THE COMPARATIVE VALUE OF A CAROTENE
CONCENTRATE, ALFALFA MEAL, AND A FISH
LIVER OIL IN MAINTAINING THE VITAMIN A
CONTENT OF THE BLOOD AND LIVER OF
FATTENING LAMBS

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It has been widely reported in the literature that carotene from different
sources varies in its biological value (Ward, 1940; Fraps and Meinke,
Rubin and Bird, 1941; Smith and Otis, 1941; Jones et al. 1944). Since much
of this work has been done with cattle, poultry, and small laboratory animals
it has little direct application to sheep nutrition. If the efficiency of utiliza-
tion of carotene and other vitamin A active substances by sheep is related
to the source of these materials, it should become apparent in a comparison
between such widely different sources as alfalfa meal, a natural source of
carotene, a carotene concentrate prepared from carrots, and fish liver oil
which supplies vitamin A per se. During the course of several experiments,
designed to study factors which might influence the concentration of vita-
mins A and C in the blood plasma of lambs, it was possible to collect data
on the storage of vitamin A in the livers of lambs which had received
carotene from different sources and vitamin A from fish liver oil.

The results of Hauge et al. (1944) indicate that dairy cows can utilize caro-
tene from alfalfa hay as well as isolated carotene. King and co-workers (1940)
had previously reported however, that the carotene of alfalfa is more readily
available to the bovine than crystalline carotene given in oil.

Rubin and Bird (1941) observed that a group of chicks fed alfalfa meal
showed consistently larger stores of vitamin A than did other groups of
chicks fed either crystalline carotene or vitamin A.

Guggenheim (1944) concluded from his work with rats that the utilization
of carotene from various plant sources, or of carotene dissolved in different
oils, varied according to the vitamin E content of these materials.

The investigations of Davies and Moore (1934) and of Gray, Hickman,
and Brown (1940) indicate that vitamin A per se is utilized much more
efficiently by the rat than carotene. Also Hilton and associates (1944) found
that the carotene of dehydrated alfalfa hay was only about one-third as

1 A part of the data presented herein was taken from a thesis presented by J. A. Hoefer to the Graduate School
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2 Now on the Animal Husbandry Staff, Purdue University, Lafayette, Ind.
3 Departments of Animal Husbandry and Agricultural Chemistry Research, Stillwater, Oklahoma.
efficient as vitamin A *per se* as a source of vitamin A in the rations of dairy cows for the production of milk fat of maximum vitamin A value. Record and associates (1937), however, were unable to find a difference between the absorption of carotene and of vitamin A by hens fed normal diets. Guilbert *et al.* (1937) make the statement that at low levels of intake, vitamin A and carotene approach biological equivalence but that the ratio of their respective efficiencies widens as the dosage is increased.

Various values have been reported for the carotene requirements of cattle depending upon the criterion used (Guilbert *et al.* 1937; Moore, 1939; Boyer *et al.* 1942; Keener *et al.* 1942; Moore, Berry and Sykes, 1943). Lewis and Wilson (1945) were able to maintain fair growth in calves on 32 units of vitamin A per kilogram of body weight. Blood levels and liver storage were low however, in all calves receiving up to 128 units.

Callison and Knowles (1944) have reported that no quantitatively measurable stores of vitamin A can be demonstrated in the livers of rats fed less than 50 to 80 units of vitamin A per kilogram of body weight per day, which is about four times the amount usually considered as the "minimum" requirement for the rat.

The generally accepted minimum carotene requirements of sheep for the prevention of night blindness (nyctolopia) are from 25 to 35 micrograms of carotene or from 6 to 8 micrograms of vitamin A per kilogram of body weight as suggested by Guilbert, Miller and Hughes (1937). Peirce (1945) has more recently reported, however, that the feeding of this minimum level brought about recovery in only one of three night-blind animals, whereas all animals which received from 50 to 55 micrograms of carotene per kilogram per day recovered from night blindness.

**Experimental Procedure**

This study, which was carried out with Fine-wool Texas feeder lambs, was divided into two parts. All lambs were drenched with phenothiazine. In addition to the usual factors considered in lotting experimental animals the initial plasma vitamin A values were also taken into account.

In the first experiment alfalfa meal and a carotene concentrate were compared at three levels of intake, 1940, 3880, and 5840 micrograms of carotene per lamb daily. Four of the six lots of lambs used in this experiment (lots 3, 4, 5 and 6) received a low-carotene basal ration for a period of 194 days prior to the feeding of the carotene supplements. Consequently, their initial plasma vitamin A values were low at the beginning of the experimental period. There were, however, no visible symptoms of a vitamin A deficiency. During the experimental period the lambs were given a constant amount of carotene with no adjustments being made for weight changes. All the lambs
were fed individually and received a "low-carotene" ration made up of 50 percent ground prairie hay, 35 percent rolled oats and 15 percent soybean oil meal. The hay used was several years old and so badly weathered that it contained no measurable amount of carotene. A mineral mixture of equal parts of dicalcium phosphate and salt was available to the lambs at all times.

