VITAMIN A CONTENT OF PASTURE PLANTS
11. TIMOTHY (*PHLEUM PRATENSE* L.) AND RED TOP (*AGROSTIS ALBA* L.) UNDER PASTURE CONDITIONS AND FED GREEN*

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The public constantly demands higher quality in dairy products, and progressive dairymen attempt to meet such demand. In recent years, because of the consumer's interest in the vitamin content of milk, investigators, and even some commercial milk producers, have tried by various methods to fortify the vitamin content of milk. After studying the problem, Krauss (12) summarized the situation as follows: "Vitaminization and mineralization of foods probably can not be justified except where natural foods fail to furnish these vital factors. This is especially true of milk."

Although there is considerable sentiment against making milk a "drug store product" by adding material to it, nevertheless, public demand will insure continued interest in milk of high vitamin content whether produced from natural feeds or treated in some manner.

Milk, butter, and cheese are important sources of vitamin A in the human diet. Many investigators have suggested that the vitamin A content of milk might be greatly influenced by the vitamin A content of the feeds consumed by the cow. In a previous paper from this station (23) some of the earlier results published on this subject were reviewed. Later investigations (1, 3, 4, 6, 13, 14) have confirmed this conclusion. It has been shown by Moore (19) that the cow is capable of converting the carotene in her ration into vitamin A, but the daily output of carotene and vitamin A in the milk fat is very small compared with the carotene intake, never exceeding that of normal summer butter regardless of the amount in the feeds consumed. Baumann, Steenbock, Beeson, and Rupel (2) report: "When the carotene intake of the cow was increased, there was an increase in both the carotene and vitamin A content of the butterfat. Calculations indicated that 3.3 per cent of the vitamin A ingested on a low carotene ration was secreted into the milk; on a high carotene ration only 1.3 per cent was secreted."

In the past, vitamin A has not been considered a limiting factor in the well-being of cattle, but more recent results indicate that under some conditions it may be. Hart and Guilbert (9) report vitamin A deficiency in range cattle under natural conditions. Deficiency developed when the dry

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feed season was unusually prolonged. Manifestation was more severe when the diet was otherwise complete and supplied in amounts above maintenance. Reproductive failure and dead, weak, or sick calves were the most common results.

Meigs and Converse (15) conclude from their experiments that “In the case of liberally milking cows fed on grain and hay or on grain, hay, and silage, without pasture, the ration is likely to be deficient in vitamin A unless it contains a large proportion of legume hay of good quality. Young calves from birth to the age of six months are highly susceptible to A deficiencies.” The same authors (14) report that when timothy hay of mediocre or low quality was fed to dairy cows reproduction was reduced, general health of the cows lowered, milk production decreased, and pregnancy was produced with more difficulty.

Woodward and Nystrom (24) in their bulletin on feeding dairy cows, point out that vitamin A is the vitamin most likely to be deficient in the ration of the dairy cow, and that as a result of this deficiency cows may give birth to weak, dead, or premature calves. They believe the quantity of milk will not be reduced so much as the vitamin A value. Because of the reserve supply in the liver, the cows themselves may go for months without showing the bad effects of the deficient ration, but calves fed the milk from such cows will cease to grow and soon will die if they are not given supplementary feeds rich in vitamin A or carotene.

Storage of vitamin A in the body of the cow may be sufficient to take care of other body functions for several months, but Hilton, Hauge, and Wilbur (11) found that as far as milk production is concerned the vitamin A value of butter responds very rapidly to changes in the vitamin A value of the rations fed to the cows and that it is possible to maintain a high vitamin A value in butter by practical feeding methods. Fraps, Copeland, and Treichler (8), however, from their study of the vitamin A requirements of dairy cows conclude that: “silage and ordinary hays and fodders apparently will not supply enough vitamin A potency to maintain a high content of the butterfat. Green growing pasture grasses appear to be needed to maintain the production of butterfat high in vitamin A.”

