THE CAROTENE AND PROTEIN CONTENT OF ALFALFA AS AFFECTED BY SOIL AMENDMENTS AND SOIL MOISTURE

by

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INTRODUCTION

For years alfalfa has been recognized as a valuable source of livestock feed. The value lies in its ability to be grown extensively under quite widely varying soils, as long as adequate calcium is supplied. It is also a valuable source of vitamin A, which is found throughout the plant in the form of carotene, the precursor of vitamin A.

Black et al. (1939) studying the effect of vitamin A in animals concluded it was essential to good health, normal growth, and lactation. They also recognized its value in the human diet. Their studies were confirmed by Meigs (1939) and also by Booher et al. (1939).

Considerable work has been done on carotene, but mostly from the standpoint of the chemist. This work has been mostly attempts to find methods whereby carotene could be preserved in the plant tissue. Chemists have worked with agriculturalists on methods of field curing, dehydration, and storage in attempts to find means whereby oxidation of carotene could be reduced.

Realizing the value of retaining high carotene content in the stored forage, much research has been carried on to find the methods of harvesting and storing that would be least destructive to the carotene content.

The object of this study is to determine, if possible, the effect of recognized fertility elements on the carotene and protein content of alfalfa when grown at different levels of soil moisture.

REVIEW OF LITERATURE

Johnson (1936), working on the effect of leaf hopper injury of alfalfa and the resulting carotene content, found that with increasing leaf hopper injury there was a decreasing carotene content. This fact was confirmed in 1946 by Ham and Tysdal. Hauge (1934) found that the leaves of alfalfa contained a larger percentage of the carotene than did the stems, also that the leaves had as much as 14 times the carotene content of the stems. Ham and Tysdal (1946) found that on an average the leaves had 77.1 percent of the total carotene of the plant, and that this varied with the plant type and variety. This variation was from 59 to 94.6 percent. From this they concluded that the percentage of leaves is an important factor in the carotene content.

Hauge (1934) concluded that young rapidly growing alfalfa, 10 to 12 inches high, is higher in carotene content than older, more mature alfalfa in the bloom stage. Douglas et al. (1933) showed that if the alfalfa was in the early bloom stage it had a higher carotene value than when it had matured to the late bloom stage. Snyder and Moore (1940) studying herbages including alfalfa, confirmed that the early stages of growth were the higher in carotene content. Ham and Tysdal (1946) found that young rapidly growing alfalfa had from two to fives times as much carotene as did older growth. Meenen (1945) studying the alfalfa leaves found that old leaves had a higher concentration of carotene than did newly formed leaves, and attributed the loss of carotene in the plant to the loss of leaves as the alfalfa matured.

The literature on the effect of fertilizer elements on vitamin content of alfalfa is very limited and that which is available is not wholly in agreement. There is so little on alfalfa that it seems pertinent to use some of the literature on some of the other forage crops as well as vegetable crops.

Smith and Wang (1941) studying grassland herbage found that carotene was higher in young grass up to flowering time, but decreases rapidly at flowering time. Dutcher (1932) studying the carotene of leafy vegetables found that it was higher in the young leaves than in the mature leaves. Hamner and Maynard (1942) in a review of literature concluded that many writers agree that carotene increases during the ripening process of tomatoe fruits.

Atkison et al. (1937) while studying pasture plants and alfalfa found that carotene content varied throughout the season being higher in the spring before seed set and higher temperatures, lowering after seed had been formed and higher again in the fall after the fall rains started new growth.

Powers (1939) found that under certain conditions boron increased the carotene as well as the yield. This was confirmed by Maynard and Beeson (1943) when applied to a boron deficient soil. Mitchell (1946) while not working directly on fertilizers found there were only slight differences due to fertilizer treatments. Honeywell and Dutcher (1930) while studying spinach found that when spinach showed a chlorotic condition the carotene was depressed. They also found that if enough manganese was withheld to give a manganese starvation the carotene content was lowered much the same as from any chlorotic conditions. This bears out the results of Johnson (1936).

