradually replaced with rapeseed expeller (RSE) or soybean expeller (SBE) at low (L) or high (H) levels of inclusion. Subsequently, abbreviations C, RL, RH, SL and SH will be used for the five diets. Daily allowances of rapeseed expeller were 2.0 (RL) and 4.0 kg d<sup>-1</sup> (RH) and those of soybean expeller 1.45 (SL) and 2.9 kg d<sup>-1</sup> (SH), respectively. All diets were supplemented with 1 g d<sup>-1</sup> of a trace mineral and vitamin premix and 100 g d<sup>-1</sup> of NaCl. No other mineral supplements were given.

### Markers and digesta sampling

To determine digesta flow entering the omasal canal, a triple-marker system (France and Siddons 1986) was used. Two external markers associated with the digesta liquid phase (CrEDTA) and small particle phase (Yb-acetate) were continuously infused into the rumen using a peristaltic pump (Watson-Marlow 502 S, Falmouth, UK). An internal marker, indigestible neutral detergent fibre, was used as large particle marker. On d 15 of each period, priming doses of CrEDTA (4.2 g of Cr) and Yb-acetate (3.1 g of Yb) were administered into

the rumen via rumen cannulas to facilitate a rapid equilibrium of marker concentrations in the rumen. Thereafter a continuous infusion of CrEDTA (2.8 g of Cr d<sup>-1</sup>) and Yb-acetate (2.4 g of Yb d<sup>-1</sup>), dissolved in 6 l of distilled water, was administered until the end of each period.

To determine digesta flow, the samples were obtained from the omasal canal using a modification (Ahvenjärvi et al. 2000) of the sampling technique described by Huhtanen et al. (1997). Digesta samples (400 ml) were collected on d 19 at 1, 4, 7 and 10 h after the morning feeding. On the two following days, the sampling time advanced 1 h each day, such that the samples represented each hour during the 12 h daytime feeding cycle. The samples were frozen immediately after sampling, and kept in -20°C until thawed. Once thawed at room temperature the samples were pooled to form one sample per cow per period, divided into liquid, small particle and large particle phases as described by Ahvenjärvi et al. (2000).

Total digestibility was determined by total faecal collections on days 18–21 of each period. Urine was separated from faeces using a light harness attached around the vulva of each cow with an adhesive. Urine was drained into a container using a flexible tube. Urine pH was kept below 3 with 10 N H<sub>2</sub>SO<sub>4</sub>.

### Chemical analysis

Ash and crude protein of the feed, digesta and faecal samples were determined with standard methods (AOAC 1990). Neutral detergent fibre, silage fermentation quality and digesta flow markers were analysed as described by Ahvenjärvi et al. (2000). Mathematically calculated reconstitution factors, based on the triple marker system, were used to reconstitute the omasal canal digesta samples subjected for determination of mineral composition. Measurements of Ca, Mg, P, S, K and Na concentrations in feed, omasal, faecal and urine samples were performed with ICP emission spectrophotometer (Thermo Jarrel Ash/Baird, Franklin, USA) as described by Luh Huang and Schulte (1985).

## Calculations and statistical analysis

The flow of minerals to the omasal canal was calculated as DM flow (kg d<sup>-1</sup>) × mineral concentration (g kg<sup>-1</sup> DM). Apparent absorption in the rumen was calculated as difference between intake and omasal flow, and in the intestines as omasal flow – faecal output. The Lucas principle was used to test if the minerals were ideal nutritional entities. According to the Lucas principle, the true digestibility of a nutritional entity is determined as the slope of the regression between the concentration of digestible nutrient (e.g. crude protein, ether extract, and cell solubles) against the concentration of the nutrient (Lucas et al. 1964). The Lucas test was not applied for Na, K and Mg because almost all Na was supplementary NaCl and the variations in dietary K and Mg concentrations were small.

Data was analysed with the GLM procedure of SAS (1999) using the following model:  $Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk}$ , where A, P and D are the animal, period and diet effects. Sums of squares of the diet effects were further divided into the following orthogonal comparisons: rapeseed vs. soybean expeller (RSE vs. SBE), linear and quadratic effects of protein supplementation and the interaction between protein source and level. The data presented

in the tables are based on LS means. Paired t-test was used to estimate the differences in flow between sampling sites. Additionally, regression equations were calculated to describe mineral absorption at different sites of the digestive tract. A mixed model regression analysis with a random cow effect was used to estimate relationships between the intake and flow parameters. The P-values <0.20 of the statistical tests were reported in the tables. P-values of 0.10–0.05 were considered a statistical trend and P-values < 0.05 were considered significant. When the P-values were smaller than 0.01, they were reported as P < 0.01 irrespective of the level of significance.

## Results

### Feed composition, DM intake and milk yield

Chemical composition of the experimental feeds is presented in Table 1. Calcium concentration of silage was relatively high due to inclusion of red clover. Rapeseed expeller was higher in Ca, Mg, P and

Table 1. Composition of the experimental feeds.

	Silage <sup>1)</sup>	Cereal grains <sup>2)</sup>	Rapeseed expeller	Soybean expeller
Dry matter (DM, g kg <sup>-1</sup> )	221	879	909	912
In dry matter (g kg <sup>-1</sup> DM)				
Ash	80	29	68	61
Crude protein	157	128	371	480
Neutral detergent fibre	498	213	312	213
Sodium	0.035	0.109	0.080	0.040
Potassium	26.8	5.3	12.7	23.3
Calcium	6.97	1.00	8.00	2.88
Magnesium	2.13	1.43	4.66	3.12
Phosphorus	2.86	4.16	10.54	6.10
Sulphur	2.12	1.63	6.39	3.91

<sup>1)</sup> Silage was prepared by mixing pure red clover silage and timothy-meadow fescue silage 1:1. Silage in vitro D-value 668 g kg<sup>-1</sup> DM, pH 3.95, ammonia-N 53 g kg<sup>-1</sup> total N, lactic acid 29 g kg<sup>-1</sup> DM, acetic acid 14 g kg<sup>-1</sup> DM and butyric acid 0.3 g kg<sup>-1</sup> DM.

