Studies on Serum Tocopherol and Selenium Levels and Blood Glutathione Peroxidase Activities in Lambs with White Muscle Disease

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ABSTRACT. For the investigation of the cause of white muscle disease (WMD), tocopherol (Toc) and selenium (Se) levels and blood glutathione peroxidase (GSH-Px) activities were examined using lambs with WMD and their ewes. Serum Se levels of 4 lambs with WMD were low under 30 ppb, lambs showing very low levels below 15 ppb. The serum Se level was correlated with the blood GSH-Px activity showing remarkably low activities in the lambs with WMD. Se contents in the organs of lambs with WMD were lower than those of control lambs, and particularly liver Se contents were deficient levels below 50 ppb. Serum Toc levels were normal, but α-Toc contents in organs showed very low levels, especially in the liver. The serum Toc and Se levels and blood GSH-Px activities of their ewes and other sheep kept in the same farm revealed similar results to those of lambs with WMD. Feedstuffs supplied on the farm showed the deficient level of the Se content below 50 ppb and a very low level of α-Toc. It was concluded that WMD of lambs in Hokkaido was nutritional muscular dystrophy resulted from deficiencies of Toc and Se to their ewes.—KEY WORDS: lamb, selenium, tocopherol, white muscle disease.


There have been a few reports on white muscle disease (WMD) in lambs in Japan, such as the pathological examination of Goto et al. [7] and Ichikawa [14], but tocopherol (Toc) and selenium (Se) levels have not been studied. Recently, Ichijo et al. [13] have reported that the Se content of soil and pasture in Hokkaido is very low and Toc in feedstuffs is decreased by oxidation during storage. Furthermore, they have reported that WMD in calves and foals occurred during a period from winter to spring due to Toc and Se deficiency [9, 11, 12]. Previously, the authors have described the clinical and clinicopathological condition of lambs with WMD on a farm in the Tokachi district in Hokkaido [18]. In the present study, we examined serum Toc and Se levels and blood glutathione peroxidase (GSH-Px) activities in lambs with WMD.

MATERIALS AND METHODS

Four Suffolk lambs with WMD and 2 of their ewes on a farm in the Tokachi district, Hokkaido, were examined in March, 1986 and simultaneously 6 lambs and 10 ewes kept on the same farm were examined (Table 1). As a control for serum Toc and Se levels and blood GSH-Px activities, 32 lambs and 10 ewes were used. In addition, 14 lambs from farms on which WMD did not occur were examined for the comparison of Toc and Se contents in organs.

Serum Toc and Se levels were determined by fluorometric analysis [1] and Olson's fluorometric method [17], respectively. Blood GSH-Px activity was assayed by the method of Paglia & Valentine [19]. Concentrations of α-Toc and Se in feedstuffs and organs were analyzed by high performance liquid chromatograph [15] and Olson's method [17], respectively.
Table 1. Sheep examined

<table>
<thead>
<tr>
<th></th>
<th>Lamb</th>
<th>Ewe</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs with WMD\textsuperscript{a)}</td>
<td>4</td>
<td>2</td>
<td>Suffolk</td>
</tr>
<tr>
<td>Ewes of lambs with WMD</td>
<td>6</td>
<td>10</td>
<td>Suffolk; 9 Corriedale; 1</td>
</tr>
<tr>
<td>Lambs kept together</td>
<td>10</td>
<td></td>
<td>Suffolk</td>
</tr>
<tr>
<td>Ewes kept together</td>
<td>32</td>
<td>14</td>
<td>Suffolk</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} White muscle disease.
\textsuperscript{b)} For the comparison of serum tocopherol, selenium level and blood glutathione peroxidase activity.
\textsuperscript{c)} For the comparison of tocopherol and selenium concentration in organ.

