EFFECT OF ADDITIONS OF POTASSIUM AND NITROGEN INTO PRESS CAKE ON MAGNESIUM UTILIZATION OF GOATS WITH RELATION TO WATER INTAKE

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Summary

In a study about minerals cycling in grassland agro-ecosystem, investigation on relations among two minerals, potassium(K) and magnesium(Mg), and nitrogen(N) was performed. Four kinds of diets different in K and N levels were fed to four goats with a Latin-square method and 2 x 2 factorial design. As the basal diet, press cake silage prepared from Italian ryegrass was used because of its uniformity and comparatively low mineral concentrations. Supplementation of K and N were made using potassium bicarbonate and urea. In the experiment, it was clearly shown that high K concentration in the forage crops is the main reason of the low utilization of Mg in ruminant animals. However, high nitrogen intake resulted in the increase of magnesium retention, urinary potassium excretion, water intake and volume of urine and in the decreases of potassium intake minus urinary potassium excretion. The results of high nitrogen intake seemed to be produced in the following order; increase of urine, increase of water intake, increase of urinary potassium excretion, and decrease of intake minus urinary potassium excretion. The amount of potassium intake minus urinary potassium excretion had significantly close relationships with magnesium utilization and serum magnesium concentration. As a conclusion, higher nitrogen intake by ruminants seemed to be preferable for magnesium utilization through increased water intake and urinary potassium excretion, if the sufficient drinking water could be supplied to ruminants. (Key Words: Goat, Press Cake Silage, Magnesium, Potassium, Nitrogen)

Introduction

As a part of the study on minerals cycling in grassland agro-ecosystem, the cycling of K and Mg, including N from forages to ruminants were investigated with an intention to know the effects of high K and high N concentrations in feed on Mg metabolism of the animals.

Until now, investigations about the mechanism of hypomagnesaemia in ruminants have been conducted by many researchers. And it has been clear that Mg metabolism in ruminants responds to K concentration in feed (Kemp, 1960; Ward, 1966; Grunes et al., 1970; Newton et al., 1972; Yano et al., 1977; Martens and Rayssiguier, 1980; Greene et al., 1983^{a,b}; Care et al., 1984). However, at present, effect of N concentration in feed on Mg metabolism of ruminants is not clear. Some of the researchers reported that high N in feed has

adverse effect on Mg metabolism relating hypomagnesaemia or hypomagnesaemic tetany (Head and Rook, 1955; Metson et al., 1966; Reid et al., 1974; Axford et al., 1982; Care et al., 1982), but the other researchers insisted that high N has no or only negligible effect on Mg metabolism (Kemp 1958; L'Estrange et al., 1967; Grace and MacRae, 1972; Moore et al., 1972; Fontenot et al., 1973).

On the other hand, the fibrous residue (press cake), left after the extraction of green juice from forage crops for preparing leaf protein concentrate, can be used as a good source of feed material for ruminants (Pirie, 1983), especially when ryegrass is used as the material crop (Houseman and Connell, 1976; Ohshima et al., 1988). And press cake is not only lower than original forage crops in mineral concentrations (Kim et al., submitted), but also very uniform in the physical state because of crushing by a crusher and mixing with a twin roll screw press at the time of processing. The above characteristics of press cake ensure the uniform feeding of the diet with same nature, while other basal diets such as fresh grass or hay can vary in their mineral concentrations. And the

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moisture of press cake silage will prevent the detachment of the added minerals when ruminant animal eats the diet.

From the above reasons, press cake silage of Italian ryegrass was used as a basal diet to study the effects of excessive addition of K and N in the forms of potassium bicarbonate powder and urea powder on Mg metabolism. The effects of K and N were analysed by a 2 x 2 factorial design. As the hypomagnesaemic tetany often breaks out in animals kept on spring pastures of high moisture content, the possible effects of water intake on the mineral balance were also discussed.

Materials and Methods

Herbage

The first growth of Italian ryegrass (*Lolium multiflorum*, Lam.), grown on the Farm of Nagoya University, was harvested on April 15, 1987 at ear emergency stage and used for the preparation of press cake silage.