<sup>2)</sup> Ground barley and oats 1:1.

S compared with soybean expeller, which contained more K. Dry matter intake was 19.7, 21.5, 21.8, 20.3 and 20.7 (SEM 0.46) kg d<sup>-1</sup> for diets C, RL, RH, SL and SH, respectively. Intake was significantly (P < 0.05) higher for the RSE diets than for the SBE diets, and it increased linearly with the level of protein supplementation. The mean energy corrected milk yield of the cows followed a similar pattern being 31.1, 34.0, 34.5, 32.2 and 32.4 (SEM 0.57) kg d<sup>-1</sup> for the diets C, RL, RH, SL and SH, respectively.

Na flow exceeded Na intake by 984 g d<sup>-1</sup> indicating an extensive contribution of Na recycled via saliva to the Na flow from the rumen. Omasal Na flow was higher (P < 0.01) in cows fed RSE diets than in those fed SBE diets, and it decreased with the level of protein supplementation (P < 0.05). The decrease was greater for SBE than for RSE diets (interaction P < 0.05). Faecal Na output and total digestibility were not significantly affected by the diet.

### Sodium digestion

The influence of the protein supplements on the flow and excretion parameters of different minerals is presented in Tables 2–4. The mean dietary Na intake (40.5 g d<sup>-1</sup>) was almost entirely derived from supplementary NaCl (Table 2). On average, omasal

### Potassium digestion

Potassium intake increased linearly (P < 0.01) with the level of protein supplementation mainly due to increased silage DM intake. Omasal K flow was similar to K intake (418 vs. 414 g d<sup>-1</sup>; P > 0.10). Ruminal and total absorption of potassium increased with the level of protein supplementation (P < 0.01).

Table 2. The influence of protein supplements on the intake, flow, site of absorption (g d<sup>-1</sup>) and digestibility of sodium and potassium.

	Control	Rapeseed expeller		Soybean expeller		SEM	Significance <sup>1)</sup> (P-value)			
		Low	High	Low	High		R vs. S	Linear	Quadr.	Prot × Lin.
<b>Sodium</b>										
Intake	40.5	40.6	40.6	40.5	40.4	0.1	0.03		0.18	0.05
Omasal flow	1046	1103	1018	994	959	17.3	<0.01	0.03	0.09	0.04
Faecal excretion	18.1	27.4	20.3	21.7	20.8	3.35			0.13	
Absorption										
Reticulo-rumen	-1006	-1062	-977	-954	-918	17.4	<0.01	0.03	0.09	0.05
Postruminal	1028	1075	997	973	938	18.2	<0.01	0.03	0.16	0.05
Total (net)	22.4	13.2	20.3	18.8	19.6	3.3			0.13	
Apparent digestibility	0.554	0.324	0.500	0.464	0.484	0.082			0.13	
<b>Potassium</b>										
Intake	379	414	437	398	442	9.6		<0.01		
Omasal flow	407	425	443	400	413	10.5	0.03	0.14		0.08
Faecal excretion	85.3	95.3	99.1	95.0	84.1	4.00	0.09		0.11	0.03
Absorption										
Reticulo-rumen	-28.6	-11.3	-6.4	-1.9	29.1	8.3	0.03	<0.01		0.02
Postruminal	322	330	344	305	329	11.2	0.12			
Total (net)	293	318	338	303	358	9.6		<0.01		0.17
Apparent digestibility	0.770	0.767	0.773	0.759	0.808	0.012		0.18	0.15	0.06

<sup>1)</sup>R vs. S = The effect of protein source (rapeseed expeller vs. soybean expeller), Linear = Linear effect of increasing protein supplementation, Quadr. = Quadratic effect of increasing protein supplementation, Prot × Lin. = Interaction between the effect of protein source and linear effect of increasing protein supplementation.

The total K digestibility averaged 0.775 and was not significantly influenced by diet (Table 2).

### Calcium digestion

Calcium intake increased ( $P < 0.01$ ) with protein supplementation the effect being greater for RSE than SBE diets (interaction  $P < 0.01$ ) (Table 3). Calcium flow to the omasal canal showed a similar pattern as the intake. Omasal Ca flow tended ( $P < 0.09$ ) to be smaller than Ca intake (106.2 vs. 108.7 g d<sup>-1</sup>) suggesting some net absorption of Ca from the rumen. Postruminal and total Ca absorption was higher in cows fed RSE than in those fed SBE. Because the differences in Ca digestibility were small, faecal Ca excretion increased ( $P < 0.01$ ) with protein supplementation, and it was higher ( $P < 0.01$ ) for RSE than SBE diets. Total Ca digestibility was not influenced by the diet.

Intercept of the regression between Ca intake and omasal flow was slightly positive and slope slightly below 1.00, but neither the intercept nor the slope were significantly different from zero or one (Table 5). The relationships between Ca intake (and flow) and net absorption were much weaker than those between intake and omasal flow vs. faecal excretion. The parameters of the Lucas test indicate that calcium is not an ideal nutritional entity.

