Experimental Studies on Hypomagnesemia in Ruminants: Effects of Varying Calcium Content in Low-Magnesium Diets on Serum Concentrations of Magnesium and Calcium, and Heart Rate in Lactating Ewes

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Abstract. In order to elucidate the relationship between hypomagnesemic tetany and Ca metabolism in ruminants, experiments were carried out on four 2-year-old lactating ewes divided into two groups (A and B) according to the order of feeding of two low-Mg diets (8.4 mg/kg of body weight/day) with varying Ca contents (normal-Ca: 154.5 and low-Ca: 26.1 mg/kg of body weight/day). Serum Mg and Ca concentrations as well as heart rate (HR) as an indicator for signs of hypomagnesemic tetany were determined every day throughout the experimental periods. On feeding either the low-Mg and normal-Ca diet or the low-Mg and low-Ca diet the decreased dietary levels of Mg resulted in a significant fall of serum Mg concentration (0.5–0.7 mg/100 ml). Serum Ca concentration gradually fell on feeding the low-Mg and low-Ca diet. On feeding the low-Mg and normal-Ca diet it temporarily fell and then returned nearly to the control level. Consequently, serum Ca/Mg ratio increased more remarkably on feeding the low-Mg and normal-Ca diet than the low-Mg and low-Ca diet. The significant increase in HR, or the onset of hypomagnesemic tetany was always preceded by a remarkable rise in serum Ca/Mg ratio on feeding the low-Mg and normal-Ca diet. It is suggested that the imbalance in dietary Ca/Mg ratio might enlarge the imbalance of Ca/Mg ratio in extracellular and intracellular fluid resulting in the hypomagnesemic tetany in lactating ewes.

Little study has been done on the mechanism by which hypomagnesemia produces a tetany in ruminants. Rook and Story [5] suggested that hypomagnesemic tetany might be induced by an impairment in cholinesterase activity at nerve endplates. Horvath et al. [2] reported that abnormalities in electrocardiograms appeared in calves fed an artificial Mg deficient diet, from 2 to 7 days before the onset of tetany and disappeared after oral supplementation with magnesium. It was also suggested that a main defect leading to tetany might be lowering of threshold for nervous impulse to cross the neuro-muscular junction, because the threshold was reduced to about one-third of normal in hypomagnesemic animals [4]. Sims et al. [10], however, observed no difference in nerve or muscle excitability between hypomagnesemic and normal control wethers.

On the other hand, the authors [8, 9] recognized that the rise of ratio of calcium to magnesium in serum and diet resulted in more striking hypomagnesemia in Mg deficient sheep and that the fall in serum Mg concentration was higher in lactating ewes, in which the availability of Ca increased remarkably, than in non-lactating ewes.

The present experiment, therefore, was carried out to determine whether or not a hypomagnesemic tetany was induced in ruminants by the imbalance between Mg and Ca metabolism. And a relationship was investigated between
hypomagnesemia and tetany in lactating ewes which were fed low-Mg diets with varying Ca contents.

MATERIALS AND METHODS

Four 2-year-old lactating ewes of the Corriedale breed weighing 31–42 kg were divided into 2 groups, A (Nos. 1 and 2) and B (Nos. 3 and 4). They were placed in metabolic cages throughout the experimental periods and fed such experimental diets as shown in Tables 1 and 2. Experimental periods consisted of 37 consecutive days; control period (10 days), Experiment I (8 days), Experiment II (5 days), Experiment III (7 days) and Experiment IV (7 days).

Control: Both groups were fed a control diet (hay cube, concentrates and wheat bran) for 10 days before each experiment.

Experiment I: Groups A and B were fed a low-Mg and low-Ca diet (hay cube and artificial feed) and a low-Mg and normal-Ca diet (hay cube and artificial feed supplemented with CaCO₃) for 8 days, respectively.

Experiment II: Groups A and B were fed a low-Mg and normal-Ca diet and a low-Mg and low-Ca diet for 5 days, respectively.