Whittemore (1934) found that the application of nitrogen, phosphorus, potassium, or manganese fertilizers had no effect upon the carotene content although there was considerable growth response when applied to soils that were deficient in these elements. Ijdo (1936) stated that application of large amounts of nitrogen fertilizers to spinach resulted in an increase of the carotene and the vitamin C content. He also stated that increasing the potassium content of the soil caused a decrease in the carotene and an increase in vitamin C. In his experiments he found that the response to phosphorus, calcium, and magnesium was very small. Scheunert and Wagner (1939) found that there was little effect on the carotene content of several vegetables regardless of an over-supply or a deficiency of the elements.

Hamner (1945) reviewing literature stated that the vitamin relationship to any one macro-nutrient element is not conclusive, but did conclude that treatments resulting in the highest forage yield per unit area also resulted in highest yield of vitamins per unit area and any chlorotic condition lowered the carotene content. Smith and Wang (1941) found that manuring with common barnyard manure had little effect on the carotene value of herbage except where ammonium sulphate had been added. This caused an increase in carotene.

Moon (1939) found that nitrogen caused an increase in the carotene content of pasture grasses. This increase under some conditions was as great as 28 percent. He also found that there was little or no effect from the application of lime or superphosphate, although there was an increase in the yield of dry matter and vitamins per unit area.

Ellis and Hamnor (1943) found that wide variations in the supply of the macro-nutrient elements produced little effect upon the carotene content of tomatoes, although the treatments did markedly effect the growth and fruitfulness of the plants. There were indications that an increase in the nitrate supply did result in an increase in the carotene content of the fruit, but the variations were only slight. They also checked the effect of the location on the carotene content and found that neither the locality or the supply of micro-elements had any measurable effect on the carotene content of the tomatoe fruit. This was confirmed by Lyon et al. (1943).

Barnes (1936) studying the effects of fertilizers on the common carrot found there was no effect from the elements nitrogen, phosphorus, potassium, manganese, and magnesium. Ott (1937) found that with the application of potassium fertilizers, there was an increase in the carotene content up to an optimum, and any further application caused a rapid decrease. Swanson et al. (1940) studied the effects of fertilizers on sweet potatoes. Their results failed to produce any significant difference between any of the fertilizers applied. Pfutzer and Pfaff (1935) worked with various plants and applied full and partial fertilization. They found a complete fertilizer increased the vitamin A-content and the yield and increased the yield of both forage and vitamin per unit area. Guthrie (1929) investigated the effect of fertilizers on the carotene content of soybeans, concluded that where an insufficient supply of nitrate nitrogen was available, the product had a decreased carotene content.

Thomas and Moon (1938) found a significant correlation between carotene and crude protein. Moon (1939) in later work found that carotene and protein were more closely associated when a fertilizer that had no direct effect on either of them was applied. Fertilizer treatments that had a direct effect on either caused the correlation to be not so close, and concluded that the effect of carotene and protein was merely a secondary effect and not a direct one. Working with immature oat leaves, Wynd and Noggle (1945) concluded the same soil factors that effect carotene percentage seemed to effect protein percentage. They also found the higher soil pH levels increased the carotene and percentage of dry matter. This seemed to be due to increased available nitrates at the higher pH values.

Later work by Wynd and Noggle (1946) on rye plants showed that with an increase in the base exchange capacity of the soil there was an increase in the carotene content of the leaves. They found that potassium was unrelated to the carotene produced although there was a vaguely positive reaction. Calcium and magnesium were found to be related to increased amounts in leaves. However, all were not as large as the effect of nitrogen alone. They postulated that this effect would probably be negligible with a leguminous plant.

Virtanen et al. (1933) found that plants grown under an optimum pH have a higher carotene content than when grown under more acid conditions and that the highest carotene content was obtained when the plants were grown with sufficient nitrogen to insure maximum growth. It has been generally postulated that because maximum carotene and maximum growth go hand in hand; carotene must be regarded as an essential growth factor in the plant. Virtanen again reported similar results in (1936). Moon (1939) reported that when enough carbonate of lime was applied to raise the pH from 5.63 to 7.31 there was no effect on the carotene content of pasture grasses.