The carotene concentrate used was Research Carrot Oil containing 14,920 micrograms of carotene per gram. This carrot oil was given to the lambs in gelatin capsules. The required amount of alfalfa meal was fed on top of the basal ration. The crude and "true" carotene contents of these supplements were determined at frequent intervals by a combination of the Peterson, Hughes, and Freeman (1937) procedure as modified by Peterson (1941), and the Wall and Kelley (1943) method. The latter method makes use of MgO to separate so-called "true" carotene from associated yellow pigments.

In the second experiment all the lambs were fed a low carotene basal ration composed of cottonseed hulls, oats, and cottonseed meal for a preliminary period of 90 days before initiating the experimental treatments. At this time four representative lambs were slaughtered and the vitamin A content of their livers determined in order to obtain some measure of the vitamin A storage of the lambs at the beginning of the experimental period.

Alfalfa meal and a fish liver oil were compared in the second experiment at two levels of intake. The lambs in two lots (lots 7 and 8) received 50 I.U. of vitamin A per kilogram of body weight per day from alfalfa meal and a fish oil while two other lots (lots 9 and 10) were given ten times this amount, or 500 I.U. from the same two sources. These intakes represent approximately minimum and ten times minimum carotene requirements for the prevention of night blindness as established by Guilbert et al. (1937). Intakes were adjusted at frequent intervals to take care of weight changes and changes in carotene content of the alfalfa meal. Variations in the guaranteed potency of the fish liver oil as determined by colorimetric methods (Gallup and Hoefer, 1946) were negligible.

In both experiments blood samples were taken at frequent intervals and the citrated plasma analyzed for vitamin A by a modification of the Kimble (1939) procedure. At the conclusion of the experimental feeding periods representative lambs from each group were slaughtered and the storage of vitamin A in the liver determined. Early in the experiment the method of vitamin A analysis used for liver was that of Benham (1943). A procedure (Gallup and Hoefer, 1946) somewhat similar to that of Kaser and Stekol (1943) was adopted later. Both methods proved to be satisfactory when conditions were carefully controlled. Color intensities were measured with an Evelyn photoelectric colorimeter in the usual manner.

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4 International or U.S.P. units, assuming .6 microgram of beta carotene or .25 microgram of vitamin A equals one unit.
TABLE 1. THE VITAMIN A CONTENT OF THE BLOOD PLASMA AND LIVER OF LAMBS WHICH HAD RECEIVED CAROTENE OR VITAMIN A FROM DIFFERENT SOURCES AT VARYING LEVELS OF INTAKE

Experiment No. 1

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>No. of lambs</th>
<th>Days</th>
<th>Source</th>
<th>Average daily carotene or vitamin A intake</th>
<th>Average plasma vitamin A</th>
<th>Livers analyzed</th>
<th>Average liver vitamin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Per lamb</td>
<td>Per kg. wt.</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>55</td>
<td>Carotene conc.</td>
<td>5,820</td>
<td>155.2 (258.7)</td>
<td>25.6±1.44</td>
<td>24.9±1.78</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>55</td>
<td>Alfalfa meal</td>
<td>5,820</td>
<td>157.3 (262.2)</td>
<td>25.8±1.34</td>
<td>30.5±1.48</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>92</td>
<td>Carotene conc.</td>
<td>3,710</td>
<td>100.5 (167.5)</td>
<td>22.9±2.62</td>
<td>23.3±1.27</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>92</td>
<td>Alfalfa meal</td>
<td>3,710</td>
<td>99.2 (165.3)</td>
<td>13.4±3.03</td>
<td>27.1±1.23</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>92</td>
<td>Carotene conc.</td>
<td>1,940</td>
<td>44.9 (74.8)</td>
<td>13.5±2.74</td>
<td>17.5±3.43</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>92</td>
<td>Alfalfa meal</td>
<td>1,940</td>
<td>50.3 (83.8)</td>
<td>13.1±2.48</td>
<td>20.8±4.04</td>
</tr>
</tbody>
</table>