Experiment III: Group A was still fed

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Table 1. Experimental design

<table>
<thead>
<tr>
<th>Experimental period (days)</th>
<th>Sheep</th>
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<tbody>
<tr>
<td></td>
<td>Group A (Nos. 1 and 2)</td>
</tr>
<tr>
<td>Control (10)</td>
<td>Control diet</td>
</tr>
<tr>
<td>Experiment I (8)</td>
<td>Low-Mg, low-Ca diet</td>
</tr>
<tr>
<td>Experiment II (5)</td>
<td>Low-Mg, normal-Ca diet</td>
</tr>
<tr>
<td>Experiment III (7)</td>
<td>Low-Mg, normal-Ca diet</td>
</tr>
<tr>
<td>Experiment IV (7)</td>
<td>Normal-Mg, normal-Ca diet</td>
</tr>
</tbody>
</table>

Table 2. Experimental diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control diet</th>
<th>Low-Mg, low-Ca diet</th>
<th>Low-Mg, normal-Ca diet</th>
<th>Normal-Mg, normal-Ca diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay cube</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Concentrates</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Purified diet*</td>
<td>—</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>—</td>
<td>0.323</td>
<td>0.323</td>
<td></td>
</tr>
<tr>
<td>MgO</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.083</td>
</tr>
<tr>
<td>Total contents Mg</td>
<td>61.7</td>
<td>8.4</td>
<td>8.4</td>
<td>58.5</td>
</tr>
<tr>
<td>Total contents Ca</td>
<td>186.8</td>
<td>26.1</td>
<td>154.5</td>
<td>154.5</td>
</tr>
</tbody>
</table>

* The composition in per cent is as follows: glucose, 26.8; corn starch, 26.8; wood pulp, 26.8; milk casein, 12.2; urea, 2.0; corn oil, 2.9; mineral mixture, 2.5; vitamins A and D.
the low-Mg and normal-Ca diet while group B was fed a low-Mg and normal-Ca diet for 7 days.

Experiment IV: Both groups were fed a normal-Mg and normal-Ca diet supplemented with CaCO₃ and MgO for 7 days.

Each diet was given twice a day, at 9:00 a.m. and 4:30 p.m., and the animals were allowed to take water ad libitum during the experiments. Daily intakes of Mg and Ca in each experiment are shown in Table 2. Suckling was done twice a day at the time of feeding.

Blood samples were obtained by jugular-vain puncture every morning before the time of feeding. Magnesium and Ca concentrations of blood serum were analysed with the atomic absorption spectrophotometer. Heart rate (HR) was determined with the stethoscope every day two hours after the evening time of feeding since ewes had been relatively quiet at that time.

Results

1. Changes in serum Mg concentration

Fig. 1 shows changes in serum Mg concentration throughout the experimental periods.

Control: An average of serum Mg concentrations during the last 5 days of the control period (2 ewes×5 days, N=10) was 2.25±0.10 and 2.39±0.20 mg/100 ml in groups A and B, respectively. There was no significant difference between these values.

Experiment I: The decrease in dietary Mg resulted in a linear decrease in serum Mg concentration in both groups. Acute hypomagnesemia occurred with a remarkable fall in serum Mg concentration (1.0 mg/100 ml) in group A on the fourth day and group B on the second day, respectively. Then the serum Mg concentration gradually fell to 0.74 mg/100 ml in group A and to 0.58 mg/100 ml in group B on the eighth day.

Experiment II: By changing the low-Mg and low-Ca diet to the low-Mg and normal-Ca diet, the serum Mg concentration fell to 0.50 mg/100 ml in group A on the fifth day. On the other hand, it remained at a level of about 0.50 mg/100 ml in group B even after changing the low-Mg and normal-Ca diet to the low-Mg and low-Ca diet.

Experiment III: In group A which was fed the low-Mg and normal-Ca diet during the periods of Experiments II and III, serum Mg concentration gradually rose to 0.88 mg/100 ml on the seventh day. In group B, it fell to 0.42 mg/100 ml on the third day and kept the level of about 0.50 mg/100 ml.

Experiment IV: When both groups were fed the normal-Mg and normal-Ca diet again, serum Mg concentration rose significantly on the first day, reaching a maximal value of 2.82 mg/100 ml in group A on the second day and 2.55 mg/100 ml in group B on the third day, and then decreased gradually to the control values.

2. Changes in serum Ca concentration

Fig. 1 shows changes in serum Ca concentration throughout the experimental periods.

Control: An average of serum Ca concentrations during the last 5 days of the control period (2 ewes×5 days, N=10) was 9.36±0.64 and 8.87±0.35 mg/100 ml in groups A and B, respectively. There was no significant difference between these values.

Experiment I: In group A which was fed the low-Mg and low-Ca diet, serum Ca concentration gradually fell to 7.02 mg/100 ml on the eighth day. On the other hand, in group B which was fed the low-Mg and normal-Ca diet it also fell to
7.99 mg/100 ml on the fifth day, but rose to 9.33 mg/100 ml on the eighth day.