Preparation of press cake

One thousand and six hundred kilograms of the herbage was disintegrated with a herbage crusher (Nihon Sharyo Ltd.) and pressed out 1,000 liters of green juice with a twin-screw press (Stord Bartz Japan Ltd., TP-24-2). The remaining fibrous residue was referred as press cake.

Preparation of silage

Silage was prepared from the press cake by packing a part of them into 30 polyethylene bags (600mm in width, 800mm in height and 0.09mm in thickness) at a rate of 15 kg per bag. The upside of bag was closed by tying with string after removing air with a vacuum pump. The bags were opened one by one after June 1, 1987 and the content of each bag was subdivided into small portions and packed again into small bags as to feed two bags to one animal per day. The small bags were kept in a refrigerator.

Animal trials

Four *Shiba* strain Japanese pygmy goats weighing about 18 kg were individually housed in metabolic cages. The press cake silage was fed to them twice daily at 9 AM and 9 PM as to offer 480 g DM per day with or without additional K and/or N. Four dietary treatments were made and they were; control(C), high potassium (high K),

high nitrogen (high N) and high potassium and nitrogen (high KN). The high K and the high N treatments were made by adding 24.6g of KHCO_3 and 15.4 g of urea per day, respectively. Two grams of salt and 10 g of CaHPO_4 were also fed to all animals everyday. All the reagents were in powder conditions and mixed well with the press cake silage immediately before feeding. Each of the four diets were offered to each goat in a Latin-square design over four periods with a 2 x 2 factorial arrangement of dietary treatments. During each period, faeces and urine were collected for 5 days after 7 days of preliminary feeding. The faeces were dried with a draft-oven at 80°C and the urine was acidified with H_2SO_4 and kept in a refrigerator before analysis. Blood samples were taken twice per goat at 1 PM on the last two days of each period from the jugular vein with multiple purpose syringe and vacuum neotube (Nipro NM-20G).

Chemical and Statistical analyses

Determinations of the pH and the dry matter content of the silages were made by the method of Ohshima and Kogure (1984). Concentrations of K, Mg and N in the feeding material, urine and faeces were analysed with a flame photometer, with an atomic absorption spectrophotometer and by Kjeldahl method, respectively. Serum Mg concentration was analysed with a method of Willis (1960). Comparisons among means of treatment were performed by using both the analysis of variance and the Studentized range Q test, and the magnitude and the direction of the main effects of K and N and the interaction on the 2 x 2 factorial design were analysed by using orthogonal comparison (Snedecor and Cochran, 1982).

Results

The pH and the dry matter content of the press cake silage were about 4.1 and 32%, respectively. No butyric acid was found in the silage. Table 1 shows the crude ash, K, Mg and N concentrations in Italian ryegrass and in its press cake silage. Press cake silage contained less K, Mg and N comparing with original grass and the difference is great in K and the smallest in N. Table 2 shows the K and N concentrations in the feed after the additions.

Table 3 shows Mg balances of goats. Though it was not significant, Mg intake on high K seemed to be less than those on other treatments. Faecal Mg

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TABLE 1. CRUDE ASH, POTASSIUM, MAGNESIUM AND NITROGEN CONCENTRATIONS IN ITALIAN RYEGRASS AND ITS PRESS CAKE SILAGE

	Crude ash (% on a DM basis)	K (mg K/g DW)	Mg (mg Mg/g DW)	N (mg N/g DW)
Ryegrass ^a	10.1 ± 1.15	41.3 ± 6.05	1.47 ± 0.085	26.8 ± 3.01
Press cake silage ^b	7.6	25.0	1.03	22.8

^a Harvested on April 15, 1987, mean ± SD of 3 replicates.

^b Mean of 2 replicates.

TABLE 2. POTASSIUM AND NITROGEN CONCENTRATIONS OF THE DIETS

	K	N
Control	2.5	2.28
High K ^a	4.5	2.28
High N ^b	2.5	3.73
High KN ^{a, b}	4.5	3.73

1) 24.6g KHCO₃ X 39.05/100 = 9.6g K/day (9.6g/480g DW = 2.0% K)

2) 15.4g urea X 45.3/100 = 7.0g N/day (7.0g/480g DW = 1.45% N).

