TOCOPHEROL (VITAMIN E) DEFICIENCY AMONG SHEEP FED NATURAL FEEDS

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Previous reports from this Station (Willman et al., 1931, 1934, 1940) have described the prevalence of nutritional muscular dystrophy ("stiff-lamb disease") among lambs of farm and range flocks, the gross and microscopic symptoms, and the feeds that have consistently resulted in a high incidence of this condition. Recently Vawter and Records (1947) reported a similar condition among range calves. Willman et al. (1945, 1946) presented evidence to show that the muscular dystrophy among lambs was probably due to a deficiency of vitamin E in the milk of the lactating ewe, since it could be prevented or cured by feeding mixed tocopherols to both the ewes and lambs, or to the lambs alone. During the course of these earlier experiments, satisfactory chemical methods were not available for determining the tocopherol content of feedstuffs, blood plasma, or milk. Since such methods are now available some of the earlier experiments were repeated during 1946-47 to ascertain whether the tocopherol content of the blood plasma and milk of the ewes, and the tocopherol content of the blood plasma of the lamb would reveal further evidence that the condition was due to a tocopherol deficiency. The values obtained are compared with those of a flock of ewes fed a ration that has not resulted in muscular dystrophy among the lambs. The tocopherol content of the rations was also determined.

The value of supplementing the prepartum deficient ration with tocopherol was also determined in order to ascertain whether sufficient body stores of this nutrient could be built up in the ewe and/or the lamb to prevent the occurrence of muscular dystrophy among the lambs.

Natural and synthetic tocopherols had proved effective in curing the stiffness in previous experiments. Since wheat germ oil is a source of tocopherols that is readily available to sheepmen, its effectiveness in curing the stiffness was tested in this study. The reports of Marsh (1946) and Willman et al. (1945) indicated that wheat germ oil might be an effective cure. Milhorat and Bartels (1945) found that cold pressed wheat germ oil produced no demonstrable change in the urinary excretion of creatine in muscle dystrophy in humans, but that wheat germ oil obtained by extraction with warm ethylene dichloride reduced the creatinuria in 4 patients tested. For these reasons both cold-pressed and solvent extracted wheat germ oils were com-

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pared with synthetic dl-alpha-tocopherol as curative treatments. Urinary creatine excretion (creatinuria) was used as an index of the effectiveness of these treatments in curing the stiffness, as it is known that a high urinary excretion of creatine is associated with nutritional muscle dystrophy in rabbits and guinea pigs, and that this excretion rapidly falls to normal when tocopherol is administered (Mackenzie and McCollum, 1940; Eppstein and Morgulis, 1942; Hove and Harris, 1947; and others).

During 1947-48 further experiments were carried out to determine whether the feeding of different types of hays was of any importance in prevention of the "stiff-lamb" condition.

Data and Discussion

1946-47 Experiment

Sixty-eight western range ewes were used in the 1946-47 investigations. They were fed daily from early December 1946, until turned out to pasture the following spring, a ration consisting of 1 to 1.5 pounds of cull beans (red kidney) and 3 to 4 pounds of second-cutting alfalfa and clover hay. A large number of "stiff" lambs have always occurred when ewes have been fed this ration (Willman et al., 1945). These ewes had had good pasture the previous summer and fall. Beginning approximately 6 weeks before parturition, 20 of these ewes, picked at random, were fed 80 mg. per head daily of mixed natural tocopherols in the form of a stabilized powdered concentrate. This concentrate was mixed with the beans each day just before feeding. Its feeding was discontinued as the ewes lambed. Lambing occurred during the latter part of March and early April. This flock is referred to as the "experimental flock" in future discussion.

Another group of ewes which served as controls was fed a ration of mixed grass hay, oat hay, corn silage and a grain mixture consisting of corn, oats, wheat bran, and brewers grain. "Stiff" lambs have never occurred when ewes have been fed this ration. This flock is referred to as the "control flock" in future discussion.

One third of all lambs showing definite signs of stiffness was treated by oral administration with 10 ml. of cold-pressed wheat germ oil daily until a decided improvement was noticed. Another one third of the "stiff" lambs was treated with a similar quantity of solvent extracted wheat germ oil. Both of these oils contained approximately 3 mg. of total tocopherol per gram of oil by chemical analyses. The other third of the "stiff" lambs was given 25 mg. of dl-alpha-tocopherol (Ephynal Acetate) in the form of a

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tablet. In the odd case that the lamb became very stiff before treatment was begun, twice the above mentioned dosage was given. No lambs were left untreated, as previous experiments (Willman et al., 1945) had shown that most lambs left untreated died.