Table 2. Serum tocopherol, selenium levels and blood glutathione peroxidase activities of lambs with white muscle disease (WMD), lambs kept together and control

<table>
<thead>
<tr>
<th></th>
<th>Lambs with WMD</th>
<th>Lambs kept together (N=6)</th>
<th>Control lambs (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1 No. 2 No. 3 No. 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum tocopherol ((\mu g/100) ml)</td>
<td>122.2 133.1 317.8</td>
<td>365.2±158.9 (230.9–655.4)</td>
<td>104.6±60.9 (48.8–206.4)</td>
</tr>
<tr>
<td>Serum selenium (ppb)</td>
<td>7.2 11.2 10.3 16.6</td>
<td>17.0±5.9 (10.3–24.0)</td>
<td>58.7±12.6 (38.2–88.5)</td>
</tr>
<tr>
<td>Blood glutathione peroxidase (E.U./g Hb)</td>
<td>— 11.0 10.3 14.0</td>
<td>14.2±5.7 (10.4–25.6)</td>
<td>58.8±6.6 (49.4–71.0)</td>
</tr>
</tbody>
</table>

The clinical symptoms of the 4 lambs with WMD were recumbency, stiffness, anorexia, marked tachycardia and cyanosis. Serum enzyme activities increased remarkably with GOT (2,900-10,970 Karmen unit), GPT (850-1,815 Karmen unit), CPK (2,013-84,700 International unit) and LDH (5,508-29,390 Wroblewski unit). LDH isoenzyme patterns showed an increase of LDH\textsubscript{1} and LDH\textsubscript{5}. Pathologically, severe degeneration of sarcoplasm with swelling, fragmentation, hyalinosis, granulation and vacuolation were observed in skeletal muscles and cardiac muscles of the endocardium of the right ventricle and interventricular septum.

RESULTS

Serum Toc and Se level and blood GSH-Px activity: Serum Toc levels of the 3 lambs (Nos. 1, 3 and 4) ranged from 122.2 to 317.8 \(\mu g/100\) ml, showing no significantly difference from control lambs (104.6±60.9 \(\mu g/100\) ml) (Table 2). Serum Se levels of the 4 affected lambs, ranging from 7.2 to 16.6 ppb, were lower than those of control lambs (58.7±12.6 ppb). Furthermore, blood GSH-Px activities ranged from 10.3 to 14.0 E.U./g Hb, which were remarkably lower compared with control lambs (58.8±6.6 E.U./g Hb).

In the 2 ewes of lambs with WMD, serum Toc levels were 206.4 and 266.2 \(\mu g/100\) ml, which were no different from control ewes (Table 3). But serum Se (11.9 and 13.9 ppb)
SELENIUM AND TOCOPHEROL IN WHITE MUSCLE DISEASE OF LAMBS

Table 3. Serum tocopherol, selenium levels and blood glutathione peroxidase activities of their ewes of lambs with white muscle disease, ewes kept together and control

<table>
<thead>
<tr>
<th></th>
<th>Ewes No. A&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Ewes kept together (N=10)</th>
<th>Control ewes (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum tocopherol (μg/100 ml)</td>
<td>266.2</td>
<td>134.2±53.5 (54.3–182.0)</td>
<td>157.6±33.3 (118.1–216.9)</td>
</tr>
<tr>
<td>Serum selenium (ppb)</td>
<td>13.9</td>
<td>14.1±4.7 (6.3–20.6)</td>
<td>105.2±30.0 (65.5–162.5)</td>
</tr>
<tr>
<td>Blood glutathione peroxidase (E.U./g Hb)</td>
<td>9.1</td>
<td>11.0±3.3 (7.3–16.7)</td>
<td>37.8±5.4 (30.2–48.0)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Ewe of Nos. 2 and 3. <sup>2</sup> Ewe of No. 4.

and blood GSH-Px (9.1 and 12.7 E.U./g Hb) were at much lower levels than those of control ewes (Se; 105.2±30.0 ppb, GSH-Px; 37.8±5.4 E.U./g Hb).

The lambs kept together in the same farm showed rather high serum Toc levels (365.2±158.9 μg/100 ml) compared to control lambs. Serum Se (17.0±5.9 ppb) and blood GSH-Px (14.2±5.7 E.U./g Hb) were at low levels just as the lambs with WMD (Table 2). Regarding the ewes, serum Toc levels averaged 134.2±53.5 μg/100 ml and showed no difference from those of ewes of affected lambs and control ewes. But serum Se (14.1±4.7 ppb) and blood GSH-Px (11.0±3.3 E.U./g Hb) were at very low levels (Table 3).

