



# The Effect of Drinking Water Temperature in Winter on Water, Magnesium and Calcium Metabolisms in Ewes

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**ABSTRACT.** An experiment was carried out with 4 non-lactating ewes to determine the effects of replacing cold (C period: about 5.4°C) drinking water with warm (W period: about 26.8°C) on water, Mg or Ca metabolisms in winter. By changing from C to W period, the water intake and urine volume increased in 60–102% ( $P < 0.05$ ) and in 65–118% ( $P < 0.05$ ), respectively. Moreover, there was a significant positive correlation between the water intake and urine volume throughout the experimental period ( $P < 0.001$ ). Serum Mg concentration rose significantly in all ewes, and urinary Mg level decreased in three ewes. In the remaining ewe, water intake, urinary Mg and Ca excretions increased remarkably. The serum Ca concentration declined significantly in 2 ewes (inclusive of No. 3). There was a significant negative correlation between serum Mg and urinary Ca concentrations ( $P < 0.01$ ) throughout the experimental period. These results suggest that the water intake in sheep decreases if cold drinking water is supplied in winter, resulting in a decrease in the absorption of Mg and a decline in the serum Mg concentration. Renal function may be important for regulation of water, Mg and Ca metabolisms in cold environment. The absorption mechanism of Mg (mainly by diffusion) might differ from that of Ca.—**KEY WORDS:** calcium, magnesium, sheep, water, winter.

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In early spring or autumn, beef cattle and sheep grazing in the pasture often develop hypomagnesemia or hypomagnesemic tetany (grass tetany) [8, 9, 12]. Housed dairy of beef cattle may also suffer from chronic hypomagnesemia [2, 11]. The occurrence of hypomagnesemia may be related to cold environment in addition to such established causative factors as Mg contents in feed, Mg absorption in the intestine, Mg secretion into milk, Mg excretion into urine, etc. Generally, exposure to cold environment has a remarkable effect on energy and water metabolism. Since Mg is an essential cofactor of enzyme systems in energy metabolism, and since the absorption and excretion of Mg relate closely to water metabolism, it is possible that a cold environment has a significant effect on Mg metabolism. Some authors [15, 16] observed that the urinary Mg excretion ratio rose in sheep exposed to cold temperature,

and that in dairy cows, especially old ones, the ratio was higher in a cold than a warm period. However, little is known about the effect of a cold environment on Mg metabolism in ruminants [19].

The present experiment was performed to study the effects of water intake in a cold environment (winter) on Mg metabolism in sheep by changing from cold to warm drinking water. At the same time, Ca metabolism, which is related closely to Mg metabolism [10, 17, 18], was studied.

## MATERIALS AND METHODS

Four non-lactating 2-year-old ewes of Corriedale breed, weighing 31–42 kg, were used. Each was housed in a metabolism cage, which was equipped for the separate collection of feces and urine.

The sheep were fed with the ration twice a day at 9:00 a.m. and 4:30 p.m., and given

Table 1. Composition of experimental ration and experimental design

Composition of ration	Daily intake (kg body weight)
Orchard hay	8.4 g
Concentrates	8.4 g
Wheat bran	4.4 g
Urea	0.4 g
Vitamin A	20 IU
Vitamin D	5 IU
Total contents	
Mg	51.2 mg
Ca	188.3 mg

Experimental period (day)	Temperature (°C)	
	Water	Room <sup>a)</sup>
Cold water period (10)	5.4±0.2 <sup>b)</sup>	2.2±1.0
Warm water period (5)	26.8±0.8	2.2±1.6

a) At 10 a.m. in winter (January).

b) Mean±S.D.

Table 2. Daily changes in the amount of water intake, daily fecal and urine excretions in ewes given warm instead of cold drinking water in winter