Urine samples were obtained for creatine and creatinine determinations from as many of the "stiff" lambs as possible before treatment was administered, again two days after the first treatment, and again 4 days after the first treatment. The method of Folin (1914) as modified by Hove (1947) was used to determine creatine and creatinine.

Blood samples were obtained approximately 6 weeks before lambing began, from the 40 "experimental" ewes (20 of which later received the tocopherol supplement), from 6 of the "control" ewes, and from the same 40 "experimental" ewes 2 weeks before lambing. Colostrum samples were obtained from 20 ewes in each of the "experimental" groups and from 10 ewes in the "control" group. Milk samples were obtained from 3 ewes in the "experimental" flock that had received tocopherol, and 9 ewes in the "experimental" flock that had not received tocopherol at the time that their lambs showed definite signs of stiffness and from 5 ewes at a comparable stage of lactation in the "control" flock. Blood samples were obtained from 9 lambs in the "experimental" flock when they showed definite signs of stiffness, and before they had received any treatment, and from 3 lambs of comparable age in the "control" flock. These samples were analyzed for tocopherol content using the method of Quaife and Biehler (1944) for blood, and the method of Quaife (1947) for milk.

Results of 1946–47 Experiment

Of a total of 68 lambs in the "experimental flock" reared to 3 weeks of age or more, 31 developed signs of muscular dystrophy between 3 and 5 weeks of age. Of these 31 lambs, 10 were from the ewes that had received the prepartum tocopherol supplements. The sheep were apparently unable to build up a reserve of tocopherol, sufficient to produce milk of high enough tocopherol content to prevent muscular dystrophy among the lambs.

The results of the blood and milk analyses are shown in table 1. The blood plasma and the milk of the "experimental" ewes and the blood plasma of the "stiff" lambs were significantly lower in tocopherol than similar samples from the "control" flock. Tocopherol supplementation increased the tocopherol content of the blood plasma of the ewes but the increase was not statistically significant. However, at this level of tocopherol supplementation (80 mg. per 100 lb. body weight daily) the tocopherol content did not reach that of the "control" ewes, which would indicate that higher levels
of tocopherol supplementation were required if the tocopherol content of the blood was to reach "normal" levels. This is also indicated by the fact that the tocopherol content of the first colostrum of the tocopherol supplemented "experimental" ewes was higher than that of the non-supplemented ewes but not as high as that of the control ewes. The tocopherol content of blood plasma of the pregnant ewes that did not receive tocopherols, increased as pregnancy advanced. This is in agreement with the work of Straumfjord and Quaife (1946) who reported the tocopherol content of the blood plasma of pregnant women increased as they approached the time of parturition.

**TABLE 1. TOTAL TOCOPHEROL CONTENT OF THE PLASMA AND MILK OF THE "EXPERIMENTAL" AND "CONTROL" EWES, AND OF THE PLASMA OF "STIFF" AND "NORMAL" LAMBS**

<table>
<thead>
<tr>
<th>Items studied</th>
<th>&quot;Experimental&quot; flock</th>
<th>&quot;Control&quot; flock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplemented</td>
<td>Non-supplement</td>
</tr>
<tr>
<td>No. of observations</td>
<td>Tocopherol content</td>
<td>Tocopherol content</td>
</tr>
<tr>
<td>Blood plasma of ewes 6 weeks prepartum</td>
<td>20</td>
<td>43±21</td>
</tr>
<tr>
<td>Blood plasma of ewes 2 weeks prepartum</td>
<td>20</td>
<td>132±68</td>
</tr>
<tr>
<td>Blood plasma of lambs</td>
<td>3</td>
<td>53±24</td>
</tr>
<tr>
<td>Colostrum of ewes</td>
<td>14</td>
<td>801±450</td>
</tr>
<tr>
<td>Milk of ewes</td>
<td>3</td>
<td>71±32</td>
</tr>
</tbody>
</table>

