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The effects of calcium and sodium loading on organic matter digestibility and mineral absorption in sheep 3. Changes in the Ca, Mg, Zn and Cu concentrations in rumen fluid

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ABSTRACT

The effect of diets containing excess calcium and sodium (5 and 10 times above ARC-recommended levels) on the concentration of Ca, Mg, Zn and Cu in rumen fluid of sheep was studied. The concentrations of Ca, Mg and Zn were closely correlated with the decline in pH after feeding.

Ca and Mg concentrations remained elevated for up to 6 h after feeding, as compared with preprandial levels. The concentrations of Zn and Cu increased immediately after feeding, but 2-3 h later rapidly decreased to pre-feeding levels. This points to high microbial uptake and/or particle association of microelements in the rumen. Hence, mineral concentration in rumen fluid not always reflects differences in mineral intake. Sodium loading increased Mg concentration, while Ca loading elevated the Cu concentration in rumen fluid. Both tended to decrease the Zn concentration, also the Zn intake in these groups was higher.

KEY WORDS: minerals, rumen, sheep

INTRODUCTION

Improving the yield of the organic matter from a unit area of cultivated land necessitates the application of a wide range of fertilizers, among them mineral fertilizers. On the other hand, the quality of lignocellulotic materials is very low, and many methods have been developed for overcoming their low intake and poor digestibility. The one of the most popular chemical methods for improving the nutritional value of high fibre roughages is treatment with sodium hydroxide or calcium hydroxide. Both approaches, fertilization and treatment, can lead in consequence to overloading of macroelements in both ruminants and other animals. Macroelement overdosing in the ruminant diet can disorder absorption, flow rate in the gastrointestinal tract and metabolism of minerals and other dietary components (Chicco et al., 1973; Underwood, 1977; NRC, 1980; Ben-Ghedalia et al., 1982; Alfaro et al., 1988). Sodium overloading increases saliva excretion (Bartley, 1976), changes the Na:K ratio in saliva (Chiy and Phillips, 1993), affects Mg absorption (Fontenot et al., 1973) and increases water requirements (Evans, 1981). The first place where some mineral disorders can be observed is the mouth and the rumen. Therefore, the purpose of the present study was to estimate the effect of calcium and sodium overloading on the dynamic Ca, Mg, Zn and Cu changes in the rumen fluid.

MATERIAL AND METHODS

Nine sheep were divided into three groups: control (I), Ca-loaded (II; 19.6% calcium lactate) and Na-loaded (III; 9.6% NaCl in ration). A detailed description is given in the first and second parts of this study (Leontowicz et al., 1995 a,b). Samples of rumen digesta were taken through a cannulae on the third day in the fourth and fifth weeks before (0 time) and 1, 2, 3, 4, 5 and 6 h after the morning feeding. Samples from three animals (one from each group) were not

TABLE 1

The mineral content of offered feeds, % of DM

Minerals	Concentrate			Meadow hay
	I control	II Ca-loaded	III Na-loaded	
g/kg				
Ash	53.7	149.4	169.7	82.2
Ca	7.99	45.28	14.46	6.67
Mg	2.81	2.77	2.90	2.16
P	3.33	4.88	5.28	3.58
Na	1.12	3.28	30.00	1.22
K	9.40	8.03	8.58	15.75
mg/kg				
Zn	49.4	96.8	98.7	40.9
Cu	17.2	33.9	25.8	22.6

taken in the fourth week, which means only five samples of rumen digesta were taken instead of six. The pH of rumen digesta was measured immediately after sampling. Two subsamples of rumen digesta were taken from each animal at a time. The samples were strained through eight layers of cheesecloth and were centrifuged at 24000 x g. Mineral elements in the supernatant were estimated directly by flame atomic absorption spectrometry (Perkin-Elmer 1100B), and the averages of two subsamples were used for statistical evaluation.

The mineral concentration in rumen fluid was also expressed as a relative change (%) against pre-feeding values (100%). One-way and two-way analysis of variance (group x time) for data analysis were carried out and the Tukey test was applied for comparison of means at $P \leqslant 0.05$. Regression analysis was also performed (Statgrafics software).