Dutcher (5) calls attention to the fact that the vitamin content of leafy plants and vegetables may be correlated with greenness, metabolic activity, and maturity. In discussing the growth response from vitamin A and its precursor, carotene, he sums up the problem as far as animal feeding is concerned as follows: “It is probable that we shall find that some of our so-called ‘vitamin A-rich’ foods are really quite deficient in this vitamin, per se, but that these foods are really rich in the parent substance, carotene. From the nutritional and clinical standpoints this fact need not cause concern so long as the fat-soluble factor is rendered available for the animal organism.”
Investigation of the vitamin A activity of the most common pasture plants seems justified because, first, the vitamin A activity of the feed consumed by the cow affects the vitamin A activity of the milk and butter, which is important in human nutrition; second, pasture represents the major portion of the cow's feed during nearly half the year; and third, significant differences in the vitamin A activity of different plants may indicate consideration of this factor along with others in formulating pasture mixtures for dairy cows.

Very little has been reported on the vitamin A activity of forages. In a previous paper from this Station (23), the vitamin A activity of white clover (200 rat units per gram) was reported to be about twice that of Kentucky bluegrass.

**EXPERIMENTAL PROCEDURE**

The method used for determining the vitamin A content of these plants was based upon the technique of Sherman and Munsell (20). Young albino rats, 21 to 28 days old and weighing from 32 to 51 grams, were taken from mothers who had received a ration consisting of 62 per cent ground whole wheat, 30 per cent whole milk powder, 4 per cent wheat germ, 3 per cent powdered egg yolk, and 1 per cent iodized salt. These rats were placed on a basal vitamin A-free diet which consisted of 67 per cent cornstarch, 18 per cent air-heated casein, 10 per cent dried yeast powder, 1 per cent sodium chloride, and 4 per cent salt mixture (Osborne and Mendel (17)).

To free the ground commercial casein of vitamin A, it was treated by the heat method suggested by Potter (18). Three hundred gram quantities were spread on shallow trays to a depth of ¼ inch and heated in a Freas oven at 110° C. for seven days. The casein was stirred twice daily to secure better exposure. After heating it was washed twice with distilled water and allowed to dry. Vitamin D was furnished by feeding one drop of Mead's Viosterol three times a week.

At the end of three weeks of the depletion period the rats were put in individual cages made of ¼ inch wire screen. From the time the rats were put in individual cages to the end of the depletion period they were weighed every other day.

As soon as there was a cessation of growth or the first sign of ophthalmia (slight swelling of the eyelids and an accumulation of exudate on the cornea), usually accompanied by a barely perceptible tendency to flabby musculature and slightly unkempt fur, the depletion period was ended and the test period begun.

Fresh green samples of timothy and red top under pasture conditions were received twice a week from the Caldwell Substation. To prevent moisture losses in shipping, the grass was wrapped in cellophane or paper sacks and placed in paper cartons. Upon arrival, each sample was placed
in a half-pint Economy fruit jar and kept in the refrigerator. Practically all the samples consisted of basal foliage and contained very few stems. Timothy had an average moisture content of 68 per cent for all feedings, while red top contained 61 per cent moisture.

The grasses were fed three times a week as supplements to the vitamin A-free diet. Timothy was fed at the rate of 10 and 12½ milligrams per day, while red top was fed at the rate of 10 and 7½ milligrams. Litter mates were used for comparisons between grasses, and a negative control from each litter was continued on the basal diet until death.

Weekly records of weights and food consumption, and careful notes on the condition of the animals were kept for eight weeks. The method of eye scoring was that used by Steenbock and Wirick (22). All animals were autopsied at death or at the end of the experimental period.

RESULTS

At the beginning of the depletion period, the average weight of the rats in all groups was 42 grams, and at the end of the depletion period 98 grams. The average length of the depletion period was 26 days.

Average growth response of the rats being fed each of the grasses is shown in Table 1 and Figure 1. The experiments here reported were planned and carried out on the basis of the eight-week test period used by Sherman and Munsell (20), but results are presented for periods of four weeks (28 days). A 1934 revision of the "Text and Assays for Cod Liver Oil" of the Pharmacopoeia of the United States prescribes an official method of assay for vitamin A (16). Specifications for the depletion period, assembling of rats in groups, and recording of data are given. Concerning the validity of data the method reads as follows: . . . "The data from an assay group shall be considered valid for establishing that an assay oil conforms with the U. S. Pharmacopoeia standard for Vitamin A in cod liver oil only when two-thirds or more but not less than six rats shall have made individually between the beginning day of the assay period and the twenty-eighth day thereafter an increase in body weight which shall equal or exceed 12 grams." This rule has been used to establish the validity of data from an assay group for vitamin A potency of pasture plants. No rat which has not made a gain of 12 grams or more in four weeks has been used to establish average performance of an assay group. The number of rats in each group and the number used in the average are indicated in the table. The results can not be expressed in International Units of Vitamin A, however, because there are no data from a group fed a "Reference Cod Liver Oil." The value is calculated, therefore, in rat units per gram from the daily dose in milligrams which would give an average gain of 12 grams in 28 days.