TABLE 3. MAGNESIUM BALANCES OF GOATS

	(mg Mg/day)			
	Control	High N	High K	High KN
Intake	494 ^a	494 ^a	464 ^a	488 ^a
Faeces (% of intake)	390 ^a (78.9 ^{ab})	383 ^a (77.6 ^a)	405 ^a (87.4 ^{bc})	419 ^a (85.8 ^b)
Urine (% of intake)	27 ^a (5.6 ^a)	24 ^a (5.0 ^a)	11 ^a (2.4 ^a)	10 ^a (2.1 ^a)
Retained (% of intake)	76 ^{ab} (15.5 ^{ab})	86 ^b (17.4 ^b)	47 ^a (10.2 ^a)	59 ^a (12.1 ^a)

^{a, b, c} Values in the same row with the same letter are not significantly different ($P > 0.05$).

excretions on high K and high KN tended to be more than those on control and high N treatments. Though there was no significant difference between them, urinary Mg excretions were higher

on control and high N than those on high K and high KN. The retentions of Mg were higher on control and high N than those on high K and high KN. And above 77% of Mg was excreted through faeces and 2 to 6% of the element was through urine, and Mg balances of the goats were positive with the retention of 50-90 mg/day.

Table 4 shows K balances of goats. The amounts of faecal K excretion were similar on all the treatments. Urinary K excretions were significantly more on high K and high KN, while percent of the urinary excretion to the intake tended to be higher on high N and high KN. Potassium retention was the highest on high K and the lowest on high N, while there was no significant difference when calculated as per cent of the intake. The per cent of faecal K to K intake ranged within 9 to 15% and that of urinary K was within 66 to 74%. And K balances of the goats were positive with the retention of 1.9-4.9 g/day.

TABLE 4. POTASSIUM BALANCES OF GOATS

	(g K/day)			
	Control	High N	High K	High KN
Intake	12.00 ^a	12.00 ^a	20.27 ^b	21.32 ^b
Faeces (% of intake)	1.76 ^a (14.7 ^c)	1.67 ^a (14.0 ^{bc})	1.84 ^a (9.1 ^{ab})	1.81 ^a (8.5 ^a)
Urine (% of intake)	7.92 ^a (66.0 ^a)	8.38 ^a (69.8 ^{ab})	13.56 ^b (66.9 ^{ab})	15.75 ^b (73.9 ^b)
Retained (% of intake)	2.31 ^{ab} (19.3 ^a)	1.94 ^a (16.2 ^a)	4.85 ^c (24.0 ^a)	3.76 ^{bc} (17.6 ^a)

^{a, b, c} Values in the same row with same letter are not significantly different ($P > 0.05$).

TABLE 5. NITROGEN BALANCES OF GOATS

	(g N/day)			
	Control	High N	High K	High KN
Intake	10.94 ^a	17.92 ^b	10.27 ^a	17.70 ^b
Faeces (% of intake)	4.11 ^a (37.5 ^b)	4.17 ^a (23.3 ^a)	4.12 ^a (40.0 ^b)	4.24 ^a (24.0 ^a)
Urinary (% of intake)	3.76 ^a (34.4 ^a)	8.60 ^b (48.0 ^b)	3.659 ^a (34.8 ^a)	8.83 ^b (49.8 ^b)
Retention (% of intake)	3.08 ^a (28.1 ^a)	5.15 ^b (28.7 ^a)	2.56 ^a (25.2 ^a)	4.63 ^b (26.3 ^a)

^{a,b} Values in the same row with the same letter are not significantly different ($P > 0.05$).

Table 5 shows N balances of goat. The faecal N excretion was nearly the same on all the treatments with the value of 4.1-4.2g/day. Urinary N excretion was significantly higher on high N and high KN than on control and high K. Nitrogen retention was also greater on high N and high KN. The percent of faecal N to N intake ranged within 23 to 40%, and that of urinary N was 34 to 50%. And N balances were positive with the retention of 2.6-5.2 g/day.

Table 6 shows the effects of K and N intake on serum Mg concentration, Mg and K balances, water intake and volume of urine. There was no significant difference among the treatments on serum Mg concentration. The addition of K to diet had significant negative effects on serum Mg concentration and the factors of Mg balances, and positive effects on those of K balances and volume of urine. And the addition of N had positive effects on Mg retention, urinary K excretion, water intake and volume of urine and had negative effects on the value obtained by subtracting urinary K excretion from K intake (K intake - Urinary K) and K retention. There was no significance on interaction effect.