RESULTS

The mineral composition of concentrates as well as of meadow hay are presented in Table 1. The control concentrate contained the lowest amounts not only of Ca, Na and ash, but also of P, Zn and Cu. The increase of mineral content in the experimental concentrates might possibly be due to extrusion. Under conditions of high temperature (150^{0}C), high pressure (8 MPa) and moisture, in the presence of calcium lactate and NaCl, some mineral elements could probably enter the extrudates. In consequence, sheep in group I consumed lower amounts ($P \leqslant 0.05$) of Ca, P, Na, Zn and Cu in comparison with groups II and III (Table 2). The Ca intake in the Ca-loaded group and Na intake in the

TABLE 2

Mean daily dry matter and mineral elements intake

Item	Groups		
	I control	II Ca-loaded	III Na-loaded
Dry matter, g	650^a	725^b	704^{ab}
Ash, g	43^a	85^b	91^c
Ca, g	5.08^a	19.66^b	7.98^c
Mg, g	1.74	1.80	1.80
P, g	2.23^a	2.55^b	2.48^{ab}
Na, g	0.79^a	1.69^b	11.85^c
K, g	7.82	8.51	8.36
Zn, mg	33.0^a	54.1^b	55.0^b
Cu, mg	12.6^a	20.6^b	17.1^b

$^{a, b, c}$ means followed by different letters withhin row, differ at $P \leqslant 0.05$

TABLE 3

The effect of group and time on pH and mineral concentration in the rumen fluid

Variation	pH	Ca		Mg		Zn		Cu	
		mg/l	%	mg/l	%	ug/l	%	ug/l	%
Group (n = 35)[1]									
Control, I	5.49^{a}	512^{a}	970^{a}	105.4^{a}	498^{a}	880^{a}	629	35.9^{a}	100^{a}
Ca-loaded, II	5.88^{b}	1791^{b}	3461^{b}	146.0^{b}	896^{a}	501^{b}	416	53.1^{b}	241^{b}
Na-loaded, III	5.60^{a}	504^{a}	1464^{a}	133.9^{b}	1834^{b}	557^{ab}	334	38.2^{a}	134^{a}
Time (n = 15)[2]									
0	6.88^{a}	81^{a}	100^{a}	23.5^{a}	100^{a}	156^{a}	100^{a}	31.2^{a}	100^{a}
1	5.56^{bc}	1198^{bc}	2498^{bc}	121.4^{b}	1019^{ab}	785^{abc}	614^{ab}	50.3^{b}	192^{b}
2	5.14^{d}	1295^{c}	2803^{c}	161.0^{bc}	1348^{b}	1194^{c}	893^{b}	49.6^{b}	192^{b}
3	5.24^{d}	1314^{c}	2714^{bc}	174.7^{c}	1579^{b}	894^{bc}	618^{ab}	49.8^{b}	185^{ab}
5	5.42^{cd}	944^{bc}	2150^{bc}	140.7^{bc}	1142^{b}	622^{abc}	393^{ab}	42.5^{ab}	163^{ab}
5	5.57^{bc}	974^{c}	1975^{bc}	138.8^{bc}	1151^{b}	529^{abc}	366^{ab}	37.7^{ab}	145^{ab}
6	5.80^{b}	745^{b}	1513^{b}	1398.9^{bc}	1193^{b}	342^{ab}	233^{a}	35.8^{ab}	129^{ab}

[1] n = 5 samples x 7 times.
[2] n = 5 samples x 3 groups; time: 0 – before feeding; 1, 2, 3, 4, 5, 6 – h after feeding
[a-d] means followed by different letters within column of the same variation differ at $P \leq 0.05$

Na-loaded group were the highest ($P \leqslant 0.05$). Lower DM intake ($P \leqslant 0.05$) was observed in the control group as opposed to group II (Table 2).

