EFFECTS OF SELENIUM AND VITAMIN E ON NUTRITIONAL MUSCULAR DYSTROPHY IN LAMBS

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NUTRITIONAL muscular dystrophy (NMD) occurs spontaneously in lambs in many areas (Allaway and Hodgson, 1964; Hartley and Grant, 1961; and Muth, 1963) and can result in substantial economic loss to flock owners. Willman et al. (1945) demonstrated that vitamin E was effective in preventing and curing NMD. However, Muth (1955) and Hartley and Grant (1961) observed that vitamin E had little effect on NMD under field conditions in some areas. After selenium was shown by Schwarz and Foltz (1957) to be effective in preventing some vitamin E deficiency conditions, Muth et al. (1958) and Hartley and Grant (1961) reported that selenium was effective in preventing NMD. Hopkins et al. (1964) fed lambs a torula yeast artificial milk deficient in vitamin E and selenium and observed that vitamin E prevented NMD but did not promote maximum growth. Selenium slowed the development of NMD and resulted in a growth response.

The studies reported here were initiated to evaluate the relationship between vitamin E and selenium and to estimate the effect of vitamin E and selenium deficiency on the growth and development of the lamb.

Experimental

Experiment 1. Forty-eight crossbred lambs were allotted at 2 days of age to 12 treatments in a 3 x 4 factorial design. The treatments were three levels of supplemental selenium (0.0, 0.1 and 1.0 ppm of the dry diet) and four levels of supplemental vitamin E (0.0, 2.2, 5.5 and 11.0 mg per kg of bodyweight of the lamb weekly). Groups of 12 lambs were placed on experiment at intervals of 1 too, with one lamb per treatment. This procedure was repeated to provide four replications per treatment. The basal diet shown in table 1 was fed to the first and fourth replications for 5 wk. before the level of stripped lard was reduced to 15%. The second and third replicates received the diet that contained 15% lard for the 8-wk.-experimental period. This diet contained 0.010 ppm of selenium.

The diet was prepared as an artificial milk (20% solids) by homogenizing it with warm distilled water in a Waring Blender. The first and third replicates were fed three to four times daily from polyethylene bottles fitted with swanbill style lamb nipples. In these replications, 1500 I.U. of vitamin A, 150 I.U. of vitamin D (approximately 3,000 I.U. of vitamin A and 300 I.U. vitamin D per kg of bodyweight per week) and the treatment level of dl-a-tocopherol were added to 100 gm. of dry diet prior to homogenization. In the second and fourth replicates, the diet was fed in automatic feeders which kept the diet warm and constantly agitated. Vitamins A, D and E were administered orally as an olive oil drench (5 to 15 ml per dose) three times weekly to provide 1,260 I.U. vitamin A, 136 I.U. vitamin D and the treatment quantity of dl-a-tocopherol per kg of bodyweight per week.

The lambs were weighed and blood samples were obtained by venipunctures at weekly intervals. The serum was analyzed for glutamic oxaloacetic transaminase (GOT) and lactic acid dehydrogenase (LDH, Amador et al., 1963). At 2-wk. intervals, the lambs were placed in metabolism cages and urine was collected for 24 hr. An aliquot of the urine was analyzed for creatine and creatinine by the method of Peters (1943).

Experiment 2. A second experiment was conducted to evaluate the effect of higher levels of vitamin E and selenium on the growth and development of young lambs. Sixteen crossbred lambs were weaned at 2 days of age and were fed the basal diet shown in table 1...
TABLE 1. EXPERIMENTAL DIETS

<table>
<thead>
<tr>
<th>Component</th>
<th>Artificial milk</th>
<th>Pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torula yeast</td>
<td>60.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>10.7</td>
<td>35.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1</td>
<td>15.0</td>
</tr>
<tr>
<td>Stripped lard</td>
<td>25.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* State Lakes Yeast Corp., Rinelander, Wisconsin.
* Distillation Products Industries, Rochester, New York.