Animals (10 rats) receiving 60 milligrams of timothy per week made
### TABLE 2

Average growth response of rats when fed fresh timothy and red top as vitamin A supplements

<table>
<thead>
<tr>
<th>PASTURE PLANT</th>
<th>SOURCE</th>
<th>DATE OF TEST PERIOD</th>
<th>AMT. OF PLANT FED WEEKLY</th>
<th>RATS FED</th>
<th>RATS USED</th>
<th>AVE. WT. AT BEGINNING OF DEPLETION PERIOD</th>
<th>AVE. WT. AT BEGINNING OF TEST PERIOD</th>
<th>AVE. WT. AT END OF 4 WEEKS TEST PERIOD</th>
<th>AVE. GAIN IN WT. AT 4 WEEKS</th>
<th>COEFFICIENT OF VARIATION</th>
<th>RAT UNITS OF VITAMIN A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timothy</td>
<td>Caldwell</td>
<td>5-16 to 7-11</td>
<td>gms. 0.060</td>
<td>12</td>
<td>10</td>
<td>83% 43</td>
<td>100</td>
<td>126</td>
<td>26 ± 1.5</td>
<td>26</td>
<td>220 ± 13</td>
</tr>
<tr>
<td>Red Top</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>gms. 0.075</td>
<td>4</td>
<td>4</td>
<td>100% 39</td>
<td>101</td>
<td>124</td>
<td>23</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>Negative Controls</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>gms. 0.060</td>
<td>12</td>
<td>10</td>
<td>83% 42</td>
<td>98</td>
<td>135</td>
<td>37 ± 1.1</td>
<td>15</td>
<td>308 ± 10</td>
</tr>
<tr>
<td></td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>gms. 0.045</td>
<td>4</td>
<td>4</td>
<td>100% 39</td>
<td>99</td>
<td>124</td>
<td>25</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
</tbody>
</table>

average survival—15 days
average loss in weight—15 grams

* Insufficient data.
Fig. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either timothy or red top as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.
an average total gain in body weight of 26 ± 2 grams in four weeks. This would indicate that this grass contained 220 ± 13 rat units of vitamin A activity. The average total gain of the 10 rats fed 60 milligrams of red top per week was 37 ± 1 gram. This would indicate 308 ± 10 rat units of vitamin A activity per gram in red top.

The coefficient of variation in the group fed timothy, 60 milligrams per week, was 25 per cent, while in the group fed red top, at the same level, was 16. The variability of these results compare favorably with the findings of Sherman and Burtis (21), who report that when the animals were classified by sexes the coefficient of variation was 32 per cent at the end of the fifth week at a vitamin intake level twice that needed for a growth response of 3 grams per week and 45 per cent at a level inducing 3 grams gain per week.

At the other level on each grass, the great variation found within the group, together with the limited number of rats (less than six), would preclude conclusions from these groups other than to substantiate the dependability of the levels used in interpreting results.

The difference between the rat units of vitamin A activity in red top, 308 units, and timothy, 220, was 88 units. The probable error of the difference was 11 units, or the reliability of the difference may be expressed as 88 ± 11. Since the difference between the rat units in the two pasture plants (88) was eight times the probable error of the difference, the difference is a true one.