Table 7 shows the correlation coefficients among the factors of the goat experiment. Serum Mg concentration had a positive relationship with urinary Mg excretion ($P < 0.01$), water intake ($P < 0.05$) and volume of urine ($P < 0.05$), and had a negative relationship with (K intake - Urinary K) ($P < 0.01$) and the retention of K ($P < 0.05$). The value obtained by subtracting

faecal Mg excretion from Mg intake (Mg intake - Faecal Mg) had a positive relationship with Mg retention ($P < 0.001$), urinary Mg excretion ($P < 0.001$), and had a negative relationship with K retention ($P < 0.05$), with (K intake - Urinary K) ($P < 0.01$), with urinary K excretion ($P < 0.01$) and with K intake ($P < 0.001$). Urinary Mg excretion had a negative relationship with (K intake - Urinary K) ($P < 0.01$), the retention ($P < 0.05$) and the intake of K ($P < 0.05$). Potassium intake had a positive relationship with urinary excretion ($P < 0.001$), with (K intake - Urinary K) ($P < 0.01$) and with the retention of K ($P < 0.05$). The (K intake - Urinary K) had a positive relationship with K retention ($P < 0.001$). And water intake had a positive relationship with volume of urine ($P < 0.001$) and with urinary N excretion ($P < 0.01$). Though it is not shown in the table, faecal Mg excretion had a positive relationship with urinary K excretion ($r = 0.6520$, $P < 0.01$).

From table 7, it is clear that (K intake - Urinary K) and (Mg intake - Faecal Mg) show more significant relationships with most of the factors of the goat experiment than K and Mg retentions, respectively.

Discussion

As a part of the study about mineral cycling in grassland agro-ecosystem, the present experiment was carried out. In this investigation, the effects of high K and high N and resultant water intakes on the Mg utilization of the ruminants were studied using Italian ryegrass press cake silage as a basal diet and *Shiba* goats as experimental animals.

In grassland, forage plants absorb nutrients from the soil and grow with the nutrients. Sufficient fertilization, especially of N and K, to the grassland advance the yield of forage plant (Mengel and Kirkby, 1978). However, the mineral imbalance in forage plants can be occurred with the fertilization, especially in intensively managed grassland, and resulted in mineral disorder of ruminants (Reid and James, 1985). The relationship between K and Mg concentrations in forage plants is opposite to each other in all the growing seasons. In the spring, the K concentrations in forage plants increase rapidly with the decrease of Mg to the level of the lowest range, and also with the significant increase of the water content by a

TABLE 6. SIGNIFICANCE OF THE DIFFERENCES OF THE DATA OBTAINED IN THE GOAT EXPERIMENT

Treatment	Magnesium				Potassium			
	In serum (mg/dl)	Intake -Faeces (mg/day)	In urine (mg/day)	Retained (mg/day)	In urine (g/day)	Intake -urine (g/day)	Retained (g/day)	Water intake (l/day)
C (control)	1.73	104	27	76	7.92	4.08	2.31	0.73
N (high N)	1.76	110	24	86	8.38	3.62	1.94	1.00
K (high K)	1.53	58	11	47	13.56	6.70	4.85	0.91
KN (high KN)	1.49	69	10	59	15.75	5.57	3.76	1.23
Significance ($P < $):								
K effect	0.01(-) ^a	0.005(-)	0.05(-)	0.005(-)	0.005(+)	0.005(+)	0.005(+)	NS
N effect	NS ^b	NS	NS	0.05(+)	0.05(+)	0.01(-)	0.10(-)	0.05(+)
Interaction	NS	NS	NS	NS	NS	NS	NS	NS

a + or - in the parenthesis indicates positive or negative direction of the main effects with an analysis of the 2² factorial experiment, and $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.005$ show the significance of magnitude of the main effects at 10%, 5%, 1% and 0.5% level, respectively.

b NS; not significant.