### Magnesium digestion

Due to the differences in Mg concentration of feeds and total DM intake, Mg intake was higher ( $P < 0.01$ ) in cows consuming RSE diets than in those consuming SBE diets and it increased ( $P < 0.01$ ) linearly with the level of protein supplementation (Table 3). Similar pattern of differences was observed for omasal Mg flow and faecal Mg out-

Table 3. The influence of protein supplements on the intake, flow, site of absorption (g d<sup>-1</sup>) and digestibility of calcium and magnesium.

	Control	Rapeseed expeller		Soybean expeller		SEM	Significance <sup>1)</sup> (P-value)		
		Low	High	Low	High		R vs. S	Linear	Prot × Lin.
<b>Calcium</b>									
Intake	96.2	115.0	131.1	96.9	104.2	2.5	<0.01	<0.01	<0.01
Omasal flow	92.8	111.9	128.1	96.1	102.2	1.2	<0.01	<0.01	<0.01
Faecal excretion	64.2	75.6	89.0	64.1	67.6	3.1	<0.01	<0.01	<0.01
Absorption									
Reticulo-rumen	3.4	3.1	3.1	0.8	2.0	2.3			
Postruminal	28.5	36.4	39.1	32.0	34.6	2.6	0.12	0.03	
Total (net)	32.0	39.5	42.1	32.7	36.5	2.5	0.04	0.04	0.15
Apparent digestibility	0.335	0.342	0.324	0.339	0.359	0.022			
<b>Magnesium</b>									
Intake	37.1	46.5	53.5	40.1	43.8	0.8	<0.01	<0.01	<0.01
Omasal flow	28.9	37.1	43.1	29.9	31.9	0.89	<0.01	<0.01	<0.01
Faecal excretion	28.4	37.4	42.8	30.6	34.1	1.19	<0.01	<0.01	<0.01
Absorption									
Reticulo-rumen	8.2	9.5	10.4	10.2	11.8	1.0		0.04	
Postruminal	0.6	-0.4	0.3	-0.70	-2.20	0.66	0.07	0.10	0.03
Total (net)	8.7	9.1	10.7	9.5	9.7	1.0			
Apparent digestibility	0.237	0.194	0.200	0.237	0.222	0.021	0.17		

<sup>1)</sup>R vs. S = The effect of protein source (rapeseed expeller vs. soybean expeller), Linear = Linear effect of increasing protein supplementation, Quadr. = Quadratic effect of increasing protein supplementation. Prot × Lin. = Interaction between the effect of protein source and linear effect of increasing protein supplementation.

put. Omasal and faecal Mg flow ( $\text{g d}^{-1}$ ) were closely related to Mg intake ( $\text{g d}^{-1}$ ) as indicated by the following regression equations:

$$\text{Omasal Mg} = -5.1 \pm 4.8 + 0.89 \pm 0.11 \text{ Mg intake} \\ (\text{Se. est.} = 2.78; R^2 = 0.80)$$

$$\text{Faecal Mg} = -4.5 \pm 3.4 + 0.89 \pm 0.08 \text{ Mg intake} \\ (\text{Se. est.} = 2.10; R^2 = 0.87)$$

Absorption of Mg from the rumen increased linearly ( $P < 0.01$ ) as the protein concentration of the supplement increased. However, because of the variable post-ruminal net absorption, the differences in total absorption did not reach significance.

### Phosphorus digestion

Phosphorus intake (Table 4) increased ( $P < 0.01$ ) with protein supplemented diets, and it was higher

( $P < 0.01$ ) with diets containing RSE than with those containing SBE. Phosphorus flow to the omasal canal and excretion in faeces showed similar patterns to those observed in the intake. As a result of ruminal secretion of P, omasal P flow was on average about twice greater than the intake. Apparent absorption in the rumen or post-ruminally was not influenced by the diet. The total (net) absorption was higher ( $P < 0.05$ ) for the cows fed the RSE diets than for those fed the SBE diets, and it increased linearly ( $P < 0.01$ ) with the level of protein supplementation.

There was a strong positive correlation between P intake, P flow into the omasum and faecal P excretion (Table 5). The relationship between P intake and omasal P flow was very similar to that based on individual cow data when calculated using treatment means:  $\text{P flow (g d}^{-1}\text{)} = 82.3 + 1.08 \times \text{P intake (g d}^{-1}\text{)}$ ;  $R^2 = 0.94$ ). Linear relationships be-

Table 4. The influence of protein supplements on the intake, flow, site of absorption ( $\text{g d}^{-1}$ ) and digestibility of phosphorus and sulphur.

	Control	Rapeseed expeller		Soybean expeller		SEM	Significance (P-value)			
		Low	High	Low	High		R vs. S	Linear	Quadr.	Prot × Lin.
<b>Phosphorus</b>										
Intake	65.3	85.0	98.3	71.3	75.1	1.7	<0.01	<0.01		<0.01
Omasal flow	150	179	187	163	163	4.8	<0.01	<0.01	0.10	<0.01
Faecal excretion	39.0	51.6	61.2	42.2	43.2	2.46	<0.01	<0.01		<0.01
Absorption										
Reticulo-rumen	-85.1	-94.4	-88.4	-91.9	-88.2	4.8			0.19	
Post-ruminal	111.3	127.7	125.5	120.9	120.1	4.06	0.17	0.05	0.10	
Total (net)	26.3	33.3	37.1	29.0	31.9	1.5	0.01	<0.01		0.04
Apparent digestibility	0.407	0.391	0.379	0.407	0.427	0.021	0.16			0.15
<b>Sulphur</b>										
Intake	38.3	51.1	61.1	42.5	46.9	0.8	<0.01	<0.01		<0.01
Omasal flow	32.0	40.8	47.4	35.7	38.8	1.27	<0.01	<0.01		<0.01
Faecal excretion	20.0	25.7	28.2	21.7	22.4	0.61	<0.01	<0.01	0.11	<0.01
Absorption										
Reticulo-rumen	6.3	10.3	13.7	6.7	8.1	1.3	<0.01	0.02		0.02
Post-ruminal	12.0	15.1	19.2	14.0	16.30	0.99	0.08	<0.01		0.08
Total (net)	18.4	25.4	32.9	20.8	24.5	0.6	<0.01	<0.01		<0.01
Apparent digestibility	0.479	0.497	0.538	0.490	0.523	0.008	0.20	<0.01	0.14	