Item	Water temperature (°C)	Experimental period (days)	Ewe			
			No. 1 31 kg <sup>a)</sup>	No. 2 34 kg	No. 3 41 kg	No. 4 42 kg
Water intake	5.4	4 <sup>b)</sup>	(ml) 1980 ±232 <sup>c)</sup> (ml/MS) <sup>d)</sup> 150.7± 17.7	1850 ±141 131.4± 10.0	3045 ±207 187.9± 12.8	1433 ±240 86.9± 14.5
	26.8	5	(ml) 3160 ±239*** (ml/MS) 240.5± 18.2***	3416 ±288*** 242.6± 20.5***	6156 ±954*** 379.9± 59.9***	2304 ±561* 139.7± 34.0*
Feces weight	5.4	4	(g) 395 ± 35 (g/MS) 30.1± 2.7	414 ± 61 29.4± 4.3	480 ± 46 29.6± 2.8	451 ± 35 27.3± 2.1
	26.8	5	(g) 440 ± 49 (g/MS) 33.5± 3.7	348 ± 23 24.7± 1.6	498 ± 30 30.7± 1.9	471 ± 35 28.5± 2.3
Urine volume	5.4	4	(ml) 1396 ±318 (ml/MS) 106.3± 24.2	1438 ± 89 102.1± 6.3	2236 ±273 138.0± 16.9	865 ±171 52.4± 10.4
	26.8	5	(ml) 2306 ±399*** (ml/MS) 175.5± 30.4**	2664 ±173*** 189.2± 12.3***	4877 ±929*** 301.0± 57.3	1634 ±546* 99.0± 33.1*

a) Body weight.

b) Latter 4 days of 10-day experimental period.

c) Mean±S.D.

d) Metabolic body size (body weight kg<sup>0.75</sup>).

\*, \*\*, \*\*\* Significantly different from other values of the cold water period in the same column, P&lt;0.05, 0.01 and 0.001, respectively.

water after feedings (Table 1).

The experiment consisted of 2 periods; the sheep were given water at room temperature (about 5.4°C) for 10 days (cold water period; C period), and warm (about 26.8°C) water for the following 5 days (warm water period; W period). Room temperature was about 2.2°C at 10:00 a.m. throughout the experimental period (Table 1).

In the last 4 of the C period and the last 5 days of the W period, water intake, feces and urine excretions were measured by weight or volume every morning before feeding. A portion of the urine sample was used for analysis, and blood (10 ml) was withdrawn from the jugular vein. The urine and serum samples were stored at -20°C.

Levels of Mg and Ca in the feed, urine or serum samples were analysed with an atomic absorption spectrophotometer (Hitachi, Ltd., type 508).

Table 3. Changes in the serum Mg concentrations, in the daily urinary Mg levels and in calcium in ewes given warm instead of cold drinking water in winter

Item	Water temperature (°C)	Experimental period (days)	Ewe			
			No. 1 31 kg <sup>a)</sup>	No. 2 34 kg	No. 3 41 kg	No. 4 42 kg
Serum Mg	5.4	4 <sup>b)</sup>	2.35±0.06 <sup>c)</sup>	2.47±0.05	2.32±0.04	2.65±0.03
	26.8	5	2.45±0.03*	2.73±0.05***	2.44±0.02**	3.03±0.14**
Urinary Mg	5.4	4	(mg/day) 270±130	437±31	358±104	267±26
			(mg/MS) <sup>d)</sup> 20.55±1.56	31.04±2.20	22.10±6.42	16.18±1.58
	26.8	4	(mg/100 ml) 18.59±5.16	30.43±1.60	16.04±4.18	32.06±8.31
		5	(mg/day) 246±74	346±64*	578±77**	235±45
Serum Ca	5.4	4	(mg/100 ml) 9.60±0.32	9.10±0.63	9.08±0.31	8.05±0.17
	26.8	5	9.53±0.32	7.42±0.12***	8.32±0.19**	7.92±0.22
Urinary Ca	5.4	4	(mg/day) 65.8±57.4	50.3±36.3	89.3±70.5	8.5±4.4
			(mg/MS) 5.01±4.37	3.57±2.58	5.51±4.35	0.52±0.27
	26.8	4	(mg/100 ml) 4.36±3.18	3.39±2.33	3.95±2.86	1.04±0.54
		5	(mg/day) 101.6±67.2	34.4±19.4	191.2±128.0	6.0±5.7
		(mg/MS) 7.73±5.11	2.44±1.38	11.80±7.90	0.36±0.35	
		(mg/100 ml) 4.31±2.80	1.29±0.74	3.97±2.30	0.32±0.22*	

a) Body weight.

b) Latter 4 days of 10-day experimental period.

c) Mean±S.D.

d) Metabolic body size (body weight kg<sup>0.75</sup>).