Experiment No. 2

<p>| | | | | | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>10</td>
<td>103</td>
<td>Alfalfa meal</td>
<td>1,164.0</td>
<td>30.0 (50)</td>
<td>26.5±3.12</td>
<td>21.0±1.79</td>
<td>4</td>
<td>209.1±43.83</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>103</td>
<td>Fish liver oil</td>
<td>403.8</td>
<td>12.5 (50)</td>
<td>25.1±1.98</td>
<td>24.1±1.69</td>
<td>4</td>
<td>664.6±235.82</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>103</td>
<td>Alfalfa meal</td>
<td>11,730.0</td>
<td>300.0 (500)</td>
<td>32.4±1.11</td>
<td>34.3±1.89</td>
<td>4</td>
<td>3,321.8±136.32</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>103</td>
<td>Fish liver oil</td>
<td>4,775.0</td>
<td>125.0 (500)</td>
<td>24.1±2.26</td>
<td>33.8±2.08</td>
<td>4</td>
<td>19,742.5±1,686.40</td>
</tr>
</tbody>
</table>

1 International or U.S.P. units, assuming .6 microgram of beta carotene or .25 microgram of vitamin A equals one unit.
2 The carotene concentrate was Research Carrot Oil obtained from Nutritional Research Associates, Inc., South Whitley, Indiana.
3 The fish liver oil was a vitamin A concentrate produced by the Borden Vitamin Company, New York, N. Y.
4 In Experiment No. 1 the lambs were given a constant amount of carotene throughout the experimental period. No adjustments were made for weight changes. The per kg. weight intake was calculated from the average weight of the lambs during the period.
5 In Experiment No. 2 the supplements were given according to body weight.
Results and Discussion

The results obtained in this experiment are summarized in table 1. It is evident from the data reported for experiment No. 1 that alfalfa meal consistently maintained a higher level of vitamin A in the blood than did the carotene concentrate, Research Carrot Oil. It is also apparent that more vitamin A was stored in the liver when alfalfa meal supplied the carotene than when the lambs were dependent on "Carrot Oil." The same relative differences were maintained regardless of the level of carotene intake or the state of vitamin A nutrition of the lambs. For example, lambs in lots 3, 4, 5, and 6 were practically depleted of their vitamin A reserves as shown by their very low initial plasma values, whereas, the lambs in lots 1 and 2 had plasma values within the normal range when the supplemental carotene feeding was started. Under both conditions the feeding of alfalfa meal resulted in an increased concentration of vitamin A in both the blood and liver.

Liver storage of vitamin A was quite variable as indicated by the standard errors. In general there was very little storage at the two lowest levels of intake, 1940 and 3880 micrograms of carotene per lamb daily. These results are in agreement with those of Peirce (1945) with sheep, Lewis and Wilson (1945) with calves, and Callison and Knowles (1945) with rats, with regard to the storage of vitamin A in the liver when the intake is limited.

Although it is recognized that the data are limited in number, it seems hardly likely that chance alone would account for the consistent differences obtained, and it must therefore be concluded that the carotene supplied as alfalfa had a higher biological value to the lambs than the carotene of Research Carrot Oil.

The lambs used in the second experiment had been placed on a low-carotene ration composed of cottonseed hulls, oats, cottonseed meal and a mineral mixture immediately after being received. After 90 days on this ration 4 representative lambs were slaughtered and their livers analyzed for vitamin A. The results of these determinations are shown in table 2. The remaining lambs were divided into four uniform lots and the alfalfa meal and fish liver oil given at two levels of intake, 50 units and 500 units per kilogram of body weight per day as described under "Procedure."

If it is assumed that the lambs which were retained for the experimental feeding period had approximately the same liver storage of vitamin A as the lambs slaughtered at the beginning of the trial, then it is evident that the feeding of either alfalfa meal or a fish liver oil in amounts sufficient to meet the minimum requirements for the prevention of night blindness (50 units per kilogram) did not maintain liver storage of vitamin A.

When the vitamin A or carotene intake was increased from 50 units to
500 units per kilogram of body weight there was a definite increase in the concentration of vitamin A in the blood. The blood levels of the lambs receiving alfalfa meal were practically the same as for the lambs receiving the fish liver oil. However, there was a marked difference between these two groups in liver storage. The four livers obtained from the lambs, which had received the oil, contained 19,742.5 ± 1,686.40 micrograms of vitamin A per 100 gm., whereas, the lambs on alfalfa meal had an average liver storage of vitamin A of only 3,321.8 ± 136.32 micrograms per 100 gm. This latter value is practically the same as for the lambs slaughtered at the beginning of the experiment which would indicate that under the condition of this experiment it took approximately 10 times the minimum requirements for carotene in the form of alfalfa meal to maintain the initial liver storage of vitamin A.