<sup>1)</sup>R vs. S = The effect of protein source (rapeseed expeller vs. soybean expeller), Linear = Linear effect of increasing protein supplementation, Quadr. = Quadratic effect of increasing protein supplementation, Prot × Lin. = Interaction between the effect of protein source and linear effect of increasing protein supplementation.



tween daily P intake or P flow and apparent absorption of P were observed. When the Lucas principle was applied for P intake, the intercept was positive ( $P > 0.05$ ) and the slope (true digestibility) was only 0.29. However, when the same test was made for the omasal P flow, the intercept was negative ( $P > 0.05$ ) and the slope markedly higher (0.80).

## Sulphur digestion

Sulphur intake increased ( $P < 0.01$ ) with the level of protein supplementation and was higher ( $P < 0.01$ ) in the cows consuming RSE diets than in those consuming SBE diets (Table 4). Similar pattern of differences between the diets was observed in omasal flow and faecal excretion. Apparent absorption of sulphur from the rumen was higher ( $P < 0.01$ ) for RSE fed cows than for SBE fed cows, and it increased linearly ( $P < 0.01$ ) with protein supplementation. The latter effect was stronger for RSE compared with SBE diets (interaction  $P < 0.01$ ). Total sulphur digestibility increased ( $P < 0.01$ ) with the level of protein supplementation.

Omasal flow and faecal excretion of sulphur were closely related ( $P < 0.01$ ) to S intake (Table 5). Regressions of faecal S output on S intake or omasal S flow had positive intercepts suggesting that faecal S had a metabolic and endogenous component. Applying the Lucas test for sulphur indicated a significantly ( $P < 0.01$ ) negative intercept and a slope with relatively low standard error. There was a close relationship between faecal N and S excretion ( $R^2 = 0.91$ ).

## Discussion

### Experimental methods

Omasal sampling technique provides some advantages compared with duodenal sampling in estimating mineral metabolism in the reticulo-rumen as problems associated with absorption from the

omasum will be avoided. However, a potential source of error in the omasal sampling technique is the difficulty in obtaining representative samples of the omasal digesta. Minerals are distributed differently between rumen digesta fractions (Joblin and Lee 1990), and therefore unrepresentative digesta sample composition will result in biased mineral flow estimates, both due to erroneous DM flow estimates and mineral concentrations. Sodium and potassium are almost entirely in the rumen liquid phase, whereas Ca, Mg, P and S are present in both liquid and solid phases of the digesta with majority of P and Mg in the liquid phase, and Ca and S in the particulate fraction, respectively (Joblin and Lee 1990).

We used a triple-marker technique (France and Siddons 1986) to reduce the possibility of biased flow estimates resulting from unrepresentative sampling. The effect of unrepresentative sampling on the marker concentration relative to individual chemical component is dependent on the association between the two (Ahvenjärvi et al. 2003). In the case that a marker is closely associated with a specific chemical component, unrepresentative sampling will influence the marker concentration in DM, but not on the ratio of the marker and chemical component. Consequently, unrepresentative sampling would influence DM flow, but not that of the chemical component to which the marker is associated with. For example, using only a liquid phase marker may result in biased DM flow estimate, but due to the close association between the marker and liquid phase minerals (Na and K), the flow can be estimated accurately.

The method used by Poutiainen (1968), i.e. determining liquid passage rate and rumen volume using polyethylene glycol and the concentrations of Na and K are less susceptible to experimental errors. In contrast, estimation of Na or K flow from the fore-stomachs by using a solid phase marker can result in severely biased flow estimates because of two reasons: unrepresentative sampling causes errors both in DM flow estimate and mineral concentrations. For example, if a single marker system based on a particle marker is used and the digesta sample contains too much particle phase, both true DM flow and Na (and K) concentrations are under-



Table 5. Regression equations for the flow and absorption of calcium, phosphorus and sulphur ( $\text{g d}^{-1}$ ), and the Lucas tests ( $Y = \text{digestible mineral content (g kg}^{-1} \text{ DM)}$  and  $X = \text{mineral content in the diet (g kg}^{-1} \text{ DM)}$ ).