\*, \*\*, \*\*\* Significantly different from other values of the cold water period in the same column, P<0.05, 0.01 and 0.001, respectively.

The water intake, feces and urine excretions were estimated on the basis of metabolic body size (MS; body weight Kg<sup>0.75</sup>), which expresses the degree of water metabolism in the body. The results were statistically analysed with the paired Student's t test or with the multiple regression.

## RESULTS

Water intake, fecal and urine excretions (Table 2): A ration was completely ingested by all the ewes, and daily intakes of Mg and Ca were 51.2 and 188.3 mg/kg body weight/head, respectively, throughout the experimental period. In the C period, average

values of water intake, fecal weight and urine volume ranged from 86.9 to 187.9 ml, 27.3 to 30.1 g and 52.4 to 138.0 ml/MS/day, respectively.

By changing from cold to warm drinking water (in W period), the water intake and urine volume in all the ewes increased in 60–102% and in 65–118%, respectively. The increases are statistically significant (P<0.05). The amount of fecal excretion, however, did not change significantly. Average values of water intake and urine excretion in ewe No. 3 were much higher than those in the other 3 ewes.

The serum concentrations and urinary levels of Mg and Ca (Table 3): Mean values

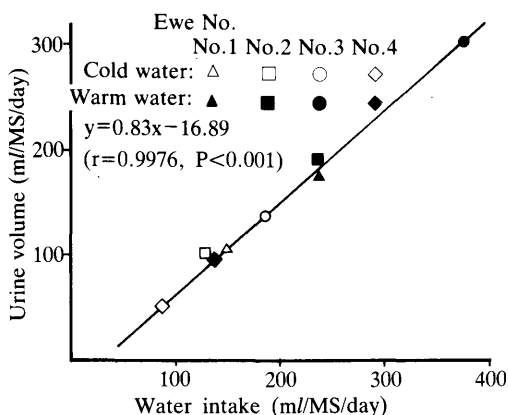


Fig. 1. Correlation between water intake (X) and urine volume (Y) in ewes throughout the experimental period. Cold water: 5.4°C. Warm water: 26.8°C. MS: Metabolic body size (body weight  $\text{kg}^{0.75}$ ). r: Correlation coefficient.

of Mg concentration in the serum and daily urinary level of Mg in the C period ranged from 2.32 to 2.65 mg/100 ml and from 6.36 to 12.86 mg/kg body weight (from 16.04 to 32.06 mg/100 ml), respectively.

In the W period, serum Mg concentration rose significantly ( $P < 0.05$ ). A urinary Mg level decreased significantly in 1 ewe (No. 2,  $P < 0.05$ ), showed a tendency to decrease in 2 (Nos. 1 and 4) and to increase significantly in the other one (No. 3,  $P < 0.01$ ), but the average of the urinary Mg concentrations declined in all the ewes (11.22–15.83 ml/100 ml).

Mean Ca concentration in the serum and daily urinary level of Ca in the C period ranged from 8.05 to 9.60 mg/100 ml and from 0.19 to 2.18 mg/kg body weight (from 1.04 to 4.36 mg/100 ml), respectively.

In the W period, mean serum Ca concentration did not rise in 2 ewes (Nos. 1 and 4), but fell significantly in the other 2 ewes (Nos. 2 and 3,  $P < 0.001$  and 0.01, respectively); In particular, ewe No. 2 showed a remarkably low concentration (7.42 mg/100 ml). Daily urinary Ca level showed a tendency to increase in 2 ewes (Nos. 1 and 3) and to decrease in the other 2 ewes (Nos.