Ca loading increased ($P \leqslant 0.05$) the rumen pH as well as the Ca, Mg and Cu contents in the rumen fluid, and decreased the Zn concentration (Table 3). Relative changes of the Mg and Zn rumen fluid contents were not significantly affected by Ca loading. In the Na-loaded group, a significant rise of Mg (in absolute and relative amounts) was noted against group I. The Zn concentrations in group III were closer to those found in group II (Table 3).

The highest pH value was observed before feeding (6.876) and remained lower ($P \leqslant 0.05$) for all times after, achieving a minimum at 2 and 3 h after feeding (Table 3). The Ca rumen fluid concentration (81 mg//l) increased ten times after feeding up to about 1300 mg/1 (2 and 3 h) and after 6 h still was 9 times higher ($P \leqslant 0.05$). The Mg content behaved similarly (Table 3) and remained higher ($P \leqslant 0.05$) than before feeding even after 6 h (138.9 vs. 23.5 mg/l). The relative values of both macroelements showed similar changes. The Zn concentration increased ($P \leqslant 0.05$) from time 0 to 2 h after feeding (156 vs. 1194 mg/l) and then its content started to decrease; no significant differences between 0 time and 4, 5, 6 h after feeding were found (Table 3). The Cu concentration in rumen fluid increased ($P \leqslant 0.05$) immediately (doubled) after feeding (31.2 vs. 50.3 mg/l) and did not change during the following two hours. Similarly as in the case of Zn, the concentration of Cu 4, 5, 6 h after feeding did not differ from its value at 0 time (Table 3).

DISCUSSION

To be absorbed from the gastrointestinal tract, mineral elements must be in soluble form, though such absorption is usually incomplete (Ivan et al., 1979). We observed a higher intake of dry matter and minerals, except K and Mg, in groups II and III (Table 2). The higher DM intake could be caused by better palatability and/or by lower energy concentration caused by loading the diets with the Ca and Na. Similarly, Chiy and Phillips (1993) and Chiy et al. (1994) reported increased uptake of Ca, P and Mg when sheep consumed increasing amounts of sodium. The mineral concentration in rumen fluid may also be influenced by the outflow rate from the forestomachs (Teller and Godeau, 1987), pH value (Bremner, 1970; Ivan et al., 1979; Keyser et al., 1985), NH_3-N concentration (Care et al., 1982), other mineral element intake, e.g. sodium (Moseley and Jones, 1974; Reffett and Boling, 1985), potassium (Wylie et al., 1985; Yano et al., 1988), calcium (Yano et al., 1978).

In this study, the VFA molar proportion (acetate : propionate : butyrate) was similar in the control (54:34:12) and in Na-loaded groups (55:32:13), while in the