Singleton et al. (1945) studying fertilizers on alfalfa in Washington State, found that phosphorus fertilizers alone or in combinations, caused increases in the yield; also any combination of fertilizers were found to be no more effective than phosphorus alone. Bernstein et al. (1945) indicated that turnip greens grown in the greenhouse during the winter time had a higher carotene value than those grown during the summer. They found no applicable effect on the carotene content by any fertilizer when the plants were grown in soil, but in sand cultures where mineral deficiencies were maintained, sulphur, nitrogen, and potassium deficiencies resulted in decreased carotene content, but a deficiency of phosphorus resulted in no decrease. Brown et al. (1947) indicated that insufficient nitrogen, magnesium or iron would cause a decrease in the carotene content of Swiss Chard, and that calcium, manganese, potassium, and phosphorus had no measurable effect upon the carotene content of Swiss Chard. Hackerott (1946) found alfalfa responded very little to fertilizer treatment. His work shows the best possibility for increased carotene content is not from a fertility, but from a breeding program. The soil moisture experiment that he conducted shows the higher percentage carotene was consistently obtained from the lower soil moisture levels, and his investigations showed the greatest abundance of carotene in the leaves with little in the stems. With the abundant production of stems from the high moisture treatments, the carotene content was depressed for the increased stem content was not equalized by increased leafiness.

Maynard and Beeson (1943) in a review of literature indicated the carotene content was due to many factors, among them are: varietal differences, seasonal and climatical variations, and to a lesser extent fertility treatments.

They stated that:

> A critical study of the mass of data dealing with the effect of soil type and the nutrition supply suggests that both have much less influence than variety and climate. It is also clear, however, that only a few of the many possible relationships have been studied.

From a review of literature it appears that the carotene content of plants has not been consistently influenced by fertilizer treatments. There seems to be no disagreement, however, in that any nutrient deficiency resulting in visible chlorosis causes a decrease in the carotene content. It is indicated that the content of alfalfa can be increased by applying boron if the soil is deficient in that element. Young rapidly growing alfalfa seems to be higher in carotene than older more mature alfalfa, and that the content fluctuates from spring to fall, being higher in the spring and fall, than in the summer. It appears, however, that a well developed breeding plan can possibly increase the carotene content of alfalfa.

MATERIALS AND METHODS

The equipment and facilities for this investigation were furnished by the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture; Departments of Agronomy and Chemistry, Kansas Agricultural Experiment Station; and the Kansas Industrial Development Commission.

The plant material analyzed in this investigation was grown either in the greenhouse or on the Agronomy Farm at Manhattan.

Greenhouse Fertility and Soil Moisture Studies

The greenhouse experiments were conducted during the spring and the fall of 1947. The two experiments were different in that the fertilizer applications were modified slightly and a different soil type from a different source was used. Pots of "high" and "low" moisture levels were maintained in both experiments. "High" approaching field water holding capacity and "low" near the wilting point of the plant. Each moisture level was subdivided into treatments of boron, calcium, magnesium, nitrogen, phosphorus, potassium and combinations of these, making a total of 18 treatments including the check. Three pots of each treatment were used in each moisture level. The soil for the experiment in the spring of 1947 was obtained from an eroded, leached slope at the Soil Conservation Nursery near Manhattan. It was light brown in color and of fine sandy loam texture. The wilting coefficient was estimated from the moisture equivalent as determined in the usual manner. The high moisture pots were maintained at 18 percent and the low at 8 percent which is slightly above the wilting coefficient. The pots received boron at the rate of 30 pounds per acre, in the form of borax, $Na_2B_4O_7$; magnesium at 150 pounds per acre as epson salts, $MgSO_4$; calcium at the rate of 250 pounds per acre as gypsum, $CaSO_4$. Nitrogen was applied as ammonium nitrate $(NH_4)NO_3$ at the rate of 75 pounds per acre; phosphorus as treble superphosphate, 43 percent P_2O_5 at 55 pounds per acre and potassium as muriate of potash, 60 percent K_2O at 30 pounds per acre. These rates were used in all treatments whether alone or in combinations. The soil for the experiment in the fall of 1947 was obtained from the Thayer Agricultural Experiment Field at Thayer, Kansas. It was grayish brown in color and a silt loam in texture. It has a pH of 4.38 with 42 pounds of available phosphorus per acre and 315 pounds of available potassium per acre. The soil had received no lime application in its known history. The high moisture pots were maintained at 20 percent moisture and the low were maintained at 12 percent. The change in the percentage of moisture from the first experiment was due to a different soil type having a different moisture capacity. The fertilizer elements and rates were the same as in the spring experiment, but the treatments were different in that N.P.K. was applied to one-half the high moisture pots and one-half the low moisture pots, giving a series of boron, calcium and magnesium treatments with and without N.P.K. in each of the high and low moisture treatments.