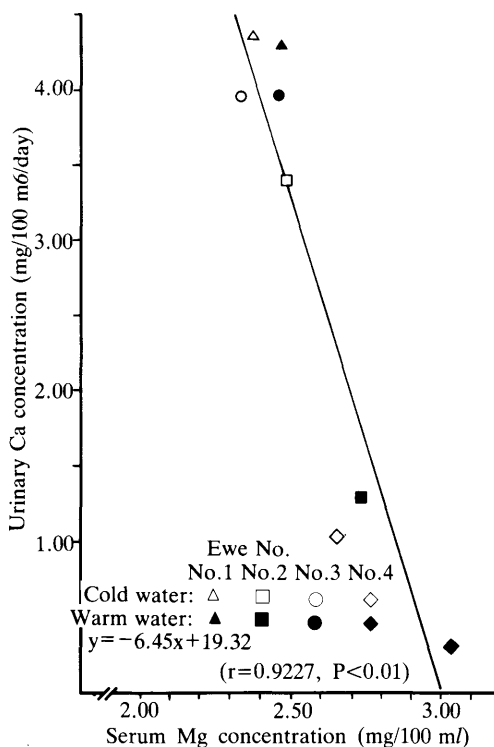


Fig. 2. Correlation between concentrations of serum Mg (X) and urinary Ca (Y) in ewes throughout the experimental period. Cold water: 5.4°C. Warm water: 26.8°C. r: Correlation coefficient.

2 and 4), and the average urinary Ca concentration did not change in 2 ewes (Nos. 1 and 3), but it declined in the other two (Nos. 2 and 4).

The increased water intake did not affect daily Mg and Ca intakes since the contents of those minerals in water were negligible.

Correlations among water intake, urine volume and the serum Mg and urinary Ca concentrations (Figs. 1 and 2): Significant correlations were obtained between the average values of water intake (X) and urine volume (Y) ( $y = 0.83x - 16.89$ ,  $r = 0.9976$ ,  $P < 0.001$ ) (Fig. 1), and of serum Mg concentration (X) and urinary Ca concentration (Y) ( $y = -6.45x + 19.32$ ,  $r = -0.9227$ ,  $P < 0.01$ ) (Fig. 2) throughout the experimental period.

## DISCUSSION

In winter, metabolic disorders associated with minerals (hypomagnesemia, urolithiasis and so on) often occur in ruminants fed on hay of poor quality or excessive concentrates with imbalanced mineral components, and their occurrence seems to be associated with water intake. It has been known that water intake is affected by various factors (dry matter; DM intake, feed quality, age of animals, environmental temperature, temperature of drinking water and so on), of which DM and environmental temperature are the most important ones. For example, water intake of housed sheep in environmental temperatures under 15°C, within 15–20°C and over 20°C was 2.0, 2.5 and 3.0 kg/kg DM, respectively, and temperature of the drinking water had little effect on it, providing that the water did not freeze [13]. In the present experiment, water intake in non-lactating ewes fed with a certain ration, however, increased significantly by changing from cold to warm drinking water in a cold environment (January). Therefore, it is concluded that the temperature of drinking water has a remarkable effect on water intake in a cold environment.

On the other hand, the daily urine excretion significantly increased in all the ewes with increased water intake, and moreover, there was a significant positive correlation between water intake ( $X$ ; ml/MS) and urine volume ( $Y$ ; ml/MS,  $y=0.83x-16.89$ ) (Fig. 1). Watanabe *et al.* [21] reported that there was a significant positive correlation between water intake ( $X$ ; ml/MS) and urine volume ( $Y$ ; ml/MS) in environmental temperatures at 10.7 or 17.6°C ( $y=0.189x+68.98$ ). The difference between the results of Watanabe *et al.* and those of the author suggested that the urinary excretion rate rises remarkably in a cold environment ( $0.83x$ ), though part of the water failure

might be supplemented by metabolic water ( $-16.89$ ). Therefore, in cold environment, extra- and intra-cellular water pools might decrease and consequently blood might become concentrated.

The daily Mg intake was constant and sufficient, and the serum Mg concentration was within the normal range (1.8–3.2 mg/100 ml) in all the ewes. Water intake increased when they were supplied with warm drinking water, and, at the same time, serum Mg concentration became elevated in all the ewes. It was proved that Mg metabolism in sheep was affected significantly not only by Mg intake [10, 17], but also by water metabolism and cold environment. The factors affecting the increased serum Mg concentration may be; 1) increased Mg absorption from the intestine; 2) increased Mg reabsorption from the nephrons in the kidney; and 3) Mg movement from intra- to extra-cellular fluid. Concerning the Mg absorption mechanism, it has been reported that Mg was absorbed from the small intestine by diffusion [5] or active transport [1, 14]. In the present experiment, the increased water intake inducing the rise in serum Mg concentration suggested that Mg absorption might be accelerated by diffusion along with increased water absorption in the intestine. On the other hand, it was suggested that Mg reabsorption might be accelerated in the kidney, because the daily Mg excretion did not increase in 3 ewes other than No. 3 in spite of the increased urinary excretion and serum Mg concentration. However, Mg movement from intra- to extra-cellular fluid might not occur in the present experiment, because the Mg intake was adequate. Therefore, the increased serum Mg concentration in the present experiment might be due to: 1) increased Mg absorption from the intestine and 2) increased Mg reabsorption from the nephrons in the kidney.