TABLE 7. CORRELATION COEFFICIENTS AMONG THE FACTORS OF THE GOAT EXPERIMENT

	Urinary N	Volume of urine	Water intake	K retained	K intake- Urinary K	Urinary K	Mg retained	Urinary Mg	Mg intake- Faecal Mg
Serum Mg	0.2188	0.5311*	0.5545*	-0.5924*	-0.6526**	-0.0949	0.1644	0.6878**	0.4816*
Mg intake-Faecal Mg	0.1822	-0.0546	0.0638	-0.5318*	-0.6398**	-0.7125**	0.8624***	0.7444***	
Urinary Mg	0.0373	0.2862	0.3687	-0.5373*	-0.6291**	-0.3485	0.3021		
Mg retained	0.2317	-0.2956	-0.1893	-0.3504	-0.4348+	-0.7522***			
K intake	0.0881	0.4075	0.3291	0.5533*	0.6513**	0.9493***			
Urinary K	0.2694	0.6208*	0.5374*	0.2711	0.3800				
K intake-Urinary K	-0.3895	-0.2985	-0.3275	0.9749***					
K retained	-0.3884	-0.2996	-0.3268						
Water intake	0.6500**	0.9483***							
Volume of urine	0.6395**								

N = n-2 = 14, +, *, **, *** indicate statistical significance at 10%, 5%, 1% and 0.1% level, respectively.

high K fertilizer application to the grassland (Kim et al., 1988).

Press cake silage was chosen as the basal diet because of lower mineral concentrations than original forage plants and of uniformity in the physical state. And these natures of press cake ensure the uniform feeding to the goat, a ruminant species smaller in body weight and less in dry matter intake than cattle or sheep, with low and uniform mineral concentrations as the basal diet. Though goat seems to have a marginal ability to compensate for low dietary Mg by reducing the rate of its excretion (NRC, 1981), 0.1% Mg in the press cake of Italian ryegrass can be considered to be useful as a control of lower Mg concentration. Because Mg concentration above 0.2% in spring grass is advisable for grazing cattle for prevention of hypomagnesaemic tetany (Kemp, 1960). The feature of the reduction of crude ash concentration of the residual press cake during the green crop fractionation was similar to that reported by Walker et al. (1982). And the K and Mg concentrations of the press cake were similar to those reported by Kim et al. (submitted), but the crude protein concentration was somewhat higher than that reported by Ohshima et al. (1988).

In this study, KHCO_3 and urea were given to the animals as K and N supplement, respectively. Potassium bicarbonate is not a neutral salt but an alkaline salt. Therefore, effect of KHCO_3 level on the rumen acid-base balance possibly existed during the experiment, because potassium bicarbonate is one of the main buffering components in the rumen (Durand and Kawashima, 1980). However, addition of KHCO_3 into diets in Mg metabolism study seems to be not problematical (Kunze et al., 1953; Newton et al., 1972; Greene et al., 1983^{a,b}), especially in the present experiment in which the silage of high acidity was used as the basal diet. There was no difference on Mg metabolism between urea and crude protein as a N source (Moore et al., 1972).

As shown in table 3, the goats fed on high K and high KN excreted less urinary Mg than those fed on the other diets as reported by Fontenot et al. (1973). Therefore, high K intake exerts a significant adverse effect on urinary Mg excretion (table 6). From the results in table 6, it is clear that K concentration in feed material has significant effect on serum Mg concentration and on the balances of Mg and K of ruminants as reported by

many workers (e.g. Kemp, 1960).

Some researchers thought that high N concentration in spring grass is the reason for the reduced Mg absorption by ruminants (e.g. Head and Rook, 1955; Martens and Rayssiguier, 1980; Care et al., 1982), while others have considered that the N level is not so significant as to be able to drop the serum Mg significantly (e.g. Kemp, 1958; Fontenot et al., 1973; Care et al., 1984). In the present experiment, high N in feed functioned for the increase of Mg retention as shown in table 6, suggesting some favorable effects of the N level on Mg utilization in ruminants. But Fontenot et al. (1973) reported that Mg absorption is a more meaningful measurement of efficiency of Mg utilization than retention. The data shown in table 4 and table 6 indicate that the high N intake increased urinary K excretion, as was reported by Moore et al. (1972). Further, the effects of high N on Mg retention, (K intake - urinary K) and K retention were opposite to those of high K.