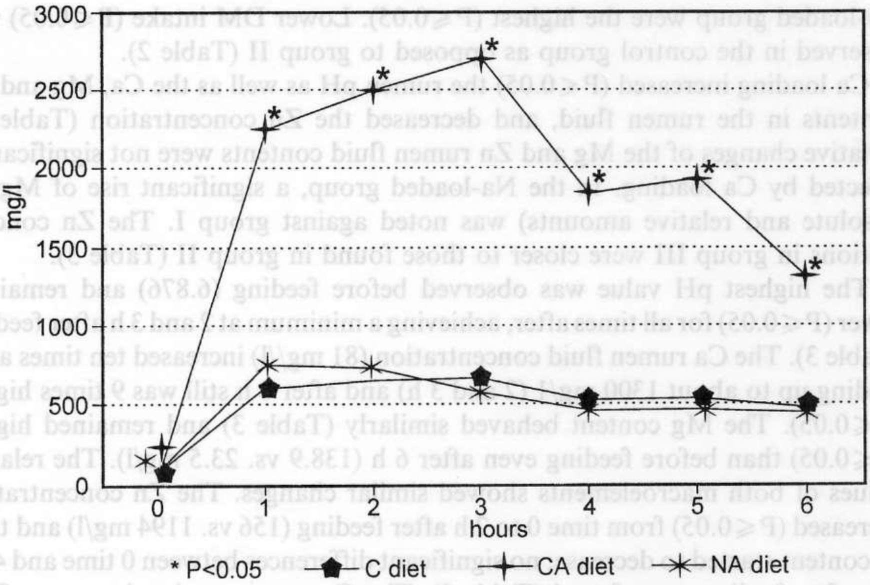

Figure 1. Changes of Ca concentration in rumen fluid after feeding

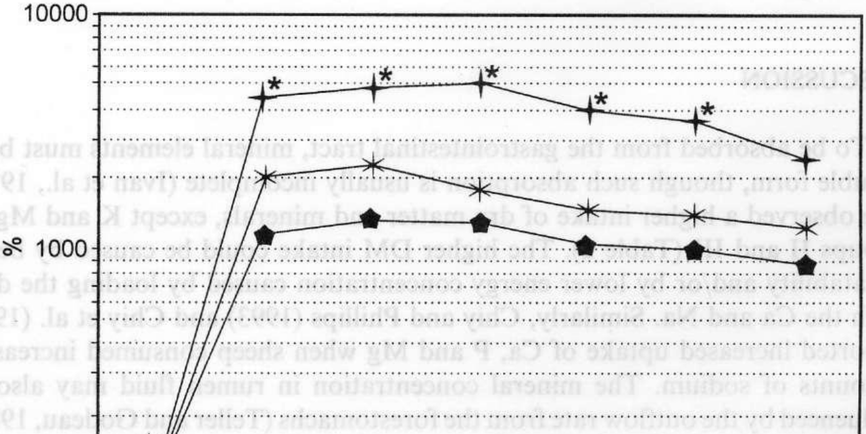

Figure 2. Relative changes of Ca concentration in rumen fluid after feeding

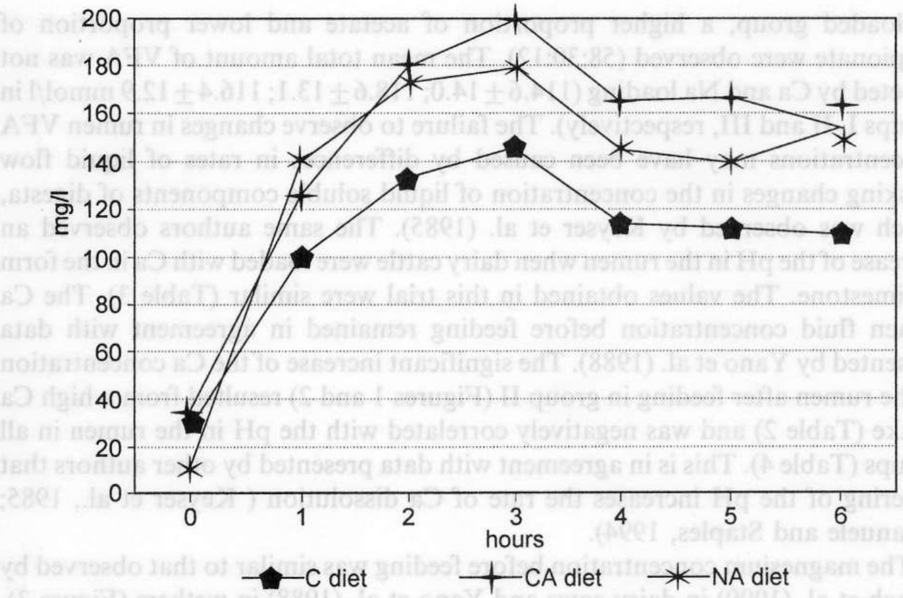

Figure 3. Changes of Mg concentration in rumen fluid after feeding

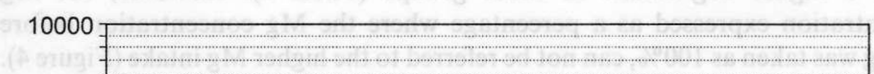

Figure 4. Relative changes of Mg concentration in rumen fluid after feeding

Ca-loaded group, a higher proportion of acetate and lower proportion of propionate were observed (58:30:12). The mean total amount of VFA was not affected by Ca and Na loading (114.6 ± 14.0; 118.6 ± 13.1; 116.4 ± 12.9 mmol/l in groups I, II and III, respectively). The failure to observe changes in rumen VFA concentrations may have been caused by differences in rates of liquid flow masking changes in the concentration of liquid soluble components of digesta, which was observed by Keyser et al. (1985). The same authors observed an increase of the pH in the rumen when dairy cattle were loaded with Ca in the form of limestone. The values obtained in this trial were similar (Table 3). The Ca rumen fluid concentration before feeding remained in agreement with data presented by Yano et al. (1988). The significant increase of the Ca concentration in the rumen after feeding in group II (Figures 1 and 2) resulted from a high Ca intake (Table 2) and was negatively correlated with the pH in the rumen in all groups (Table 4). This is in agreement with data presented by other authors that lowering of the pH increases the rate of Ca dissolution (Keyser et al., 1985; Emanuele and Staples, 1994).