The equipment used in this experiment was designed by Grandfield (1941) for soil moisture studies. It consists of

7-inch glazed pots each fitted with stoppers in the bottom through which is placed a coiled copper tube with numerous perforations. Water is applied through the lower end of the copper tube and forced through the numerous small openings to insure uniform moisture distribution throughout the soil mass. To maintain the proper moisture level the plants were weighed daily, on scales weighing with an accuracy of ±10 grams. Each day the plants were moved in a rotation system to eliminate as much as possible the effect of variation in light due to location. Each pot made a complete rotation every 10 days.

The plants used in this experiment were obtained by making cuttings from a single clone to eliminate genetic variability. The plants were allowed to reach bloom stage in order to become well established before applying the fertilizer. The top growth was then removed, and the fertilizers were applied and worked into the soil. At this time the soil moisture was established at the previously planned levels. After the plants had developed to one-tenth bloom stage they were sampled by cutting off all the top growth and combining the three plants in each treatment as a single sample; thus yielding sufficient dry matter for duplicate carotene analyses. The plants produced only enough dry matter for carotene analyses, for this reason no protein determinations were made on greenhouse material.

Field Fertility Studies

The field experiment was conducted on plots located at the Agronomy Farm at Manhattan.

The experimental area selected was on a uniform east slope of two to four percent in a field that had been seeded to Buffalo alfalfa in the fall of 1946, following a crop of Pawnee wheat.

The soil is Sharpsburg silty clay loam. This soil has calcarious concretions below 18 inches and the subsoil is mottled with red and yellow.

The experiment block consisted of 18 treatments replicated 6 times, making a total of 108 plots. The plots were 6 feet wide and 25 feet long. They were randomized in such a manner that all treatments including the check appeared only once in each replication. The fertilizers and rates were the same as those employed in the greenhouse experiment.

The difficulty of applying small quantities of fertilizers to small areas was overcome by adding dry sand to the fertilizer, making a total weight of sand and fertilizer uniform for all plots. The sand and fertilizer were mixed in a rotatory drum mixer until a uniform mixture was obtained. A top dress drill was then calibrated, and all plots received 25 pounds of top dressing (sand and fertilizer), the checks receiving no fertilizer.

The plots were allowed to grow normally until approximately one-tenth bloom when they were sampled for analysis by picking stems at random throughout the plot as a representative sample, and taking it immediately to the laboratory where it was blanched in an autoclave at five pounds pressure for five minutes. This was done to inactivate the carotene destroying system that is found in alfalfa (Mitchell and Hauge, 1946).

Immediately after autoclaving, the samples were dried in a force draft oven at 65° C. ± 2°, from two to three hours. After the samples were dry they were removed from the oven and ground in a Wiley mill using a #20 screen, all the ground material was thoroughly mixed and placed in a light proof envelope and stored at 3° F. until ready for analysis. The analyses were made by the method of Silker, Schrenk, and King (1944), which consists of weighing duplicate one gram samples of the dry ground material, extracting with a solution made of two parts Skelly Solv "B" and one part acetone, filtering with a suction filter and concentrating to about 60 ml over a steam bath. The sample is then ready to chromatograph in a differentially absorptive filter consisting of a column of magnesia and hyflo super cel. The sample is then transferred to the absorptive filter. The carotene pigments are eluded by a four percent acetone-skelly solv B solution.

A sample of this filtrate is taken and the color intensity of the pigment read on a Beckman quartz Spectrophotometer at 4360 A°. Carotene content is herein reported as milligrams per one hundred grams dry weight. All samples were handled in this manner unless otherwise stated.

The protein determinations were made by the Kjeldahl method for total nitrogen (Assoc. of Off. Agr. Chemists, 1940).