It is interesting to note that at the lower level of vitamin A intake, 50 units per kilogram of body weight, the lambs receiving the fish liver oil maintained a liver storage of vitamin A approximately three times greater than that of the alfalfa meal lambs. However, when intake was increased ten times, or to 500 units per kilogram of body weight, the difference in liver storage of vitamin A was approximately six times in favor of the lambs receiving the fish liver oil. This result substantiates the earlier statement of Guilbert and associates (1937) that at low levels of intake carotene and vitamin A approach biological equivalence, but as the dosage is increased the comparative value of the vitamin A per se is increased.

It should also be pointed out that in spite of the great difference in liver storage of vitamin A between these two groups there was practically no difference in the vitamin A level in the blood. When blood values are normal there seems to be little relationship between the concentration of vitamin A in the liver and in the blood. Braun (1945) has found in cattle that a tendency toward a direct relationship between these two variables exists only when the values fall below normal.

<table>
<thead>
<tr>
<th>Lamb No.</th>
<th>Vitamin A in blood plasma, micrograms per 100 ml.</th>
<th>Vitamin A in liver, micrograms per 100 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>230</td>
<td>44.3</td>
<td>3520.2</td>
</tr>
<tr>
<td>232</td>
<td>29.7</td>
<td>2693.1</td>
</tr>
<tr>
<td>235</td>
<td>35.8</td>
<td>3718.3</td>
</tr>
<tr>
<td>236</td>
<td>26.3</td>
<td>1421.8</td>
</tr>
<tr>
<td>Average</td>
<td>34.0</td>
<td>2845.9</td>
</tr>
</tbody>
</table>
The results obtained in this experiment and in others (Davies and Moore 1934; Gray et al. 1940; Wise et al. 1946) suggest that there is a limit to the ability of certain species to convert carotene to vitamin A. There is evidence indicating that even when animals are on green pasture and receiving an unlimited supply of carotene, they will not store as much vitamin A in the liver as animals which are given a relatively large amount of vitamin per se in the form of fish liver oil. During the period this second experiment was in progress an extra lamb was allowed to run on green pasture. He was killed along with the rest of the experimental lambs and a liver and blood sample taken for analysis. This lamb had 47.9 micrograms of vitamin A per 100 ml. of plasma and 7460 micrograms of vitamin A per 100 gm. of liver. The carotene intake of this lamb was very high and his plasma vitamin A was the highest of any of the lambs killed. However, liver storage of vitamin A was only moderately high and failed to approach that of the lambs receiving 500 units of vitamin A per se per kilogram of body weight. This inability to utilize large amounts of carotene efficiently also seems to hold true for rats. Davies and Moore (1934) failed in attempts to induce hypervitaminosis A in rats by feeding excessive amounts of carotene. Liver storage of vitamin A was only moderate. Wise and associates (1946) made observations with cattle which may be related to this problem. They found that the feeding of large quantities of vitamin A to dairy cows in the latter stages of gestation augmented significantly the vitamin A concentration in the blood and livers of their newborn calves, but pasture grazing, providing an abundance of carotene in the prepartum diet of the dams, failed to effect an increase over that observed in calves from dams restricted to a standard winter ration.

There was little variation in the average feed consumption and rate of gain between the lots of lambs receiving the various carotene and vitamin A supplements. Neither the kind of supplement or the level of feeding had a significant influence on the rate and economy of gain. This is in keeping with the report of Jones et al. (1943) that carotene in the ration of cattle has no effect upon gain or fattening so long as there are body reserves of vitamin A.

Summary and Conclusions

Fine wool feeder lambs, uniformly distributed with respect to vitamin A nutrition between comparable groups, were individually fed a low carotene ration with supplements of alfalfa meal, a carotene concentrate (Research Carrot Oil) and a fish liver oil. Alfalfa meal and the carotene concentrate were compared at levels of intake equivalent to approximately 79, 166 and 260 international units of vitamin A per kg. body weight, daily. The meal and fish liver oil were compared at accepted biological equivalent levels of 50 and 500 international units of vitamin A per kg. body weight, daily.
Values for the vitamin A content of the blood plasma, and liver storage of vitamin A at time of slaughter, were used as measures of the relative value of these supplements as sources of vitamin A for lambs.

The data collected in these experiments support the following conclusions:

1. Alfalfa meal is superior to a carotene concentrate from carrots as a source of vitamin A for lambs.
2. Fish liver oil is superior to alfalfa meal when fed on an equivalent unit basis, especially in the promotion of liver storage of vitamin A.
3. There is little direct relationship between values for blood plasma vitamin A and liver vitamin A when the blood plasma values are above 20 micrograms percent.
4. Values for the vitamin A content of the plasma of sheep between 20 and 45 micrograms percent may be considered within the normal range.
5. Additional vitamin A is without effect on the gaining ability of sheep that have appreciable body reserves of this vitamin.

Literature Cited


Maintaining Vitamin A Level in Lambs