Relationship between serum Se level and blood GSH-Px activity: Among the 41 lambs (3 lambs with WMD, the other 6 lambs living in the same farm and 32 control lambs), there was a high correlation (r=0.88) between serum Se level (X) and blood GSH-Px activity (Y) (Y=1.02X−7.77, p<0.001) as shown in Fig. 1.

<sup>1</sup> α-Toc and Se contents in organs: α-Toc contents in the skeletal muscle (M.longissimus cervicis), heart, liver, adrenal gland and kidney of No. 1 were at remarkably low levels (Table 4). In particular, the levels in liver and adrenal gland were below marginal detection (<0.1 μg/g) by analysis. However, there was no clear correlation between Toc concentrations in organs and serum Toc levels.

Se contents in each organ of the 2 lambs with WMD showed lower levels than control lambs, especially the liver being below 50 ppb (No. 1; 43.7 ppb, No. 2; 40.3 ppb) (Table 4). Serum Se levels and Se contents in liver showed a significantly positive correlation (r=0.86, p<0.001) (Fig. 2).

<sup>1</sup> α-Toc and Se contents in feedstuffs: The
Table 4. α-Tocopherol and selenium contents in organs of lambs with white muscle disease and control lambs

<table>
<thead>
<tr>
<th>Organs</th>
<th>α-Tocopherol (μg/g)</th>
<th>Selenium (ppb)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1</td>
<td>Control lambs (N=10)</td>
<td>No. 1</td>
<td>No. 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control lambs (N=14)</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle a)</td>
<td>0.15</td>
<td>0.85±0.65 (0.36-2.55)</td>
<td>16.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Heart</td>
<td>0.71</td>
<td>1.57±1.41 (0.64-4.92)</td>
<td>19.1</td>
<td>25.7</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;0.10</td>
<td>1.38±0.97 (0.59-3.50)</td>
<td>43.7</td>
<td>40.3</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>&lt;0.10</td>
<td>6.72±5.75 (0.80-15.30)</td>
<td>171.1±74.5 (72.3-290.4)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.32</td>
<td>0.77±0.59 (0.37-2.17)</td>
<td>232.6</td>
<td>231.6</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td></td>
<td></td>
<td>502.6±192.7 (248.0-874.3)</td>
<td></td>
</tr>
<tr>
<td>Kidney medulla</td>
<td></td>
<td></td>
<td>40.7</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>160.1±72.1 (75.9-355.2)</td>
<td></td>
</tr>
</tbody>
</table>

a) M. longissimus cervitis.

![Graph showing relationship between serum selenium levels and liver selenium concentrations.](image)

Fig. 2. Relationship between serum selenium levels and liver selenium concentrations.

α-Tocopherol levels in the feedstuffs supplied to the affected lambs and ewes were 1.8 and 4.7 mg/100 g of dry matter in hay, and below the limit of detection by analysis in corn silage (<0.1 mg/100 g DM). Se contents in these feedstuffs were at very low levels under 50 ppb DM (26.1–38.4 ppb DM) (Table 5).

DISCUSSION

Andrew et al. [6] and Sheppard et al. [24] suggested that the normal and deficient Se level in the blood of sheep were over 20 and below 10 ppb, respectively. It has also been determined that the normal Se concentration in the ovine liver, an organ of storage, is over 100 ppb and the deficient level is below 50 ppb without the serum Se level [5]. From the result of a close relationship between serum Se levels and Se contents in organs observed in this study, serum Se levels of 30 and 15 ppb were calculated to correspond to liver Se contents of 100 and 50 ppb, respectively. It was suggested that serum Se levels of the all 4 lambs with WMD were below normal (lower than 30 ppb) and 3 of them showed deficient levels. Furthermore, Se contents in the liver of the 2 affected lambs were deficient levels of below 50 ppb, and those in the muscle and the kidney were markedly lower than the levels in control lambs.

Se is an essential trace element contained in GSH-Px which protects cell membranes from oxidative damage [23]; therefore the examination of blood GSH-Px activity is an effective tool for blood Se levels [9, 11, 16].
Then the relationship between serum Se level and blood GSH-Px activity was examined, and the high correlation (r=0.88, p<0.001) was found; GSH-Px in lambs with WMD showed markedly low activities with low levels of serum Se.