During certain phases of experiment 1 (see the text), the level of glucose monohydrate was increased to 20.7% and the level of sucrose reduced to 15% to reduce the cost of the experimental diet.

Supplemented with vitamin E (0.0 or 22.0 mg. per kg. of bodyweight per week) or selenium (0.0 or 1.5 ppm of the dry diet) in a 2 x 2 factorial design. The lambs were hand fed the artificial milk (20% solids) three to four times daily. An olive oil drench was given four times per week to provide 1,260 I.U. of vitamin A, 136 I.U. of vitamin D and the treatment level of dl-a-tocopherol per kg. of bodyweight per week.

The lambs were weighed and blood samples were obtained by jugular venipunctures at weekly intervals. Heparin was used as an anticoagulant and the plasma was used for the determination of GOT *.

The packed red blood cells were used in the determination of hemolysis induced by dialuric acid. The packed red blood cells (0.2 ml.) were diluted to 10 ml. with phosphate-saline buffer (equal parts of saline and sodium phosphate buffer, 0.1 M, pH 7.4). The diluted red cells (2 ml.) were added to each of four tubes that contained the following: tube 1, 8.0 ml. of phosphate-saline buffer; tube 2 and 3, 7.8 ml. of phosphate-saline buffer and 0.2 ml. of phosphate-saline buffer containing 1.0 mg. of dialuric acid per ml.; and tube 4, 8.0 ml. of distilled water. The tubes were incubated at 37°C for 3 hr. and centrifuged. The optical density (O.D.) of the supernate of all the tubes was determined using tube 1 as the blank and the data were expressed as follows:

\[
\text{% Hemolysis} = \frac{\text{O.D. tube 2} + \text{O.D. tube 3}}{\text{O.D. of tube 4}} \times 100
\]

In both experiments, the analysis of variance for factorial experiments and correlations were calculated by the methods outlined by Steel and Torrie (1960). In experiment 1, the replicate effects were removed as blocks prior to calculation of the significance of treatment effects.

**Results and Discussion**

A summary of lamb performance is presented in table 2. When the lambs were fed the unsupplemented diets, most of them showed the clinical symptoms of NMD which included stiffness and an inability to rise or stand. They survived an average of 26.8 and 38.3 days in experiments 1 and 2, respectively. With similar diets, Hopkins et al. (1964) and Erwin et al. (1961) reported ranges of 16 to 36 days and 24 to 50 days, respectively, before the symptoms of NMD appeared.

When the basal diet was supplemented with vitamin E, the average survival time and the number of survivors increased. This effect was significant in the second experiment while it approached significance in the first experiment. One lamb in the group receiving the highest level of vitamin E in experiment 1 lost the ability to stand, but with continued administration of vitamin E it recovered and survived the experiment.

When selenium was added to the diet, survival of the lambs increased significantly in the first experiment. Most lambs appeared normal and showed no clinical symptoms of NMD. In the second experiment, two lambs receiving selenium alone died without any clinical symptoms. Both lambs died suddenly and autopsy revealed pale muscles typical of NMD as well as degeneration in the cardiac muscle (table 2).

Highly significant increases in gain were observed when selenium was added to the diet while vitamin E had little effect on growth. Oldfield et al. (1960, 1963) observed that injections of selenium to suckling lambs improved gains. McLean et al. (1959), Drake et al. (1960) and Andrews et al. (1964) have reported significant improvement in gains when selenium was administered to growing lambs. Hopkins (1964) observed growth responses to selenium when lambs were fed a similar artificial milk containing torula yeast. In contrast to these results Hintz and Hogue (1964) were not able to improve the weight gains of suckling lambs by increasing the level of selenium in the ewe’s diet from 0.04 ppm to 0.21 ppm. Young and Hawkins (1962) injected 3-wk.-old lambs with 1.0 mg. of selenium as selenite and found that gains were not
TABLE 2. EFFECTS OF SELENIUM AND VITAMIN E ON PERFORMANCE OF LAMBS* FED TORULA DIETS