Y variable	X variable	Intercept	s.e.	P-value	Regression coefficient	s.e.	P-value	RMSE	R <sup>2</sup>
<b>Calcium</b>									
Omasal flow	Intake	5.1	9.44	0.62	0.93	0.084	<0.01	4.77	0.907
Faecal excretion	Intake	-15.8	8.46	0.14	0.81	0.077	<0.01	4.90	0.874
Faecal excretion	Omasal flow	-10.0	8.76	0.32	0.77	0.078	<0.01	4.31	0.897
Absorbed									
Total	Intake	15.8	8.46	0.14	0.19	0.077	0.03	4.90	0.254
Postruminal	Omasal flow	10.0	8.76	0.32	0.23	0.078	<0.01	4.31	0.413
<b>Phosphorus</b>									
Omasal flow	Intake	85.1	12.50	<0.01	1.06	0.152	<0.01	7.26	0.762
Faecal excretion	Intake	-11.2	4.61	0.07	0.74	0.058	<0.01	3.11	0.897
Faecal excretion	Omasal flow	-42.4	12.67	0.03	0.53	0.074	<0.01	4.33	0.897
Absorbed									
Total	Intake	11.2	4.61	0.07	0.26	0.058	<0.01	3.11	0.500
Postruminal	Omasal flow	42.4	12.67	0.03	0.47	0.074	<0.01	4.33	0.745
<b>Sulphur</b>									
Omasal flow	Intake	7.4	4.33	0.16	0.66	0.088	<0.01	2.97	0.762
Faecal excretion	Intake	4.8	1.78	0.06	0.39	0.037	<0.01	1.34	0.897
Faecal excretion	Omasal flow	5.7	2.77	0.05	0.46	0.070	<0.01	1.99	0.897
Absorbed									
Total	Intake	-4.8	1.78	0.06	0.61	0.037	<0.01	1.34	0.936
Postruminal	Omasal flow	-5.7	2.77	0.11	0.54	0.070	<0.01	1.99	0.754
Faecal S	Faecal N	-8.2	2.53	0.03	0.16	0.013	<0.01	1.05	0.914
Lucas tests									
Digestible Ca	Ca	0.16	0.587	0.80	0.31	0.111	<0.01	0.246	0.308
Digestible P	P	0.40	0.329	0.29	0.29	0.086	<0.01	0.165	0.360
Digestible S	S	-0.40	0.095	<0.01	0.68	0.041	<0.01	0.056	0.936

RMSE is an estimate of the standard deviation of the error term in the model.

estimated, which can lead to severe underestimation of Na (and K) flow. These methodological aspects have very seldom been considered in studies investigating mineral absorption from different sites of the digestive tract.

## Sodium

The mean Na flow of  $1024 \text{ g d}^{-1}$  to the omasal canal was almost 25-fold compared with the sodium intake ( $40.5 \text{ g d}^{-1}$ ) reflecting net salivary Na secre-

tion into the rumen. In the present study, omasal Na flow was markedly higher than in studies using duodenal sampling. At the sodium intake of  $61 \text{ g d}^{-1}$  the duodenal flow was 4.5-fold (Rahnema et al. 1994) and at the intake of  $106 \text{ g sodium per day}$  the duodenal flow was 2.1-fold (Khorasani et al. 1997). Using omasal sampling the Na flow to the omasum was 17-fold at the intake of  $34 \text{ g d}^{-1}$  in ruminating calves (Edrison et al. 1986). Although the higher DM intake may have contributed to the higher Na secretion in the rumen in the present study, most of the difference between the studies can be attributed

to different sampling sites. Appreciable absorption of sodium from the omasum of both sheep and cattle has been reported. Engelhardt and Hauffe (1975) measured omasal absorption of sodium in sheep to be 26% and Edrisc et al. (1986) reported a range of 40–60% in young cattle. Faecal Na output was not related to omasal Na flow [Faecal (Na g d<sup>-1</sup>) = 14.0 ± 19.2 + 0.008 ± (0.0187) × Omasal Na (g d<sup>-1</sup>)] indicating that Na secreted via saliva into the rumen was completely reabsorbed. The omasal Na flow was greater in cows receiving RSE compared to those on SBE diets, but the difference was quantitatively relatively small and possibly related to differences in feed intake.

Salivary Na concentration seems to be much less variable than that of phosphorus and therefore saliva production may be estimated from net Na secretion and Na concentrations in saliva. Using the mean salivary Na concentration of 160 mmol l<sup>-1</sup> (Poutiainen 1968, Van Soest 1994, Sjaastadt et al. 2003) and salivary Na secretion of 982 g d<sup>-1</sup> gives an estimate of 267 l d<sup>-1</sup> for the saliva production, which is comparable of the measured saliva flow of 239 l d<sup>-1</sup> when the cows were eating 18.2 kg DM d<sup>-1</sup> at forage:concentrate ratio of 60:40 (Maekawa et al. 2002). In the present study, salivary Na flow per kg DM intake was slightly higher (47.2 g) than the corresponding value calculated from the data of Poutiainen (38.3 g).

Assuming that the rumen volume of these cows was similar to that measured in our recent studies in cows fed similar diets (80 l; n = 13, unpublished data from MTT), the mean liquid outflow rate would be 0.138 h<sup>-1</sup>. However, Na concentration in the rumen fluid is lower than in saliva due to dilution by water drunk and contained in feeds so that total liquid outflow and outflow rate would be higher. Assuming that Na concentration in rumen fluid was 20% lower than in saliva (Poutiainen 1968) liquid outflow and passage rate had been 334 l d<sup>-1</sup> and 0.173 h<sup>-1</sup>, respectively.

## Potassium

On average, no net K absorption from the rumen was observed in the present study. However, Rah-

nema et al. (1994) and Khorasani et al. (1997) reported pre-intestinal absorption of K. This difference may be attributed to absorption from the omasum (Engelhardt and Hauffe 1975, Edrisc et al. 1986). Ruminal outflow of K was higher with RSE than SBE supplementation, which could be related to increased silage DM intake. Total K absorption was strongly related (R<sup>2</sup> = 0.95) to K intake. Ruminal and postruminal K absorption was only moderately related to K intake (R<sup>2</sup> = 0.37 and 0.28), whereas postruminal K absorption was strongly (R<sup>2</sup> = 0.94) influenced by omasal K flow.

In addition to dietary intake, K is secreted in variable concentrations into the rumen via saliva (see Van Soest 1994). Using a concentration of 10 mmol l<sup>-1</sup> (Van Soest) would result in a salivary K secretion of about 100 g d<sup>-1</sup>. Because the apparent absorption of K from the rumen was close to zero, true absorption of K was equal to salivary secretion. Salivary K concentration depends on Na status (Sjaastadt et al. 2003). Poutiainen (1968) reported that salivary K secretion decreased from 227 g d<sup>-1</sup> in cows receiving no supplementary NaCl to 40–50 g d<sup>-1</sup> when 50–100 g d<sup>-1</sup> of NaCl was given.