Since the Se intake of suckling lambs was considered to depend on that of their ewes, serum Se levels of the ewes and the Se content in feedstuffs supplied to them were examined. The serum Se levels of the ewes were deficient with very low activity of blood GSH-Px. The Se content of feedstuff showed the deficient level below 50 ppb in DM [2, 4]. In this study, the ewes on the farm where WMD occurred were supplied with forages of hay and corn silage showing deficient Se content level (26.1–38.4 ppb in DM). The above results indicated that the Se deficiency of lambs with WMD resulted from an insufficient supply of Se to their ewes. In addition, the same findings were observed in the sheep kept together and supplied with the same feedstuffs. Accordingly, it was suggested that the outbreak of WMD may be influenced by other factors such as age, physiological condition and Toc levels.

Toc, which interacts with GSH-Px, is antioxidant and plays a particularly important role in the stabilization of cellular membranes [21]. There have been some reports on the relation between WMD and Toc level [3, 8, 20]. However, serum Toc levels of lambs with WMD and their ewes were within normal ranges in this study. Since serum Toc levels in sheep are influenced by total serum lipid levels, the measurement of serum Toc levels in stored organs is not a good indicator for WMD [10, 22]. In this study, α-Toc in muscle, liver, adrenal gland and kidney of the affected lamb (No. 1) showed remarkably lower levels than control lambs, especially that in liver being below 0.1 μg/g. Rammel et al. [22] have suggested that the critical level at which deficiency disease may occur is under 2 μg/g. There was also no correlation between serum Toc levels and Toc concentrations in organs. These results suggested that Toc levels in organs should be determined.

Feedstuffs, such as green grass and fresh hay, contain a high concentration of Toc [21], but Toc content is decreased by oxidative damage during long storage [13, 21]. In general, the required amount of Toc is considered to be over 3 mg/100 g DM [25]. In this study, α-Toc contents of feedstuffs supplied to their ewes were at the low levels and 2 of the 3 samples showed the deficient level below 3 mg/100 g DM. Accordingly, the low levels of Toc in lambs with WMD were caused by the lack of Toc supply to the ewes.

Ichijo et al. have clarified that WMD in calves is caused by the deficiency of Toc and Se [11, 12], and that in foals results from a deficiency in the two nutrients [9]. Additionally, they have reported not only very low levels of Se contents in the soil and pasture in Hokkaido but also the decrease in Toc levels in the forage during long storage [13]. WMD in lambs in Hokkaido was also a nutritional muscular dystrophy caused by Toc and Se deficiencies.

REFERENCES


15. Katsui, G. 1981. Determination method of vita-


要 約

子羊白筋症における血清トコフェロール、セレニウム値および血液グルタチオンペルオキシダーゼ活性値：類似・大谷拓部・一条 茂（帯広畜産大学家畜内科学教室）—北海道十勝地方の1羊舎で発生した子羊の白筋症について、発病子羊と母羊および同居羊の血清トコフェロール（Toc）、セレニウム（Se）値並びに血液グルタチオンペルオキシダーゼ（GSH-Px）活性値の検討を行った。発病子羊4例の血清Se値は30 ppb以下の値を示し、とくに3例では15 ppb以下の欠乏値を示した。血清Se値と血液GSH-Px活性値との間には相関が認められ、かつ発病子羊の血液GSH-Px活性値は欠乏値を示した。臓器中のSe濃度は対照羊に比べて低値であり、とくに肝臓では50 ppb以下の欠乏値であった。血清Toc値は正常範囲内であったが、臓器中のα-Toc濃度は低値であり、とくに肝臓で顕著であった。母羊および同居羊の血清Se、Toc値並びに血液GSH-Px活性値も発病子羊と同様の所見を示した。さらに発生羊舎における給与飼料中のSe含量は50 ppb以下、欠乏値であり、α-Toc含量も著しい低値であった。以上の成績から、子羊白筋症の原因は母羊に対するTocとSeの給与不足による栄養性筋変性症と判断された。