<table>
<thead>
<tr>
<th>Se level ppm</th>
<th>Vit. E level mg./kg./wk.</th>
<th>Av. gain b kg./day</th>
<th>Av. survival a days</th>
<th>Deaths no.</th>
<th>Stiff lambs no.</th>
<th>Heart lesions no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.054</td>
<td>26.8</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>0.0</td>
<td>2.2</td>
<td>0.058</td>
<td>40.5</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>0.0</td>
<td>5.5</td>
<td>0.091</td>
<td>34.3</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.0</td>
<td>11.0</td>
<td>0.109</td>
<td>46.0</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>0.109</td>
<td>39.3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.1</td>
<td>2.2</td>
<td>0.154</td>
<td>45.0</td>
<td>2</td>
<td>1</td>
<td>1</td>
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<tr>
<td>0.1</td>
<td>5.5</td>
<td>0.095</td>
<td>56.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>11.0</td>
<td>0.145</td>
<td>56.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0</td>
<td>0.181</td>
<td>49.0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>2.2</td>
<td>0.186</td>
<td>56.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>5.5</td>
<td>0.159</td>
<td>50.0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>11.0</td>
<td>0.181</td>
<td>56.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.054</td>
<td>38.3</td>
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<td>4</td>
<td>3</td>
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<tr>
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<td>0.177</td>
<td>56.0</td>
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<td>0</td>
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<tr>
<td>1.5</td>
<td>0.0</td>
<td>0.227</td>
<td>39.0</td>
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<td>2</td>
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<td>0.167</td>
<td>56.0</td>
<td>0</td>
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</table>

* Four lambs per group.

a Calculated from the gain in weight over the number of days the lamb survived.

b The experimental period was 56 days.

improved when the dams were fed a hay known to produce NMD.

The enzyme, GOT, rose rapidly within 2 wk. after the lambs were placed on the deficient diet (table 4). When vitamin E or selenium were supplemented in the diet, the average weekly enzyme levels were significantly decreased in the first experiment, but in the second experiment only vitamin E significantly reduced the level of GOT. A rise was noted in the GOT level at approximately 2 to 3 wk. in all treatments and since more clinical outbreaks of NMD occur prior to 6 wk. of age (Willman et al., 1940), this may suggest that there is poor utilization of dietary vitamin E or selenium by the young lamb.

Kuttler and Marble (1960), Erwin et al. (1961) and Hopkins et al. (1964) have reported that selenium delayed but was not effective in preventing the increase in serum GOT. The results from this study are similar and further suggest that the addition of vitamin E or selenium to a diet that supplies marginal quantities of these nutrients may result in a decrease in enzyme levels. This may explain the results of Young and Keeler, (1962), Oldfield (1963) and Hintz and Hogue (1964) in which the supplementation of natural diets with selenites decreased the serum GOT levels. The LDH values were highly correlated with GOT determined in the same sample (r = 0.82; P < 0.001, 354 d.f.). Vitamin E significantly decreased the level of LDH in serum while the effect of selenium was not significant.

Supplementation of the diet with vitamin E and/or selenium resulted in significant reduction in the ratio of creatine to creatinine excreted in the urine. Draper et al. (1956) and Bacigalupo et al. (1952) have also reported elevated creatine excretion by vitamin
### TABLE 4. EFFECT OF SELENIUM AND VITAMIN E ON BLOOD ENZYMES OF LAMBS FED TOLULA DIETS