The magnesium concentration before feeding was similar to that observed by Lough et al. (1990) in dairy cows and Yano et al. (1988) in wethers (Figure 3). Peak Mg values were reached 2 and 3 h after feeding, which is earlier than the 4 and 6 h reported by Xin et al. (1989). We found higher Mg concentrations in groups II and III than in the control group (Table 3; Figure 3), which could reflect a higher Mg intake in those groups (Table 2). However, the Mg concentration expressed as a percentage where the Mg concentration before feeding was taken as 100%, can not be referred to the higher Mg intake (Figure 4). While the relative Mg concentration in the Ca-loaded group did not differ from the control group level, it was significantly higher in the Na-loaded group ($P \leqslant 0.05$) than in either of the remaining groups. This can be explained on the basis of antagonism between Na and K. It was observed that intraruminal infusion of K decreased Na and Mg rumen fluid concentrations (Wylie et al., 1985; Yano et al., 1988). In our experiment the Na:K ratio increased from 1:10 (group I) to 1:5 and 1.5:1 in groups II and III, respectively. Care et al. (1982) also observed that a low ruminal Na:K ratio reduced the net efflux of Mg and Na from the rumen, meaning their solubility was reduced. So, it is possible that high Na and Ca intake diminished the detrimental influence of K on Mg rumen fluid concentration. That is why we found a very good correlation between Mg concentration and Ca concentration as well as with the pH value (Table 4).

The Zn concentration in rumen liquid in the control group and time of peak value (2 h after feeding) were similar to results presented by Ivan et al. (1979), who also reported that the concentration of the soluble Zn increased when there was a considerable decrease in the pH, which is also in agreement with our findings (Table 4). Despite a higher Zn intake in groups II and III (Table 2), the