**Experiment II:** In group A in which the low-Ca diet was changed to the normal-Ca diet, serum Ca concentration rose to 9.70 mg/100 ml on the fifth day. In group B in which the normal-Ca diet was changed to the low-Ca diet serum Ca concentration fell to 6.95 mg/100 ml on the third day and rose to 7.96 mg/100 ml on the fifth day.

**Experiment III:** In group A fed the normal-Ca diet during previous and this experimental period, serum Ca concentration fell temporarily till the third day and rose to 10.96 mg/100 ml on the seventh day. In group B in which the low-Ca diet was changed to the normal-Ca diet, it reached a maximal value of 11.30 mg/100 ml on the second day and fell to 8.49 mg/100 ml on the seventh day.

**Experiment IV:** In both groups serum Ca concentration remained essentially at the control level.
3. Changes in HR and other clinical signs

Fig. 2 shows changes in HR throughout the experimental periods.

Control: An average HR during the last 5 days of the control period (2 ewes×5 days, N=10) was 75.9±4.0 and 69.6±5.9 beats/min in groups A and B, respectively. There was significant difference between these values (p<0.05). No abnormal clinical signs were observed in any ewes.

Experiment I: In group A HR increased to 87 beats/min on the first day, and then decreased gradually to the control level on the seventh day. In spite of remarkable hypomagnesemia it was only for the first three days that such clinical signs as pricking-up ears and staring eyes appeared. On the other hand, in group B
HR increased remarkably over a period from the first day (85 beats/min) to the fourth day (101 beats/min) and was maintained at a high level of about 94 beats/min up to the eighth day. Such clinical signs as rapid respiration, pricking-up ears, staring eyes, and a twitching of muscles of the face and ears appeared after the fourth day. At the same time the ewes began to respond nervously to a sound and the appearance of human, and to act violently at the suckling.

Experiment II: In group A HR did not change until the third day. It increased remarkably on the fourth day and attained to 96 beats/min on the fifth day. No clinical signs were seen until the third day, but, thereafter, staring eyes and pricking-up ears appeared. On the other hand, in group B HR was high, remaining at an equable level throughout this experimental period. The clinical signs gradually disappeared, in spite of remarkable hypomagnesemia.

Experiment III: In group A HR was about 94 beats/min constantly. Such clinical signs as rapid respiration, pricking-up ears, staring eyes, and a twitching of muscles of the face and ears were noticed throughout this experimental period. In group B HR was about 93 beats/min until the third day and thereafter increased remarkably to 109 beats/min on the sixth day. The degree of nervous tension was stronger in group B than group A.

Experiment IV: In both groups HR decreased gradually to the control level on the seventh day. The clinical signs with nervous tension disappeared almost completely in both groups on the second day.

4. Relationship between HR and serum Ca/Mg ratio

An average of serum Ca/Mg ratios during the last 5 days of the control period (2 ewes×5 days, N=10) was 3.95±0.11 and 4.20±0.14 in groups A and B, respectively (Fig. 2). Prior to the onset of hypomagnesemic tetany, or the increase in HR and the occurrence of other clinical signs, the rise in serum Ca/Mg ratio was always recognized, as shown in Fig. 2 (group A in Experiment II and group B in Experiments I and III). On the other hand, the recovery of HR was gradually done with a fall in serum Ca/Mg ratio after oral supplementation with Mg (groups A and B in Experiment IV).

Discussion

In the authors' previous studies [8, 9], it was recognized that a rise in the ratio of Ca to Mg in diet or serum made hypomagnesemia more striking in Mg-deficient ewes, that the body retention of Mg was lower in lactating ewes than in non-lactating ewes, and that the difference in Mg metabolism between lactating and non-lactating ewes were related to the difference in Ca metabolism between these ewes. The present study, therefore, was carried out to clarify the effects of varying Ca contents (normal-Ca: 154.5 and low-Ca 26.1 mg/kg of body weight/day) on the intensity of hypomagnesemia and on the severity of clinical signs of tetany in lactating ewes fed a low-Mg diet.

In the present study, when fed a diet containing about 8.4 mg Mg per kg of body weight per day, young lactating ewes were affected with remarkable hypomagnesemia, showing a serum Mg concentration of 0.5 to 0.7 mg/100 ml. In the previous study [7], when non-lactating young ewes were fed a diet containing about 2.9 mg Mg per kg of body weight per day, their serum Mg concentration fell from 2.5 to 1.39 mg/100 ml. These results made it possible to confirm that Mg retention was less in
lactating ewes than in non-lactating ones.