<table>
<thead>
<tr>
<th>Se level ppm</th>
<th>Vit E level mg/kg/wk.</th>
<th>GOT 8 Weekly av. Units</th>
<th>LDH 8 Weekly av.</th>
<th>Creatine ratio a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 2 4 6 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>52 1536 1495</td>
<td>838</td>
<td>2.36</td>
</tr>
<tr>
<td>0.0</td>
<td>2.2</td>
<td>49 643 660 1600 659</td>
<td>611</td>
<td>3.06</td>
</tr>
<tr>
<td>0.0</td>
<td>5.5</td>
<td>45 930 1247 760 239</td>
<td>624</td>
<td>3.21</td>
</tr>
<tr>
<td>0.0</td>
<td>11.0</td>
<td>55 540 1138 309 195</td>
<td>490</td>
<td>1.35</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>41 587 935 790 1393</td>
<td>552</td>
<td>1.75</td>
</tr>
<tr>
<td>0.1</td>
<td>2.2</td>
<td>39 112 373 810 83</td>
<td>235</td>
<td>1.05</td>
</tr>
<tr>
<td>0.1</td>
<td>5.5</td>
<td>69 670 130 398 104</td>
<td>244</td>
<td>0.89</td>
</tr>
<tr>
<td>0.1</td>
<td>11.0</td>
<td>41 152 56 521 53</td>
<td>132</td>
<td>0.86</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0</td>
<td>42 600 136 702 1653</td>
<td>580</td>
<td>2.79</td>
</tr>
<tr>
<td>1.0</td>
<td>2.2</td>
<td>39 356 108 725 108</td>
<td>263</td>
<td>1.09</td>
</tr>
<tr>
<td>1.0</td>
<td>5.5</td>
<td>44 187 269 106 120</td>
<td>199</td>
<td>0.48</td>
</tr>
<tr>
<td>1.0</td>
<td>11.0</td>
<td>32 70 160 151 71</td>
<td>84</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>40 627 1096 813 740</td>
<td>639</td>
<td>1.05</td>
</tr>
<tr>
<td>0.0</td>
<td>22.0</td>
<td>66 609 121 59 68</td>
<td>184</td>
<td>1.05</td>
</tr>
<tr>
<td>1.5</td>
<td>0.0</td>
<td>45 1715 441 345 575</td>
<td>643</td>
<td>1.05</td>
</tr>
<tr>
<td>1.5</td>
<td>22.0</td>
<td>47 327 69 51 69</td>
<td>120</td>
<td>1.05</td>
</tr>
</tbody>
</table>

a Four lambs were used in each group.

b The creatine ratio was calculated by dividing the 24-hr. urinary excretion of creatine by the excretion of creatinine over the same period.

c Weekly determinations for each lamb were averaged over the number of weeks the lamb was in the experiment. These averages were summarized for the treatment totals.

d Determinations made every other week were averaged as outlined in footnote c.

e The units were defined for LDH and GOT as that amount of enzyme which results in a change of 0.001 O.D. unit per ml. of serum (expt. 1) or plasma (expt. 2) under the conditions of assay (Sigma Chemical Co. Bull. 410).

E deficient lambs. The creatinine-creatinine ratios were significantly correlated (P<0.001) to the enzyme determinations made at the same time (GOT, r=0.54; LDH, r=0.53).

A significant effect of replication was observed in rate of gain, survival time and creatine-creatinine ratios. This was primarily due to the improved response observed when the lambs were hand-fed and receiving a higher level of vitamin A and D (replicate I and III).

The data do not allow determination of whether this response was due to the higher level of vitamins A and D or to the method of feeding.

In experiment 2, the red blood cells were tested for susceptibility to hemolysis by dialuric acid each week. The values for each lamb were averaged over all weeks observed and these values combined for comparison. The hemolysis values were 32% in the deficient lambs, 3% when vitamin E alone was supplemented, 60% when selenium was fed and 2% when the combination was fed. A highly significant decrease in hemolysis was noted when vitamin E was added to the diet. Selenium significantly increased hemolysis which is probably related to the increased survival of these lambs and the increased depletion of their vitamin E stores. Hopkins et al. (1964) reported that selenium did not prevent hemolysis by dialuric acid with lambs as did Gitler et al. (1938) with rats.