EXPERIMENTAL RESULTS

The results reported in this paper are concerned with studies on the effect of fertilizer elements on the carotene and protein content of alfalfa when grown at different moisture levels.

Greenhouse Fertility and Soil Moisture Studies

These studies were undertaken to determine what effect, if any, plant food elements had upon the carotene content of alfalfa forage. The effect of various fertilizer treatments and soil moisture levels under greenhouse conditions on the carotene content of alfalfa in the first experiment is reported in Table 1, and the results of the analysis of variance of these data are given in Table 2.

In Table 1 the data of both high and low moisture represent the mean of two duplicate analyses whose readings varied less than ten units as read on the Beckman quartz Spectrophotometer.

There was no statistically significant response in carotene due to the application of nitrogen, phosphorus, potassium, boron, magnesium or calcium. Similarly there was no response from any combination of these fertilizers. There was, however, a significant difference between soil moisture levels. The low soil moisture treatment resulted in consistently higher carotene content than did the high moisture treatment.

The fall greenhouse experiment gave much the same results, with moisture levels causing the greatest variation. However, the design was changed so that two series of boron, magnesium and calcium in all possible combinations were applied, one with the application of N.P.K. and the other without N.P.K. The presence of N.P.K. resulted in a higher carotene content. The analysis

Table 1. Effect of soil moisture and fertilizer treatments on carotene content of alfalfa grown in pots in the greenhouse.[1]

Treatment	Mg/100 gm dry wt. High	Low	Mean
Boron	41.2	42.2	41.7
Ca	40.2	41.5	40.8
Mg	42.3	45.2	43.7
N	42.7	43.2	42.9
P	42.0	44.3	43.1
K	39.9	42.8	41.3
NP	39.5	43.3	41.4
NK	40.9	45.2	43.0
PK	41.6	44.4	43.0
NPK	39.0	42.4	40.7
NPK-B	43.8	45.2	44.5
NPK-Ca	40.4	38.9	39.6
NPK-Mg	46.5	41.5	44.0
NPK-B-Ca	42.5	45.6	44.0
NPK-B-Mg	34.4	44.9	39.6
NPK-Mg+Ca	39.9	47.1	43.5
NPK+B+Mg+Ca	48.0	43.8	45.9
Check No treatment	42.3	42.9	42.6

[1] Conducted spring 1947.
LSD - between means of moisture levels equals 5.0 at the 5% level of significance.

Table 2. Analysis of variance of carotene content of alfalfa plants produced under soil moisture and fertility treatments in the greenhouse.[1]

Factors	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	35	247.77				
Between soil moisture levels	1	38.64	38.64	5.95#	4.45	8.40
Between fertilizer treatments	17	98.62	5.80	.89	2.32	3.36
Interaction moisture levels, fertility treatments (i.e.) Error	17	110.51	6.50			

[1] Conducted spring of 1947.
Significant at 5% level.

of variance showed a 5 percent level of significance between these two series. The pots receiving N.P.K. gave higher yields than those from which it was withheld. From the data in Table 3, this variation cannot be attributed to any one element, but it probably is due to the combined effect of the three elements.

Table 3. Effect of soil moisture and fertilizer treatments on the carotene content and yield of alfalfa grown in pots in the greenhouse.[1]

Elements	Carotene mg/100 gm dry wt.				Yield total gms dry wt.			
	High moisture		Low moisture		High moisture		Low moisture	
	With N.P.K.	Without N.P.K.	With N.P.K.	Without N.P.K.	With N.P.K.	Without N.P.K.	With N.P.K.	Without N.P.K.
Check	47.2	44.0	50.4	51.5	19.5	21.0	16.0	16.0
B	50.3	47.5	56.4	46.7	19.0	19.5	15.5	15.0
Ca	48.3	46.0	50.6	46.1	21.0	20.5	17.5	14.5
Mg	52.4	47.7	54.0	55.7	18.5	16.0	14.5	13.5
B-Ca	48.0	44.2	52.9	48.3	22.5	20.0	17.5	16.0
B-Mg	47.6	48.2	53.6	46.6	23.0	20.0	16.0	15.0
Ca-Mg	48.5	48.6	56.7	52.3	22.5	18.5	15.5	14.5
B-Ca-Mg	47.8	49.6	49.3	53.6	17.5	18.5	16.5	15.0
Average	48.8	47.0	53.0	50.1	20.4	19.3	16.1	15.0