In table 2 are presented the creatinuria ratios before and after treatment of all lambs that lived more than 4 days after the first treatment. The numerical expression of creatinuria in this paper is the same as that used by Hove and Harris (1947), which is the ratio of total creatinine (creatinine plus preformed creatinine) to preformed creatinine. A numerical creatinuria ratio exceeding 1.3 is usually considered indicative of active muscular dystrophy. Some lambs were found with creatinuria ratios as high as 8.0, but only one lamb with an initial value higher than 4.0 was cured by the treatment used. However, some lambs died that did not show a high creatinuria ratio, but upon autopsy showed either skeletal or heart muscle lesions (Zenkers Degeneration). It is apparent from table 2 that the three treatments were
TABLE 2. THE AVERAGE CREATINURIA RATIOS OF LAMBS WITH MUSCLE DYSTROPHY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of observations</th>
<th>Before treatment</th>
<th>Two days after treatment</th>
<th>Four days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ oil (cold pressed)</td>
<td>7</td>
<td>2.0 (1.6-2.7)*</td>
<td>1.9 (1.4-3.0)</td>
<td>1.4 (1.1-2.0)</td>
</tr>
<tr>
<td>Wheat germ oil (solvent extracted)</td>
<td>6</td>
<td>2.1 (1.8-3.6)</td>
<td>1.8 (1.3-2.8)</td>
<td>1.4 (1.1-1.9)</td>
</tr>
<tr>
<td>Synthetic d,l,alpha tocopherol</td>
<td>5</td>
<td>2.5 (1.7-4.3)</td>
<td>1.6 (1.2-3.2)</td>
<td>1.5 (1.0-2.3)</td>
</tr>
</tbody>
</table>

* The figures in parenthesis show the range of values obtained.

equally effective in reducing the creatinuria ratio. However, it should be mentioned in connection with the wheat germ oil treatments that there is considerable danger of getting the oil into the lamb’s lungs and causing pneumonia unless it is administered with the utmost care. Most of the lambs treated were fully recovered 4 to 7 days after treatment began.

Table 3 shows the total tocopherol content of the hays and beans fed to the “Experimental” and “Control” ewes during the 1946-47 study. Individual tocopherols (i.e. alpha, beta, gamma and delta) were not determined on these feeds due to the presence of interfering pigments, except in the case of the beans, in which it was found that approximately 60% of the total tocopherol was made up of the gamma and delta forms with alpha tocopherol presumably representing the remainder.

TABLE 3. THE TOTAL TOCOPHEROL CONTENT OF THE FEEDS FED TO THE “EXPERIMENTAL” AND “CONTROL” FLOCKS*

<table>
<thead>
<tr>
<th>Feed</th>
<th>Total tocopherol content (mg/100 gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>“Stiff-lamb” ration</td>
</tr>
<tr>
<td>Alfalfa hay, and cutting</td>
<td>6.25</td>
</tr>
<tr>
<td>Clover hay, and cutting</td>
<td>6.71</td>
</tr>
<tr>
<td>Oat hay</td>
<td>—</td>
</tr>
<tr>
<td>Mixed grass hay</td>
<td>—</td>
</tr>
<tr>
<td>Corn silage</td>
<td>—</td>
</tr>
<tr>
<td>Concentrate grain mixture</td>
<td>—</td>
</tr>
<tr>
<td>Cull beans (fed before lambing)</td>
<td>1.85</td>
</tr>
<tr>
<td>Cull beans (fed after lambing)</td>
<td>0.91</td>
</tr>
<tr>
<td>Calc. content of total ration as fed</td>
<td>5.16</td>
</tr>
</tbody>
</table>

* We are indebted to Distillation Products, Inc., Rochester, N. Y., for these tocopherol analyses.
On the basis of the analyses shown in table 3, the ewes in the “experimental” flock were receiving approximately 2.0 mg. of total tocopherols per kilogram of body weight daily, while the ewes in the “control” flock were receiving approximately 2.3 mg. of total tocopherols daily. It seems unlikely that the small difference in the total tocopherols supplied in these two rations is sufficient to cause a large number of “stiff” lambs in one flock and none in the other. Differences in the forms of tocopherols present or in their utilization in the two rations may account for the differences in the value of these two rations.

From the results of many feed analyses Quaife (1947) has found that the tocopherols in legumes contain a smaller percentage of alpha tocopherol than non-legumes. Since only alpha-tocopherol is of high value in preventing muscle dystrophy (Baxter et al., 1943; Joffe and Harris, 1943; Harris et al., 1944; Hove and Harris, 1947; Weisher et al., 1945) it appears likely that the ration fed to the “control” flock was higher in alpha-tocopherol than the ration fed to the “experimental” flock.

Practical experience of New York farmers has indicated that when grass hays were fed in combination with cull beans the incidence of “stiff” lambs was less than when legume hays were fed. An experiment was therefore conducted during 1947-48 to determine whether a ration of cull beans and grass hay would result in as high an incidence of stiff lambs as a ration of cull beans and alfalfa hay and cull beans and mixed legume and grass hay.