Concerning the Ca metabolism, the daily

Ca intake was constant and sufficient in all the ewes throughout the experimental periods. In the C period, serum Ca concentration was within the normal range (9.0–11.0 mg/100 ml) in 3, but not in No. 4 ewes. But in the W period, serum Ca concentration fell below the normal range in 3 of the 4 ewes in spite of no significant change in the urinary Ca level. The increased water absorption inducing the decreased serum Ca concentration might be caused not only by the competitive absorption of Ca and Mg in the small intestine [3, 6], but also by dilution of extracellular Ca. Calcium ions may be absorbed in the small intestine mainly by active transport [20] independent of increased water absorption. Thus, it was suggested that the intestinal absorption mechanism of Ca might differ from that of Mg [4, 5, 7]. On the other hand, since there was the significant negative correlation between the serum Mg and urinary Ca concentrations throughout the present experiment (Fig. 2), there might be a positive relationship between Mg and Ca in renal reabsorption; the rise of serum Mg concentration might be caused partially by enhanced Mg reabsorption in the kidney. The renal reabsorption of Mg as well as that of Ca might be regulated by parathyroid hormone. In the present experiment, the ewe No. 3 might have had a kidney disorder which affected its regulation of water and mineral metabolism. The ewe No. 4 which showed little urinary Ca and Mg excretion might be hyperparathyroidismic.

The energy which was necessary to raise the temperature of ingested cold and warm drinking water to body temperature (about 39.4°C) was  $70.6 \pm 23.3$  and  $48.5 \pm 21.5$  Kcal/day/head, respectively, and the amount of consumed energy was more in the C period than in the W period ( $P < 0.01$ ). But this energy might be small in a comparison with the daily total digestive energy (TDN; 2,000–3,000 Kcal/day/head)

ingested in the present experiment. Hence, there might be little difference in energy balance between the periods C and W.

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## 要 約

冬季における羊の水、Mg および Ca 代謝に及ぼす温水給与の影響：志賀隴郎（岩手大学農学部家畜生理学教室）——冬季（1月）に、4頭の非泌乳雌羊に対し、一定飼養条件下で自然状態の飲水（5.4℃：冷水）から加温飲水（26.8℃：温水）へ切りかえ給与し、水、Mg および Ca 代謝の変化とそれらの相互関係を調べ、以下の成績を得た。1) 1日の飲水量および尿排泄量は、全例で有意に増加し（60-102%および65-118%）、実験期間を通じ、飲水量（ $X: \text{ml}/\text{kg}^{0.75}$ 体重/日）と尿排泄量（ $Y: \text{ml}/\text{kg}^{0.75}$ 体重/日）との間に有意の正の相関（ $y=0.83x-16.84$ ,  $P<0.001$ ）が認められた。2) Mg は、全例で血清濃度が有意に上昇し、消化管からの吸収量および腎臓での再吸収量の増加が示唆された。3) Ca は、血清濃度が2頭で有意に低下し、2頭で変化しなかったことから、水の吸収量の増加はCaの吸収に影響しないことが示唆された。4) 実験期間を通じ、血清Mg濃度（ $\text{mg}/100\text{ml}$ ）と尿中Ca濃度（ $\text{mg}/100\text{ml}$ ）との間に有意の負の相関（ $y=-6.45x+19.32$ ,  $P<0.01$ ）が認められた。5) 羊No. 3は、飲水量、尿排泄量、Mg および Ca の尿中排泄量が他の3頭に比べ著しく多く、腎臓の機能障害がうたがわれた。6) 冬季の飲水温度低下による水摂取の抑制はMg 吸収量を減少させること、Mg と Ca の吸収様式が異なること、水、Mg および Ca の代謝調節に腎臓機能が重要であること、などが示唆された。