## Calcium

The proportional Ca absorption will decrease as dietary Ca intake exceeds the requirements of the tissues for absorbed Ca (NRC 2001). In the present study, the supply of Ca was below the requirement (MTT 2006) for the cows consuming the control diet and SBE diets, which would allow to truly determine the efficiency of Ca absorption. In spite of this, the intercept of the regression between the intake and faecal output was negative which has no biological meaning (faecal output can not be negative). The negative intercept between Ca intake and faecal output (or positive intercept between intake and absorption) suggests that the incremental Ca derived from increased silage DM intake and from protein supplements was absorbed with a lower efficiency. The mean Ca digestibility of 0.34 was slightly higher than that of 0.30 in the study of Khorasani et al. (1997).

The mean apparent absorption of Ca from the rumen was 2.5 g d<sup>-1</sup>, but the true absorption is higher due to salivary secretion. According to Yano et al. (1991), Ca concentration in mixed saliva ranges from 16 to 30 mg l<sup>-1</sup>. Therefore, we could speculate that approximately 6 g of Ca were secreted into the rumen via saliva, when calculated using salivary secretion estimated from omasal Na flow (267 l d<sup>-1</sup>). The slope of the regression between Ca intake and omasal flow (0.93) suggested that proportionally 0.07 of incremental Ca was absorbed from the rumen. In the study of Khorasani et al. (1997), the corresponding slope was markedly lower (0.51), which in addition to possible absorption of Ca from the omasum was related to a greater absorption in stomachs of cows fed lucerne silage high in Ca. There is some disagreement in the literature on the site of Ca absorption. In the study of Khorasani and Armstrong (1992) the major site of Ca absorption was prior to the small intestine, whereas Yano et al. (1991) concluded in their review that the small intestine is the main absorptive site for Ca, which is also supported by our data.

## Magnesium

Apparent Mg digestibility (mean 0.218) compares well with the values of 0.244 and 0.202 reported by Rahnema et al. (1994) and Khorasani et al. (1997). The strong positive relationships between Mg intake and omasal and faecal outputs indicate that intake rather than feed characteristics regulated Mg excretion. Magnesium absorption occurred in the rumen, which is in accordance with earlier reports (Khorasani and Armstrong 1990, 1992, Rahnema et al. 1994, Khorasani et al. 1997).

The net secretion of Mg into the small intestine (0.5 g d<sup>-1</sup>) was marginal compared with the values of 3.7 and 4.5 g d<sup>-1</sup> in earlier studies using duodenal sampling (Rahnema et al. 1994, Khorasani et al. 1997). The difference may be attributed to different sampling sites, since considerable Mg absorption was found in the omasum of young cattle (Edrison and Smith 1986, Edrison et al. 1986). The relationship between omasal Mg flow (X) and fae-

cal Mg excretion ( $Y = 3.6 \pm 2.6 + 0.91 \pm 0.07 X$ ;  $R^2 = 0.87$ ) indicates that 3.6 g d<sup>-1</sup> Mg was secreted post omasum and that proportionally 0.09 of Mg flowing into the omasum was absorbed, most likely in the omasum.

## Phosphorus

In the present study, the intercept of the regression between P intake and omasal flow was almost equal to the mean P intake (85 vs. 79 g d<sup>-1</sup>) whereas in the studies of Rahnema et al. (1994) and Khorasani et al. (1997) the intercept of regression was markedly lower than intake. The difference of 89 g d<sup>-1</sup> between P flow and intake was markedly higher than the corresponding values of 28 and 48 g d<sup>-1</sup> in other studies (Rahnema et al. 1994, Khorasani et al. 1997). This discrepancy may be associated to digesta sampling sites. Appreciable absorption of P between the reticulum and duodenum, presumably from the omasum, has been demonstrated (Edrison and Smith 1986). It has been suggested that daily salivary secretion might be calculated by the difference between daily P intake and flow into the duodenum (Scott and McLean 1981), but the data presented by Yano et al. (1991) indicated that the sum of dietary and salivary P considerably exceeds the P flow at the duodenum, probably reflecting P absorption from the omasum. Therefore omasal sampling may allow a more accurate estimation of salivary flow than duodenal sampling due to absorption of P between reticulum and duodenum.

The range in salivary P concentration reported in the literature is rather wide ranging from <100 to 700 for cattle and from 400 to 700 for sheep as reviewed by Van Soest (1994), Karn (2001) and Pfeffer et al. (2005), and therefore analysis of P concentration in mixed saliva is a prerequisite for an estimation of salivary flow from the rumen. However, salivary P concentrations from 312–359 mg l<sup>-1</sup> (10.1 to 11.6 mmol l<sup>-1</sup>) calculated from salivary secretion estimated from the net Na and P secretion into the rumen are within the range reported for salivary P concentration of dairy cows (Karn 2001, Valk et al. 2002, Pfeffer et al. 2005).

The present results do not support the suggestion of Khorasani et al. (1997) that P flow from the rumen is rather constant and that as P content of the diet increases, the salivary P secretion decreases. In contrast, increased P intake with protein supplemented diets resulted in small increases (slope 1.06) in the omasal P flow indicating that salivary P secretion was not influenced by P intake. The slope slightly above 1.00 may reflect increased saliva production in response to increased silage DM intake when the protein supplements were fed.