In general, the higher the proportion of minerals including NH_3 in the diet, the greater the volume of urine (ARC, 1980). Potassium intake had positive effect on volume of urine, but did not have significant effect on water intake (table 6), which was similar to the results of Kawagoe and Kayama (1981). On the other hand, high N in feed influenced significantly water intake and volume of urine (table 6) as reported by ARC (1980). Volume of urine increased because of diuretic effect of urea and presumably it might be followed by the increase of water intake. Table 7 shows that water intake had a positive relationship with serum Mg concentration contrary to the hypothesis made by Suttle and Field that water intake increased might contribute to the development of hypomagnesaemia or hypomagnesaemic tetany of ruminants grazed on fresh grass (1966) but water infusion into the rumen did not alter significantly the serum Mg concentration (1967). However, the intraruminal infusion of water resulted in significant increase of urinary Mg excretion (1966), which is considered as an index of Mg absorption and in grazing condition the excretion fell significantly (Kemp et al., 1961).

At present experiment, with high N feeding, urinary K excretion increased, which might be followed by the increase of urine volume (table

6). Subsequently, (K intake - Urinary K) decreased significantly with high N feeding as shown in table 6, and the value had a significantly negative relationship with serum Mg (table 7). From the results, high N in feed advanced the volume of urine, urinary K excretion, and decreased the (K intake - Urinary K). There was a considerable lag in K excretion of the supplementary K, and presumably the K not excreted was retained largely in the gut of sheep (Suttle and Field, 1967). And at present experiment, the remained K from urinary K excretion in the animal body seemed to function negatively for Mg absorption. From the above, the effects of N on Mg utilization can be summarized as follows.

$$\begin{array}{ccccccc} \text{N} & \uparrow & \text{Volume} & \uparrow & \text{Water} & \uparrow & \text{Urinary K} & \uparrow & \text{(K intake} & \downarrow & \text{serum} \\ \text{intake} & \downarrow & \text{of urine} & \downarrow & \text{intake} & \downarrow & \text{excretion} & \downarrow & \text{-Urinary K)} & \downarrow & \text{Mg} \end{array}$$

where $\uparrow$ and $\downarrow$ shows the increased and decreased amounts or concentration of the factor, respectively.

L'Estrange and Axford (1966) reported that volume of urine increased with the abrupt change of diet into fresh cut grass in the early spring. Here, a consideration is required, because there is a big difference between the water of the spring grass and the water of the present experiment or the infused water by Suttle and Field (1966). In the spring grass much more K is contained than that of other growing seasons (Bohman et al., 1983) and increased K fertilization raised still further the water content (Kim et al., 1988). Therefore, grazing animals have to consume "plant water" and K above the requirement of them. As the volume of urine and the urinary excretion of K increase with the water intake, the value of subtracting urinary K excretion from K intake (K intake - Urinary K) may remain in large quantity in grazing animals compared with those given water at the requirement level.

Furthermore, (K intake - Urinary K) showed more close relationship with serum Mg, urinary Mg excretion and Mg retention than K intake, urinary K excretion or K retention did. And the value obtained by subtracting faecal Mg from Mg intake (Mg intake - Faecal Mg) has a significant relationship with K intake, urinary K excretion, (K intake - Urinary K) and K retention, as shown in table 7. Kemp et al. (1961) and Chicco et al. (1972) also reported that (Mg intake -

Faecal Mg) had close relationship with Mg utilization. If we say that (Mg intake - Faecal Mg) is good indicator for Mg utilization of ruminant in positive direction, the value of (K intake - Urinary K) possibly indicates the utilization in negative direction.

As a conclusion, not only lower K intake but also higher N intake seemed to be good for Mg utilization of ruminants. And the effects of a higher N intake for Mg utilization seemed to be produced with increasing the volume of urine accompanying urinary K excretion through the increase of water intake. But favorable effects of N can be obtained only when sufficient water is supplied to ruminants for excreting excessive K from the animal body in addition to the "plant water" in fresh grass especially in the spring season. Furthermore, it was made clear that (K intake - Urinary K) and (Mg intake - Faecal Mg) are the good indicators for predicting the Mg utilization of ruminants.

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