In order that the results obtained may be compared with data reported on the basis of 24 grams gain in eight weeks, the average gain made by each group in eight weeks is given. When the average gain for the longer period is the basis used for calculating the rat units per gram, all animals in a group are usually included whether or not they are gaining at the end of the test. All animals, therefore, are used in summarizing the eight-week averages. On the 60-milligram per week level the 12 animals fed timothy gained 39 ± 1.2 grams in eight weeks, indicating a value of 163 ± 5 rat units per gram; while the 12 rats on red top gained 48 ± 3.4 grams, or 200 ± 15 rat units per gram. All the animals receiving 60 milligrams of red top per week were free of xerophthalmia at the end of four weeks and also at end of eight weeks. Only one of the animals of the group used with the timothy showed eye infection at four weeks, and this condition failed to clear by the end of eight weeks. One of the other rats included in the longer test group on timothy also failed to develop normal eyes. Post-mortem examinations of the animals at the end of eight weeks revealed some signs of infection, especially in the ear and nasal sinuses. The eight animals used as negative controls survived an average of 15 days beyond the end of the depletion period and lost an average of 15 grams in body weight. Post-mortem examination of these animals showed that 3 had pus in the
nasal sinuses, all had pus in the inner ear, 2 had pus at the base of the tongue, 2 had congestion in the intestinal tract, and 1 had pus in the lymph glands.

**DISCUSSION**

White clover and Kentucky bluegrass previously have been reported to contain 200 and 100 rat units, respectively, of vitamin A activity when computed from data covering a feeding period of eight weeks (23). When computed from gains made in the first four weeks, as used in this paper, the white clover contained 242 ± 19 rat units and the Kentucky bluegrass 175 ± 11. In comparable units, timothy with 220 ± 13 would rank between bluegrass and white clover. Red top with 308 ± 10 units contained about one-third more than even white clover. These results indicate that different pasture plants may vary considerably in their content of vitamin A activity. Although the 4 weeks test period seems to have some advantages over the 8 weeks period, it appears that higher vitamin A rating may be expected from the same feed level.

The fact that red top contained about 50 per cent more rat units of vitamin A activity than timothy may be attributed to the possibility of a higher carotene content. Although no tests for carotene were conducted, and each grass was green when fed, some justification for this assumption is indicated by the fact that red top takes on a reddish cast when mature while timothy does not.

Although many writers have suggested that fresh green grass is probably a potent source of vitamin A no other bio-assays have come to the notice of the writers. Fraps and Treichler (7) reported that samples of bur clover and sudan grass dried in vacuum contained 200 and 150 units per gram, respectively, but no data were presented on the fresh green grasses as the cows consume them.

Any loss of moisture would tend to concentrate other constituents in the grass samples and would probably indicate a higher vitamin A content than the original grass contained. Although some moisture was lost from the samples studied, the grass was still fresh and green when fed. The average moisture content of the timothy samples was 67.9 per cent and of the red top 60.5. These averages compare well with the average of 60.7 per cent for 16 analyses of red top and 62.5 per cent for 88 samples of timothy, all analyses, and 75.8 for 5 samples, before bloom, reported by Henry and Morrison (10).

The vitamin A activity of each of these four pasture plants is much higher than has been reported for most feeds or foods except some samples of fish liver oils.

Fraps, Copeland, and Treichler (8) were unable to maintain high vitamin A activity in butter when cows were fed 116,000 rat units daily. They
suggested that fresh green grass was necessary in the diet of the cow in order to keep the vitamin content of the butter high.

A dairy cow can easily consume 100 pounds of fresh green grass daily. If the grass contained 200 rat units of vitamin A activity per gram, the total intake of vitamin A activity per day would be over nine million rat units. It is not surprising that summer butter produced by cows fed unlimited quantities of fresh grass has been found to be usually higher in vitamin A activity than winter butter.

CONCLUSIONS

Results of this study indicate that the vitamin A activity of the two pasture plants, timothy (*Phleum pratense* L.) and red top (*Agrostis alba* L.), was 220 ± 13 and 308 ± 10 rat units respectively, considering a gain of 12 grams in 4 weeks as representing one unit. Statistically, this is a significant difference. Compared with white clover (242 ± 19 units) and Kentucky bluegrass (175 ± 11 units) previously reported, timothy would rank between them while red top contained a third more units than even white clover. The content of vitamin A activity in these pasture plants is higher than has been reported for any feeds or foods.

LITERATURE CITED


(16) Pharmacopoeia of the United States, Tenth Decennial Revision, Interim Revision Announcement No. 2. A 1934 revision of the text and assays for cod liver oil.