The Lucas test indicated that P is not an ideal nutritional entity. The intercept was positive, which has no biological meaning, and the slope of regression had a high standard deviation. Positive intercepts may be interpreted as a low marginal digestibility of additional P and that the supply exceeds the requirements in cows consuming RSE diets, which resulted in reduced absorption (Wu et al. 2000, Pfeffer et al. 2005). Wu et al. (2000) suggested that apparent digestibility of P will be in the range of 0.45 to 0.50 when P is fed close to requirements. Lower values for apparent P digestibility would occur when P is fed in excess to requirements, because the gut attempts to regulate P uptake according to requirements and to maintain homeostasis.

The Lucas equation estimated using only the SBE containing diets was biologically more meaningful than the overall equation: Digestible P ( $\text{g kg}^{-1} \text{DM}$ ) =  $0.62 \times \text{P} (\text{g kg}^{-1} \text{DM}) - 0.72$  ( $R^2 = 0.35$ ). Although  $R^2$  value was low, the values for the intercept ( $-0.72$ ) and slope of regression (0.62) are only slightly different from the NRC (2001) recommendation for the maintenance requirement (1  $\text{g kg}^{-1} \text{DM}$  intake) and true absorption coefficient of dietary P (0.70), suggesting that these diets (P concentration 3.3 to 3.6  $\text{g kg}^{-1} \text{DM}$ ) met the P requirements of cows producing on average 33 kg milk per day.

Dietary P concentration and P intake (3.3  $\text{g kg}^{-1} \text{DM}$  and 65  $\text{g d}^{-1}$ ) of the cows fed the control diet were below the requirements calculated according to NRC (2001) system, but P concentration was higher than 3.0–3.1, which is considered marginal (Satter 2003). Although the control diet was slightly marginal in the P supply, the P availability from

soybean expeller (0.62) was clearly lower than the values (0.90) found in animals fed below the requirements (Kincaid and Rodehutsord 2005, Pfeffer et al. 2005).

A low true digestibility of incremental P mainly derived from rapeseed could be related to the low availability of P in rapeseed. However, in the study of Rodehutsord and Pfeffer (unpublished, ref. Kincaid and Rodehutsord 2005) faecal P output remained unchanged indicating a high availability of rapeseed P.

True digestibility of omasal P estimated as a regression between the phosphorus absorbed in the lower tract and omasal P flow was markedly higher than the corresponding slope between total P absorption and P intake (0.46 vs. 0.26). This suggests that salivary P was absorbed with greater efficiency than dietary P (Challa et al. 1989, NRC 2001, p. 110). The regression for the omasal P absorption ( $\text{g d}^{-1}$ ) in the lower tract, which included P intake and salivary P (omasal P flow – P intake), was as follows:  $\text{P absorbed} (\text{g d}^{-1}) = 15 \pm 8.0 + 0.26 \pm 0.058 \text{ Feed P} + 0.96 \pm 0.073 \text{ Saliva P}$  (RMSE=3.2;  $R^2 = 0.91$ ). This equation suggests that salivary P is efficiently reabsorbed. However, it is uncertain whether these coefficients are unbiased, because the intercept was positive.

## Sulphur

The dietary requirement of sulphur for the ruminants is primarily to provide substrate to ensure maximal microbial protein synthesis. Sulphur intake was higher in cows fed RSE diets than in those fed SBE diets, which partly reflects higher concentrations of S-containing amino acids and other compounds in rapeseed than in soybean.

Dietary S is absorbed as sulphate and sulphide anions. Dietary S concentration of 1.94  $\text{g kg}^{-1} \text{DM}$  in cows fed the control diet was marginally below the NRC (2001) recommendation of 2.0  $\text{g kg}^{-1} \text{DM}$ . Net absorption of S from the rumen even in cows fed the control diet suggests that NRC (2001) recommendation may overestimate the S requirement of rumen microbes. In the present study, the regression equation between S intake and omasal S

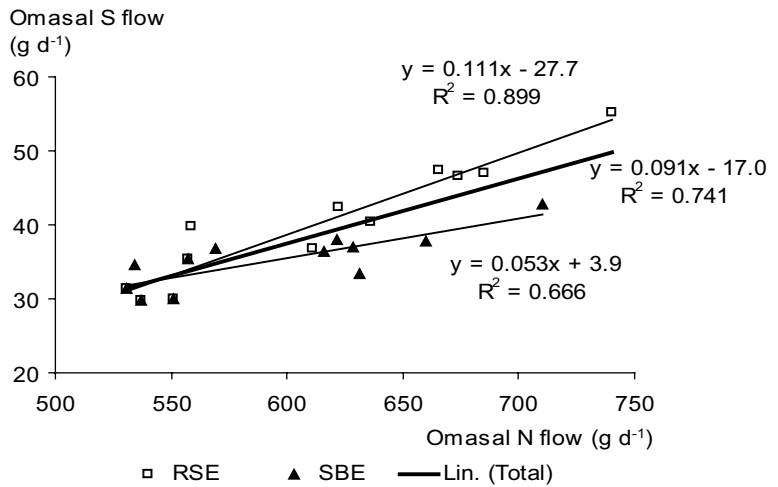


Fig. 1. Relationships between omasal nitrogen and sulphur (S) flow ( $\text{g d}^{-1}$ ) for the total data and separately for rapeseed (RSE) or soybean expeller (SBE).

flow showed that proportionally 0.34 of S intake was absorbed in the rumen.

Omasal S and N flows were closely correlated (Fig. 1) indicating that a major proportion of S flow from the rumen is associated with the protein flow. The slope of regression was markedly higher for the RSE diets than for the SBE diets (0.111 vs. 0.052;  $P < 0.01$ ). This is partly attributed to the higher content of S containing amino acids in rapeseed than in soybean (MTT 2006), but most likely to the high concentration of non-amino S compounds in rapeseed.