1 Conducted fall 1947.

Table 4. Analysis of variance of carotene content of alfalfa plants produced under soil moisture and fertility treatments in the greenhouse.[1]

Factors	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P = .05)	(P=.01)
Total	31	350.98				
Between moisture levels	1	108.04	108.04	19.19**	4.30	7.94
Between N.P.K. treatments	1	43.71	43.71	7.76*	4.30	7.94
Between fertilizer treatments	7	75.32	10.76	1.91	2.47	3.59
Interaction, i.e. Error	22	123.91	5.63			

[1] Conducted fall 1947.
* Significant
** Highly significant.

LSD Moisture levels 6.68 at 1%
4.74 at 5%

LSD N.P.K. treatments 4.74 at 5%

Table 5. Analysis of variance of yield of alfalfa plants produced under soil moisture and fertility treatments in the greenhouse.[1]

Factor	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	31	220.50				
Between moisture levels	1	148.90	148.90	119.12**	4.30	7.94
Between N.P.K. levels	1	11.41	11.41	9.13**	4.30	7.94
Between fertilizer treatments	7	32.75	4.67	3.73**	2.47	3.59
Interaction in Error	22	27.44	1.25			

[1] Conducted fall 1947.
** Highly significant

LSD moisture levels = 3.06 at 5% 4.28 at 1%
LSD N.P.K. levels 3.06 at 5% 4.28 at 1%
LSD Treatments 1.96 at 5% 2.74 at 1%

Field Fertility Studies

The field fertility studies were conducted to test fertilizer application under natural environmental conditions. The field experiment differed from those conducted in the greenhouse in that soil moisture could not be controlled. Data were obtained, however, under conditions of high and low soil moisture, as data from the first cutting were obtained under ample moisture while those of the second cutting were produced under stresses of low moisture.

An examination of data in Table 6 shows that the samples taken in the first cutting contained a relatively low carotene and a high protein, while those taken from the second cutting had a higher carotene and were low in protein. The difference in the carotene data between the first and the second cutting bears out the soil moisture relationship, as found in the greenhouse experiment, in that high soil moisture of the first cutting shows a lower carotene content than those samples taken from the second cutting grown under low soil moisture.

The protein content of these two cuttings varied with the moisture supply, being high in the first cutting when the soil moisture was ample and being lower when the plant was growing under stress of moisture deficiency.

The data in Table 6 are the averages of four replications of each treatment. Each replication was sampled at random and duplicate analyses were made upon each plot sample. The yield

Table 6. Effect of fertilizer treatments on alfalfa grown in the field. Means of four replications.

Treatment	Carotene mg/100 gms dry wt. Cuttings I	Carotene mg/100 gms dry wt. Cuttings II	Protein % dry wt. Cuttings I	Protein % dry wt. Cuttings II	Yield Lbs/plot wet wt. Cutting II
B	21.1	27.8	17.5	15.2	7.33
Ca	21.6	27.0	18.0	16.2	8.07
Mg	20.5	26.6	18.5	15.5	7.56
N	21.8	26.8	17.9	14.8	8.01
P	21.4	29.2	18.2	15.5	8.39
K	22.7	26.6	18.7	15.4	7.70
N-P	19.6	26.4	17.4	15.9	7.34
NK	21.7	27.9	16.2	15.6	7.50
P-K	21.7	27.1	17.4	15.4	8.05
N.P.K.	20.7	27.8	18.0	16.4	8.34
N.P.K.-B	20.7	26.9	18.6	16.1	7.85
N.P.K.-Ca	19.7	27.2	18.0	15.4	8.52
N.P.K.-Mg	20.6	27.9	17.3	15.5	8.34
N.P.K.-B-Ca	20.4	27.9	16.8	15.6	8.66
N.P.K.-B-Mg	20.5	27.1	17.5	15.8	8.38
N.P.K.-Mg-Ca	19.3	27.0	16.4	15.5	7.85
N.P.K.-B-Ca-Mg	19.3	27.3	16.7	16.4	8.68
No treatment	20.3	26.4	18.1	15.4	7.42

Table 7. Analysis of variance of carotene content of alfalfa produced under fertilizer treatment grown in the field.