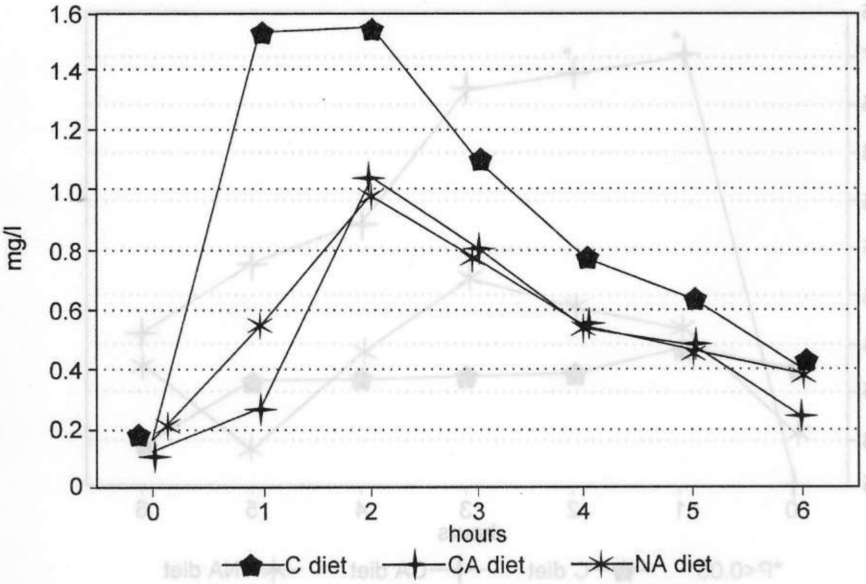

Figure 5. Changes of Zn concentration in rumen fluid after feeding

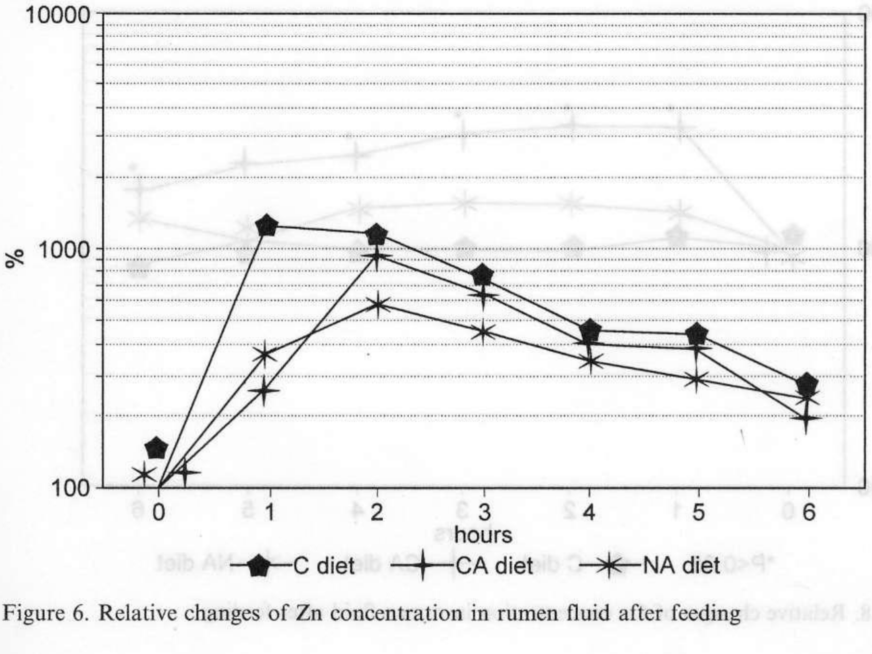

Figure 6. Relative changes of Zn concentration in rumen fluid after feeding

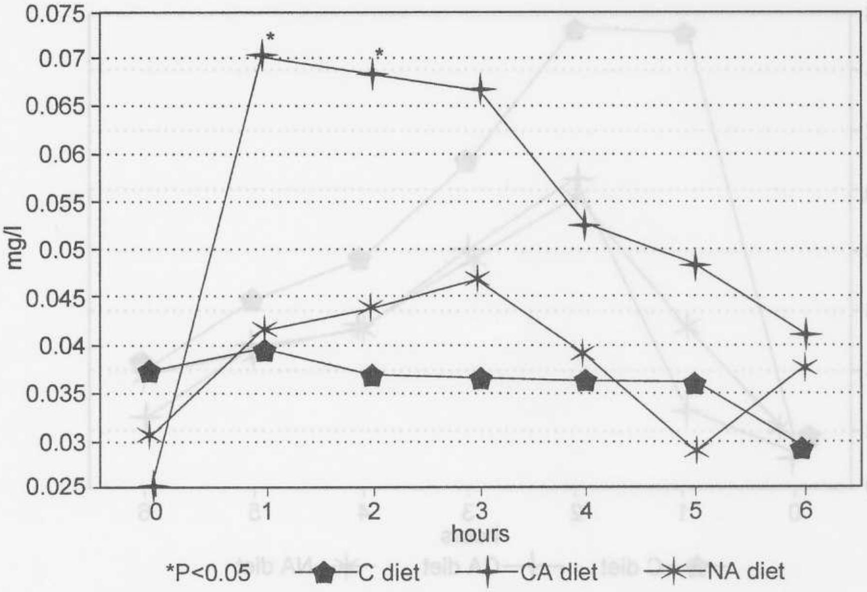

Figure 7. Changes of Cu concentration in rumen fluid after feeding

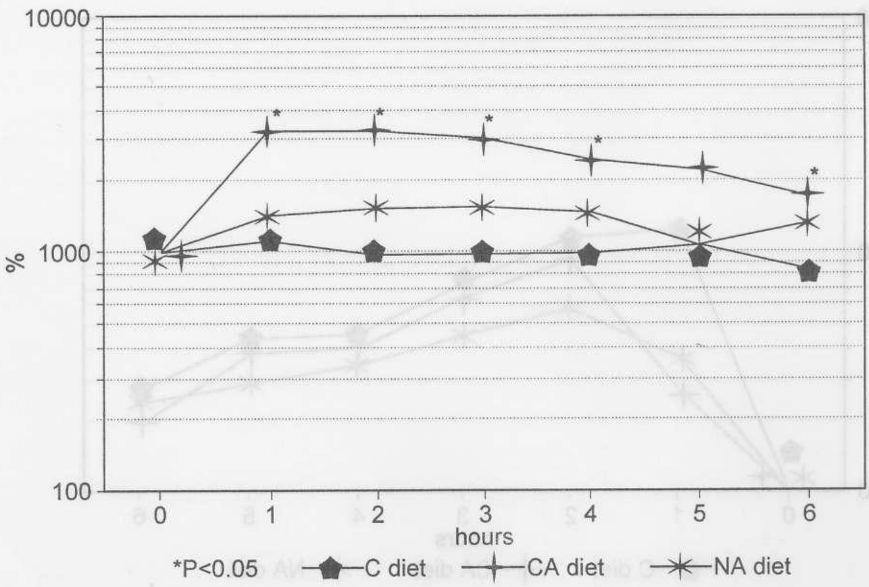

Figure 8. Relative changes of Cu concentration in rumen fluid after feeding

TABLE 4

Correlation coefficients between pH and rumen mineral concentration

Variation	pH	Ca		Mg		Zn		Cu	
		mg/l	%	mg/l	%	ug/l	%	ug/l	%
				Exponential model – $y = e^{(a*x+b)}$					
pH	–	−0.60***	−0.53***	−0.73***	−0.58***	−0.77***	−0.75***	−0.27**	−0.05NS
Ca mg*l^{-1}	−0.58***	–	0.68***	0.48***	0.30**	0.19NS	0.37***	0.56***	0.61***
%	−0.52***	0.83***	–	0.44***	0.29**	0.26**	0.25**	0.32***	0.59***
M g*l^{-1}	−0.71***	0.77***	0.64***	–	0.66***	0.43**	0.60***	0.47***	0.47***
%	−0.56***	0.53***	0.40***	0.68***	–	0.18NS	0.14NS	0.19*	0.01NS
Zn mg*l^{-1}	−0.78***	0.41***	0.39***	0.46***	0.41***	–	0.84***	0.20*	−0.01NS
%	−0.76***	0.52***	0.40***	0.56***	0.35***	0.90***	–	0.29*	0.12NS
Cu mg*l^{-1}	−0.27**	0.52***	0.24*	0.36***	0.21***	0.19NS	0.38***	–	0.58***
%	−0.04NS	0.55***	0.56***	0.37***	0.17NS	−0.04NS	0.13NS	0.52***	–
				Multiplicative model – $y = a*x^b$					

NS > 0.05; * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$

overall mean Zn concentration was lowered by Ca ($P \leqslant 0.05$) and Na ($P \leqslant 0.15$) loading (Table 3). However, there were no significant differences between particular means (Figures 5 and 6). Yano et al. (1978) indicated that high levels of Ca do not interfere with the utilization of zinc. Most probably this was also affected by the fact that only 5-10% of the Zn is in the soluble form in the rumen of sheep (Bremner, 1970; Kennedy et al., 1993) despite the fact that about 50% of Zn is water soluble in the diet (Bremner, 1970). The rapid decrease of the Zn concentration in rumen fluid (Table 3) may have been the result of microbial uptake, adhesion on feed particles or Zn outflow from the rumen. Kennedy et al. (1993) reported that most of the Zn is attributed to microorganisms and particle fraction of the rumen.