Sulphur was the only mineral fraction that met the criteria of ideal nutritional entity, i.e. low standard deviation of the slope and zero or negative intercept, when the digestible amount was regressed against the total amount in the diet. In the present study based on individual data, the standard deviation of the slope was 0.040 and  $R^2$  was 0.945. Using treatment mean data from published studies and from the present study showed a very close relationship between the concentrations of S and digestible S in DM (Fig. 2) when a mixed model regression analysis was used to exclude the variation resulting from differences in experimental and analytical techniques between the laboratories (see St-Pierre 2001). The true S digestibility was  $0.807 \pm 0.018$  and faecal metabolic and endogenous

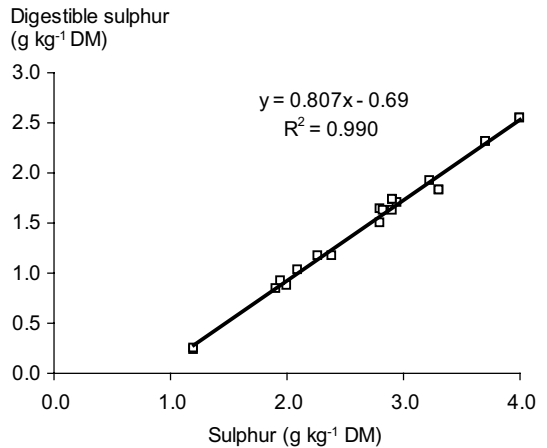


Fig. 2. Lucas test for sulphur ( $N = 20$ ). The values are adjusted for random study effect. Data from Muntifering et al. (1985), Spears et al. (1985), Puoli et al. (1991), Khorasani et al. (1997) and present study.

S fraction was  $0.69 \pm 0.051$  g. The studies were conducted using sheep (Muntifering et al. 1985, Puoli et al. 1991), growing steers (Spears et al. 1985) and lactating dairy cows (Khorasani et al. 1997, present study). The dietary S concentrations were manipulated by elementary S (Muntifering et al. 1985), sodium sulphate (Puoli et al. 1991), sul-

phur fertilization (Spears et al. 1985), forage type (Khorasani et al. 1997) and protein supplementation (present study). Even when the random study effect was excluded from the model, the  $R^2$  value remained high (0.96) and the standard deviation of true S digestibility relatively low (0.030).

## Conclusions

Omasal sampling technique provides an experimental tool to investigate mineral metabolism in the reticulo-rumen, and if used in duodenally cannulated animals, net pre-intestinal mineral absorption can be separated between reticulo-rumen and omasum-abomasum. Appropriate marker techniques are required for estimation of mineral metabolism in the fore-stomachs. Both Na and P secretions into the rumen were markedly higher than in studies conducted using duodenally cannulated animals due to marked absorption of these minerals from the omasum. Magnesium was mainly absorbed from the rumen while K and Ca from the small intestine. The Lucas test demonstrated a uniform behaviour of sulphur over a wide range of diets. Net absorption of sulphur from the rumen suggests that sulphur is unlikely to be limiting factor for rumen microbial synthesis when diets based on silage and cereal grains are fed. The results indicate that no supplementary P and Mg are required in cows fed diets similar to the current experiment, especially if rapeseed feeds are used as protein supplements.

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## SELOSTUS

### Satakertanäytteenottomenetelmä soveltuu lypsylehmien kivennäisaineiden sulatuksen tutkimiseen

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Fysiologisessa kokeessa selvitettiin rypsi- ja soijapuristeruokinnan vaikutusta lypsylehmien kivennäisaineiden eli natriumin, kaliumin, magnesiumin, fosforin ja rikin sulatukseen. Lehmien päivittäinen väkirehuannos oli 9 kg, ja se koostui ohran ja kauran seoksesta, jota korvattiin rypsi- tai soijapuristeella kahdella käyttömäärällä siten, että väkirehujen valkuaispitoisuudet olivat 130, 180 ja 230 g/kg kuiva-ainetta. Koeruokintoja oli yhteensä viisi. Lehmät söivät vapaasti säilörehua, joka sisälsi puolet puna-apilaa ja puolet timotei-nurminataa. Lisäksi lehmille annettiin suolaa ja hivenainelisiä, mutta ei muita kivennäisliisiä. Pötsisulavuus määritettiin satakertanäytteenottomenetelmällä, ja kokonaissulavuus sonnan kokonaiskeruun avulla.

Muiden kivennäisten paitsi natriumin saanti lisääntyi valkuaislisän myötä, ja rypsipuristetta saaneiden leh-

mien kivennäisten saanti oli yleensä suurempi kuin soijapuristetta saaneiden. Magnesiumin imeytyminen tapahtui pääasiassa pötsi-verkkomahasta, mutta kalsium, fosfori, natrium ja kalium imeytyivät pääasiassa pötsi-verkkomahan jälkeen. Rikkiä imeytyi sekä pötsi-verkkomahasta että sen jälkeen. Rikin nettoimeytyminen pötsi-verkkomahasta osoittaa säilörehusta ja viljasta koostuvien ruokintojen sisältäneen riittävästi rikkiä pötsimikrobien tarpeeseen. Suuri satakertaan virtaava natriumin ja fosforin määrä kertovat näiden kivennäisten runsaasta erityksestä syljen mukana. Satakerta on tärkeä kivennäisten, erityisesti natriumin ja fosforin imeytymispaikka. Tulokset osoittavat myös, että lisäfosforin ja -magnesiumin tarvetta ei ollut kokeessa käytetyillä vilja-säilörehuruokintoilla käytettäessä rypsirehuja valkuaislähteenä.