Factor	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	143	2321.17				
Between replications	3	175.07	58.35	1.08	2.70	3.98
Between cuttings	1	1545.15	1545.15	28.66**	3.94	6.90
Between fertilizer treatments	17	54.03	3.18	.01	1.77	2.24
Interaction cutting x replication	3	161.44	53.81	13.18**	2.70	3.98
Error	119	485.48	4.08			

** Highly significant.

LSD between replication means, 4.13 at 5% level of significance, 6.86 at the 1% level of significance.

LSD between cuttings means, 9.12 at 5% level of significance, 21.04 at the 1% level of significance.

data are the weights of green forage produced on the plots, as it was weighed immediately after cutting. These figures are the means of four plots.

The analysis of variance of the carotene content is found in Table 7. The fertilizer applications caused no significant differences. There was a highly significant difference between cuttings which was attributed mainly to the differences of soil moisture in which the plants were grown.

Table 8 is the analysis of variance for the protein content. It is interesting to note that fertilizers did not cause a significant variation. Between cuttings there was a highly significant difference. The first cutting was produced by ample moisture, and the protein content was high. The protein content was low in the second cutting when the soil moisture was low. There was also a significant difference between replications.

The response to fertilizer treatments as expressed in yield showed considerable difference between the greenhouse and field experiments. The greenhouse yield was affected significantly by fertilizers, but as shown in Table 9, the analysis of variance of yield of the field plots, the fertilizer applications did not show sufficient variation to be significant. This is probably due to the amount of phosphorus and other elements already in the soil.

Because of the wide variation between cuttings, it was decided to consider each cutting separately for further investigation. Even though there was no significance obtained from fertilizer treatment, co-variance analyses were made in an attempt to find out if carotene, protein or yield were affected independently or if the same factors influenced two or more components.

Tables 10, 11, 12, and 13 are the analysis of co-variance tables. It will be noted that in them there is no significant correlation coefficient. This indicates that the yield, carotene content and the protein content are affected by separate sets of factors and that these factors are effective only for the particular component. None of the correlation coefficients had sufficient magnitude to reach the 5 percent level of significance.

Table 8. Analysis of variance of protein content of alfalfa produced under soil fertility treatments grown in the field.

Factor	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	143	278.62				
Between replications	3	10.46	3.48	4.19**	2.70	3.98
Between cuttings	1	144.00	144.00	173.70**	3.94	6.90
Between fertilizer treatments	17	22.93	1.34	1.61	1.77	2.24
Error	122	101.23	.829			

** Highly significant.

LSD between replication means, 1.19 at 5% level of significance, 1.91 at 1% level of significance.

LSD between cutting means, 3.93 at 5% level of significance, 9.00 at 1% level of significance.

Table 9. Analysis of variance of yield of alfalfa produced under fertility treatments grown in the field.

Factor	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	71	167.6				
Between fertility treatment	17	20.2	1.18	.803	1.86	2.40
Between replication	3	73.6	24.53	16.67**	2.79	4.20
Error	51	73.8	1.47			

** Highly significant.

LSD between replication - 5% level = 2.37 1% level = 3.94

Table 10. Analysis of co-variance of protein and carotene content of alfalfa produced under fertilizer treatment in the field (Cutting I).

Factor	Sum of squares		Sum of product	Degrees of freedom	r	Significance of r	
	Protein	Carotene				(P=.05)	(P=.01)
Total	134.62	541.58	60,04	70	.765		
Between fertilizer treatment	37.08	59.75	17.92	16	.38	.468	.590
Between replications	14.29	305.43	.02	2	.002	.950	.990
Error	83.25	176.40	42.11	50	.347		

r = Correlation coefficient.

Table 11. Analysis of co-variance of protein and carotene content of alfalfa produced under fertility treatments in the field (Cutting II).

Factor	Sum of squares		Sum of product	Degrees of freedom	r	Significance of r	
	Protein	Carotene				(P=.05)	(P=.01)
Total	40.30	234.44	13.75	70	.141	.232	.302
Between fertilizer treatment	12.28	34.54	.54	16	.026	.468	.590
Between replications	.86	31.09	3.33	2	.644	.950	.990
Error	27.16	168.81	9.88	50	.146		

r = Correlation coefficient.