A little studies have been down about the relationship between hypomagnesemia and hypomagnesemic tetany. Kiesel and Alexander [3] reported that when their serum Mg concentration was 0.46 mg/100 ml, sheep showed no clinical signs of tetany. Sims et al. [10] also mentioned that wethers showed no clinical signs when their plasma Mg concentration was 0.95, 0.79, or even 0.28 mg/100 ml. In the present study the lactating ewes showed remarkable hypomagnesemia (about 0.5 mg/100 ml) and affected with tetany. Some of them, however, did not suffer from tetany. It was when the low-Ca content was changed to the normal-Ca content in the low-Mg diet that these ewes manifested the clinical signs of hypomagnesemic tetany.

Little attention has been paid to the effect of Ca metabolism on hypomagnesemic tetany in ruminants. Sjollema [11] reported that a low blood level of Ca was a feature in grass tetany and that the Ca/Mg ratio was raised as the loss of Mg was greater than that of Ca. On the other hand, the authors [6, 8] recognized that acute lowering of serum Mg concentration in non-lactating ewes was accompanied with lowering of plasma parathyroid hormone (PTH) and serum Ca concentration, that this lowering of serum Ca concentration had no relation to Ca intake, and that an old ewe had an attack of tetany following the increase in serum Ca/Mg ratio due to the relatively high rise of plasma PTH and serum Ca concentration. The ewe died at the time when the Ca content of the low-Mg diet was increased from low to normal level [6, 8]. As shown in the present study, hypomagnesemic tetany occurred in lactating ewes more readily than in non-lactating ewes at the time when the Ca content of the low-Mg diet was increased to a normal level. These results suggested that the enlargement of imbalance between Mg and Ca ratio might induce tetany in hypomagnesemic ewes. If the imbalance between Mg and Ca metabolism is a cause of hypomagnesemic tetany in ruminants, it will be possible to explain that hypomagnesemic tetany may be induced more readily in lactating or old animals than in non-lactating or young ones.

A few attempts have been made to elucidate the cause of hypomagnesemic tetany by means of electrophysiological measurement [1–3, 10]. Nothing is known, however, about the mechanism which makes tetany occur in Mg-deficient ruminants. This mechanism may be the abnormal function of nerve fibers caused by an alteration of ion distribution across the neuronal plasma membrane [10]. From the present study it is suggested that the imbalance between Ca/Mg ratio in extracellular and intracellular fluid may induce the abnormal function of nerve fibers in the peripheral and/or central nervous system, which causes an increase in HR, rapid respiration, pricking-up ears, staring eyes, and twitching of muscles of the face and ears.

References
反芻動物の低マグネシウム血症に関する実験的研究：低マグネシウム飼料中のカルシウム含量の変化が泌乳羊の血清マグネシウムおよびカルシウム濃度ならびに心拍数に及ぼす影響: 志賀聡郎・藤尾修 (岩手大学農学部家畜生理学講座) ——反芻動物の低Mg血症性テナーの発現に及ぼすCaの影響を調べるため, 4頭の泌乳羊(年齢2歳)を2頭づつ2群(A群とB群)に分け, 両群に低Mg (8.4 mg/kg体重/日)・正常Ca (154 mg/kg体重/日)と低Mg・低Ca (26.1 mg/kg体重/日)の2種類の低Mg飼料を順番で隔てて交互に切りかえ給与した. 実験期間を通じて毎日, 血清MgおよびCa濃度ならびにテナー発現の指標として心拍数を測定した. 血清Mg濃度は, 低Mg飼料の給与により, 低Mg・低Ca飼料時および低Mg・正常Ca飼料時とも著明に低下し, 正常Ca飼料時は, 低Mg飼料時には低下し, 低いレベルを維持したが, 低Mg・正常Ca飼料時には一時低下したのち, ほぼ対照群のレベルまで上昇した. 血清Ca/Mg濃度比は, 低Mg飼料の給与により上昇したが,上昇の程度は低Mg・低Ca飼料時大であった. 心拍数は, 血清Ca/Mg濃度比の上昇があったあとに増加したが, ときに低Mg・正常Ca飼料時には心拍数の著しい増加とともに呼吸促迫, 眼球突出などの一連のテナー症状が認められた. 以上の成績から, 羊の泌乳時においては, MgとCaの要求量が著しく異なり, Mg欠乏は非泌乳時に比べ低Mg血症をより顕著にすること, 飼料中Ca含有率がMgのそれに比べ著しく高いときは, 体内でのMgとCaの不均衡を増大させ, 低Mg血症性テナーが誘発されやすいことが示唆された.