1947-48 Experiment

Fifty-seven ewes were divided into 3 comparable groups. Group one received alfalfa hay and cull beans, group 2 received mixed legume and grass hay and cull beans, while group 3 received non-legume hay and cull beans. The alfalfa hay and the mixed grass hay were of good quality, while the mixed clover hay was of only medium quality. These rations were fed until the ewes were turned to pasture in May, 1948. Blood samples were taken from 8 ewes in each group approximately 2 weeks before lambing.

Table 4 shows the tocopherol content of the plasma of the ewes, number of lambs raised to 3 weeks of age in each group and the number that became stiff or showed muscular lesions upon autopsy. The results indicate that there was a higher incidence of “stiff” lambs when the ewes were fed cull beans and alfalfa or mixed hays than on beans and grass hay, thus supporting the observations of Quaife (1947) that legumes are low in alpha tocopherol. It should be pointed out in this connection, that the occurrence of stiffness is almost entirely limited to thrifty, rapidly growing lambs (Willman et al., 1940). Lambs from the grass hay ration gained less weight, as an average, than those on the other treatments and it is possible that this difference in
the growth rate may be related to the differing incidence of "stiff lambs" on the hays studied. But, until satisfactory methods are available for determining the various forms of tocopherols in feedstuffs, this problem cannot be finally solved. It is clear that the use of grass hay did not entirely prevent the trouble.

It is possible to estimate, within limits, the tocopherol requirements of the suckling lamb. Willman (unpublished data), Bonsna (1944) and Wallace (1944) have found that a ewe, on the average, produces approximately 70 to 90 pounds of milk during the first 5 weeks after parturition. It has also been found that the colostrum of the ewe contains on the average 9 times as much total tocopherol per unit of volume as milk, but that this value rapidly falls to normal levels a few days after parturition (Whiting et al., 1949). Using these data it can be calculated that the lambs from the "experimental" ewes received approximately 0.23 mg. of total tocopherols per kilogram of body weight daily, while those from the "control" ewes received approximately 0.37 mg. per kilogram of body weight. The average daily minimum requirements probably lie between these two values. Eppstein and Morgulis (1941) found that the requirements of d, l-alpha tocopherol acetate (calculated as free tocopherol) for the rabbit as determined by the cure of muscle dystrophy was probably about 0.32 mg. per kg. of body weight. Earlier studies at this Station showed that 25 to 50 mg. of alpha tocopherol was an effective curative dose for a 10 kg. lamb, unless the muscle dystrophy was very severe before treatment was begun. Hove and Harris (1947) found that the minimum curative dose of d, alpha tocopherol for muscle dystrophy in rabbits was 1.1 mg. per kg. of body weight, and of synthetic d, l-alpha tocopherol 1.4 mg.

**TABLE 4. THE INFLUENCE OF TYPE OF HAY UPON THE INCIDENCE OF MUSCULAR DYSTROPHY IN LAMBS**

<table>
<thead>
<tr>
<th>Hay fed</th>
<th>Tocopherol content of ewe's plasma (µg/100 ml.)</th>
<th>Lambs reared to 3 weeks of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Dystrophic lambs</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>83</td>
<td>24</td>
</tr>
<tr>
<td>Mixed alfalfa, clover, timothy and grass</td>
<td>63</td>
<td>23</td>
</tr>
<tr>
<td>Mixed grasses</td>
<td>73</td>
<td>19</td>
</tr>
</tbody>
</table>

Summary

Further studies of tocopherol (vitamin E) deficiency among sheep fed a ration of cull beans (red kidney) and alfalfa, mixed or grass hay are reported.
It is shown that the low level of total tocopherols in the milk of the ewes fed the ration of alfalfa and beans is the cause of the muscle dystrophy observed among the lambs. The condition was cured by administering tocopherols to the lambs as judged by the fall in creatinuria and the disappearance of the stiffness, but it was not prevented by prepartum supplementation with tocopherols.

Data are presented on the total tocopherol content of the blood plasma, colostrum and milk of ewes that produced normal and dystrophic lambs, on the blood plasma of “normal” and “stiff” lambs, and on the feeds fed to the ewes.

The incidence of “stiff lambs” was greater when alfalfa or mixed hay was fed than with non-legume hay, but feeding grass hay did not entirely prevent the deficiency.

**Literature Cited**


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Quaife, Mary L. 1947. Personal communication.


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