Table 12. Analysis of co-variance of carotene and yield of alfalfa produced under fertility treatments in the field (Cutting II).

Factor	Sum of squares		Sum of product	Degrees of freedom	r	Significance of r	
	Carotene	Yield				(P=.05)	(P=.01)
Total	234.44	167.60	85.09	70	.429	.232	.302
Between fertilizer treatment	34.54	20.20	11.14	16	.422	.468	.590
Between replications	31.09	73.60	27.34	2	.571	.950	.990
Error	168.81	73.80	46.61	50	.417		

r = Correlation coefficient.

Table 13. Analysis of co-variance of protein and yield of alfalfa produced under fertility treatments in the field (Cutting II).

Factor	Sum of squares		Sum of product	Degrees of freedom	r	Significance of r	
	Protein	Yield				(P =.05)	(P=.01)
Total	40.30	167.60	12.72	70	.154	.232	.302
Between fertilizer treatment	12.88	20.20	5.50	16	.349	.468	.590
Between replications	.86	73.60	2.03	2	.255	.950	.990
Error	27.16	73.80	9.25	50	.206	.273	.354

r = Correlation coefficient.

DISCUSSION

The carotene content of alfalfa does not seem to be affected by applications of fertilizer.

Many workers have found that carotene content of grassland herbages have been increased by the application of nitrogenous fertilizers. Smith and Wang (1941), Thomas and Moon (1938), and Moon (1939) have postulated that with a leguminous crop these increases would probably not be evident. The experimental work of this report seems to bear out the latter statement. Alfalfa, being a leguminous plant, would not be expected to have the carotene content increased by the application of nitrogenous fertilizers.

Plants grown in the pots in the greenhouse did not respond with increased carotene content to any of the fertilizer applications. These same plants, however, showed marked differences in carotene content between the two soil moisture levels, the lower soil moisture level produced the higher carotene content. This is similar to results obtained by Hackerott (1946) using a similarly designed experiment.

The plant series receiving fertilizer elements N.P.K. were found to have significantly higher carotene than the series from which they were withheld.

At the time of the harvest there was no distinguishable difference in the stage of maturity, as influenced by fertilizer applied or by soil moisture treatments.

In field plots the carotene and protein content of alfalfa was not influenced by fertilizer applications. There was, however, great variation between the two cuttings. Atkeson (1937) and Moon (1939) report that the normal seasonal variation of carotene content is higher in the spring followed by a drop with the approach of hot weather and a gradual rise again in the fall as the weather becomes cooler. This was also found to be true by Hackerott (1946). In this experiment the above pattern did not hold true, due primarily to the severe summer drought encountered by the second cutting. The first cutting grew under ample moisture supply and the second cutting grew under limited moisture supply, which resulted in the second cutting having higher carotene than the first cutting. The climatic conditions that prevailed and the data obtained in the greenhouse soil moisture studies seem to indicate that moisture relationships were responsible for the increase in the carotene content of the second cutting. The protein content was affected in the opposite manner. It was higher the first cutting with adequate soil moisture, and decreased the second cutting as the soil moisture decreased.

The material herein presented is not to be confused with total production, for as Hamner (1945) stated, the highest yield of forage is likely to also have the highest vitamin yield per unit area, which seems to hold true in these experiments also.

From the results of this investigation there appears to be no direct relationship between carotene and protein. They react independently to various stimuli, hence the protein content is not a reliable index of the carotene content.

SUMMARY

This experiment was conducted to determine, if possible, the effect of soil amendments and soil moisture on the carotene and protein content and forage yield of alfalfa.

It was found that the carotene content and the protein percentage were not affected by the application of fertilizers.

There was a marked and consistently higher carotene value obtained from plants receiving inadequate moisture for maximum growth.

Total yield was greatly increased by adequate moisture over the yield from inadequate moisture, and to a lesser degree by the presence of N.P.K. High moisture and N.P.K. application resulted in the highest yield. There was no relationship of the magnitude of statistical significance found among yield, carotene and protein.

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