The concentration of soluble Cu in group I decreased 2 h after feeding when there was a considerable decrease in the pH (Figures 7 and 8). Similar changes of the Cu concentration were also observed by Ivan et al. (1979). Low Cu concentration was probably connected with low solubility of copper in a acidic environment. Copper exists in the rumen in an association with the microbial material present there. This pool is proportionally greater than the associated pool of, e.g. Zn (Ivan et al., 1979), and less than 20% of the Cu in the rumen is in soluble form (Bremner, 1970). The same author also indicated that this association is exceptionally stable and maintains the Cu in insoluble form at a pH as low as 1.7. Copper may also form insoluble complexes with other dietary components, as has been suggested by Emanuele and Staples (1994). Based on the above, the significantly higher ($P \leqslant 0.05$) Cu rumen fluid concentration in the Ca-loaded group was probably caused by an elevated pH related to Ca loading (Table 3). This seems not to be tied to the higher Cu intake in group II because the similar Cu intake observed in group III (Table 2) did not increase the concentration of copper in the rumen fluid (Table 3; Figures 7 and 8). In contrast with Ca, Mg and Zn, the copper concentration was not affected by decreasing the pH (Table 4); this was also observed by Emanuele and Staples (1994). In the current experiment, Cu concentration was correlated with the Ca concentration. Three hours after feeding, a clear decrease in the rumen fluid Cu concentration was observed in groups II and III, which can be explained in the same way as in the case of Zn, i.e. by microbial uptake, particle adhesion of Cu and/or the outflow rate from the rumen.

CONCLUSIONS

Mineral element concentration in rumen fluid was a function of mineral element intake, and the extent and rate of its solubilization in rumen digesta was closely correlated, in the case of Ca, Mg, Zn, but not Cu, with pH decrease. While the concentration of Ca and Mg remained elevated up to 6 h after feeding, as compared with baseline levels, the concentration of Zn and Cu rapidly decreased 2-3 h after feeding, which indicates high microbial uptake and particle association of microelements in the rumen. Loading sheep with Ca increased the Cu concentration, while Na loading increased the Mg concentration in the rumen fluid, and both tended to decrease the Zn concentration.

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STRESZCZENIE

Wpływ nadmiaru wapnia lub sodu w diecie na trawienie składników organicznych i wchłanianie związków mineralnych u owiec. 3. Zmiany koncentracji Ca, Mg, Zn i Cu w płynie żwaczowym

W doświadczeniu przeprowadzonym na owcach badano wpływ dawek pokarmowych zawierających w nadmiarze Ca i Na (5 i 10-krotnie więcej niż zapotrzebowanie wg ARC) na koncentrację Ca, Mg, Zn i Cu w płynie żwaczowym. Była ona skorelowana, w przypadku Ca, Mg, Zn, lecz nie Cu, ze spadkiem wartości pH. Koncentracja Ca i Mg w ciągu 6 godzin po karmieniu była wyższa niż przed podaniem paszy. Zawartość Zn i Cu wzrastała początkowo po karmieniu, ale w 2-3 godziny po karmieniu szybko spadała do poziomu przed podaniem paszy. Wskazuje to na wysokie pobranie mikroelementów przez mikroorganizmy lub/i na ich połączenie z cząsteczkami pokarmu w żwaczu. Dodatek mleczanu wapniowego (19,6% s.m.) do ekstrudowanej mieszanki treściwej wpłynął na zwiększenie zawartości Cu w płynie żwaczowym, natomiast nadmiar chlorku sodowego (9,6% s.m.) zwiększył koncentrację Mg. Obciążanie owiec Ca lub Na miało na celu obniżenie zawartości Zn w płynie żwaczowym, chociaż w tych grupach zwierzęta pobierały więcej cynku niż kontrolne.