A score was devised to give each lamb a
TABLE 6. SUMMARY OF MUSCULAR DYSTROPHY SCORES

<table>
<thead>
<tr>
<th>Level of selenium ppm</th>
<th>Mg. of vitamin E/kg./wk.</th>
<th>0</th>
<th>2.2</th>
<th>5.5</th>
<th>11.0</th>
<th>22.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>(1.44)*</td>
<td>1.05</td>
<td>1.35</td>
<td>1.38</td>
<td>1.84</td>
<td>(2.85)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.16</td>
<td>2.12</td>
<td>2.24</td>
<td>2.80</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1.0</td>
<td>1.91</td>
<td>2.64</td>
<td>2.70</td>
<td>3.04</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1.5</td>
<td>(1.37)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>(3.11)</td>
</tr>
</tbody>
</table>

* Scores were computed by dividing the individual data by the best response obtained within experiments for survival, and rate of gain. Individual enzyme values were divided into the lowest values obtained from individual data and 10% hemolysis was considered normal so that values of less than 10% were scored as 1.0 while higher values were divided into 10 for the composite score.

The scores for experiment 1 were computed for each lamb by the following formula:

\[
\text{Survival} \times \text{Daily gain} \times \text{Av. weekly} \times \text{Got} \times \text{Ldh}
\]

The scores for experiment 2 (in parenthesis) were computed for each lamb by the following formula:

\[
\text{Survival} \times \text{Daily gain} \times \text{Av. weekly} \times \text{Got} \times \text{Hemolysis}
\]

rating from 0 (least effective) to 4.0 (most effective) based on the most desirable response to each of the measures used in the experiments. The treatment averages and the method of calculation of this score are shown in Table 6 and further reflect the over-all additive response of the lambs to supplementation with vitamin E and selenium. The best responses were obtained when vitamin E was given at 22 mg. per kg. of bodyweight when no supplemental selenium was fed (the basal diet contained 0.01 ppm), at 11.0 mg. per kg. when 0.1 ppm of selenium was fed and at 2.2 mg. per kg. when 1.0 ppm of selenium was added to the basal diet. Even when 1.5 ppm of selenium was added to the diet, the scores of the lambs were not as high as the scores of lambs receiving both nutrients at lower levels.

In the experiments reported here, there appeared to be an improvement in the condition of the lamb in the presence of both vitamin E and selenium that was not obtained when either one was added at comparable levels. This relationship was noted in the enzyme levels, creatine-creatinine ratio and survival time. Similar additive responses have been noted with muscular dystrophy (Calvert et al., 1962; Desai and Scott, 1965) and encephalomalacia in chicks (Jenkins et al., 1965). Schwarz (1965) has reported that levels of vitamin E and selenium that partially prevent liver necrosis are more effective when combined. In contrast to the additive response of the above measures, the rate of gain of lambs responded to the level of selenium in the diet while resistance to erythrocyte hemolysis was normal only when vitamin E was present. These results suggest that while vitamin E and selenium may share some biological functions, each still has an independent biological role.

Summary

Sixty-four lambs were weaned at two days of age and were used in two factorial studies with vitamin E and selenium. A semi-purified diet in which Torula yeast was the source of protein was prepared and fed as a milk substitute. When this diet was fed, selenium and vitamin E had an additive effect on reduction of blood levels of GOT, increasing survival time, and decreasing the ratio of urinary creatine to creatinine excretion. Vitamin E reduced LDH values and prevented hemolysis of red blood cells by dialuric acid. Selenium enhanced hemolysis which was probably related to increased survival and resulting depletion of vitamin E stores. Selenium increased the growth rates of lambs in one experiment while vitamin E had no effect in either experiment.

Literature Cited


Gitler, C., M. L. Sunde and C. A. Bauman. 1958. Effect of certain necrosis-preventing factors on
hemolysis in vitamin E deficient rats and chicks, J. Nutr. 65:397.


