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The Carotene (Provitamin A) Content of Oklahoma Feeds

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FOREWORD

To the livestock feeder, a vitamin A deficiency means less gain in weight per pound of feed, more sick animals, greater mortality, a lower grade of finished stock, and less tractable animals. The net result is, of course, a lower profit, or even a loss. The problem of what feeds are high in carotene (provitamin A) and how the carotene is best maintained during storage is thus of considerable practical importance.

The results of investigators in other areas are not directly applicable to the forage crops of this state, because of Oklahoma's extremely variable type of climate, with long growing seasons, relatively long hours of sunshine, high temperatures, and dry atmosphere.

Therefore an investigation of the carotene content of Oklahoma feeds has been undertaken, and the results are summarized below:

SUMMARY

1. The carotene content of several Oklahoma feeds has been found to have about the same range of values as found in other sections of the country, with a somewhat greater variation due to the more varied type of climate characteristic of Oklahoma.

2. The carotene content of a plant varies with the state of maturity in accordance with the rapidity with which the plant is growing; i. e., the carotene content increases to the time of blooming, and then decreases.

3. The carotene content of a plant is influenced by the season in accordance with the flavorableness of that season for growth; i. e., in the spring and fall, when the moisture and temperature are right for rapid growth, the carotene content of plants will be relatively high, while in the summer, when growth is retarded by the hot dry weather, carotene values will be low.

4. The exposure of a forage to the sun and weather in field curing should be as brief as possible, as these are the chief factors in the destruction of carotene in a feed.

5. For a hay correctly made, the carotene value will be about half that of the green forage and will drop to about one-third of that value at the end of six months storage under good conditions.

6. The carotene of silage appears to be quite stable over long periods of time, and may be expected to be not less than half the original value at the end of six months storage.

7. The green chlorophyll color of a feed is a useful qualitative index of the carotene content of that feed.

8. The methods and principles proposed for the preparation of a feed of high carotene value are identical with those uniformly recommended by agricultural experts for the curing of roughages with the minimum loss of nutritive value and palatability. Thus the feeder who follows the precautions recommended for the preparation of feeds of high carotene content will reap two-fold benefit; in addition to having a feed of higher carotene value he will also have a feed of higher actual food value.

9. The carotene content of a dried feed is subject to so many factors contributing to its variability that it is very difficult to predict accurately what it will be. Besides the influence of the stage of growth, climatic conditions and soil fertility have an effect upon the concentration of carotene in a green forage. The most important factor in determining what the carotene content of a hay will be is the curing of the forage, with the protection given the hay in storage and the time of storage subsequently entering in.

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INTRODUCTION

The mass of literature dealing with the subject of vitamins which has been written during the past twenty-five years is undisputed evidence of the popularity that this subject has found in the minds of scientific workers, doctors, farmers, feeders, and the public as a whole. Behind this literature is an almost incredible amount of research defining the physiological significance, occurrence, and chemistry of vitamins. It is obvious that a class of food material which has received so much widespread attention must be of great nutritional importance. This significance lies not in the energy-giving or body-building properties of vitamins (combined they comprise only a ten-thousandth part or less of the diet), but rather in their control and regulation over the body processes by which the proteins, carbohydrates, fats, and minerals which comprise the energy-giving and body-building foods of the organism are absorbed and utilized. Without the regulation afforded by the vitamins, growth is stunted and abnormal. The body is deformed, intelligence is dulled, nervous disturbances are encountered, the resistance to disease and infection is much lessened, and specific deficiency diseases occur. To the livestock feeder, a vitamin deficiency means less gain in weight per pound of feed, more sick animals, greater mortality, a lower grade of finished stock, and less tractable animals. The net result is, of course, a lower profit, or even a loss.

The field of vitamin study was opened twenty-five years ago with the discovery of vitamin A; and in succeeding years none of the additional vitamins subsequently discovered has been proven to be of greater importance. Long before the existence of vitamins was even suspected, carotene was known as an intensely yellow plant pigment occurring in close association with chlorophyll; but its nutritional significance as the plant form of vitamin A, and as the cause of the intense growth-promoting activity observed in green plants having little or no vitamin A, was not definitely proven until ten years ago. Now it is known that carotene is the form in which vitamin A occurs in plants almost exclusively. It is transformed into the vitamin in the liver of an animal by an enzyme, and all its physiological activity occurs by reason of the vitamin A into which it is transformed.

Carotene Essential for Livestock Profits

Vitamin A, or carotene, is essential for growth, vision, and the maintenance in normal condition of certain specialized tissues of the body, and has considerable effect upon the maintenance of a high body resistance to disease and infection, thereby keeping an animal in a healthy condition. Deficiency will result in night blindness, a temporary condition resulting from lack of sufficient vitamin A to meet the requirements of the visual process; and this, in turn, will progress into xeropthalmia, which involves a morbid degeneration of the tissue of the eyes, with permanent blindness as the final result.

An adequate supply of carotene in the feed of livestock is therefore of primary importance to the feeder. It assures the efficient utilization of a feed and rapid and normal growth of healthier livestock, thereby giving both a greater gain in weight per pound of the relatively costly rations used in preparing livestock for market, and a better grade animal commanding a

higher price on the market. It also provides the indirect saving attendant upon the greater resistance to disease and infection and the prevention of specific conditions characteristic of avitaminosis A. By keeping the exposure of a forage to the action of weather and sunshine as small as possible in the preparation and preservation of a roughage, a livestock feed of fully adequate carotene content can be secured without added expense and with very little additional trouble.

The problem of what feeds are high in carotene and how the carotene is best maintained is thus of considerable practical importance. This problem has received much attention in New Jersey (9), Texas (2), Kansas (6), California (4), and at other agricultural experiment stations. Workers have determined the carotene content of the various feeds characteristic of their state, and the reaction of this carotene content to various methods of curing and storage has been investigated.

Oklahoma Conditions Require Special Study

The results of investigators in other areas are not directly applicable to the forage crops of this state, because of Oklahoma's extremely variable type of climate, with long growing seasons, relatively long hours of sunshine, high temperatures, and dry atmosphere. For these reasons it has seemed appropriate to make a study of the carotene content of those naturally occurring hays, grasses, and forage crops which our feeders are dependent upon. In this study, attention was given to the effect upon carotene content of the season of the year, the method of cutting and drying, and the manner of preservation, in an effort to find what conditions are productive of high initial content and good maintenance of carotene in the feeds of the state.

Method of Analysis

Carotene was determined by the Peterson, Hughes and Freeman revision of the Guilbert method (7). In this method, the plant material is heated with a strong ethyl alcoholic solution of a caustic alkali to disintegrate the tissue and thus insure a complete removal of the carotene in the subsequent extraction with petroleum ether. The petroleum ether is then washed free of coloring material other than carotene by repeated extraction with aqueous methyl alcohol, and finally dried over anhydrous Na_2SO_4 and made to volume.

This method was selected from the literature as being the simplest and most rapid of the methods described. Its accuracy has been substantiated by the investigation of Munsey (5). The concentration of carotene in the petroleum ether solution was read in a Bausch and Lomb spectrophotometer, giving a marked advantage over a colorimeter in both ease and accuracy of reading.

A spectrophotometer is a special type of colorimeter designed to measure the intensity of a color in a single narrow band. As the yellow color of carotene is formed by the absorption of blue, it is evident that a given quantity of carotene will produce a much greater effect in the blue than in the red, yellow, and green portions of the spectrum, and that an instrument, the spectrophotometer, which can selectively estimate this effect in the blue will be more sensitive and accurate than the usual colorimeter, which depends upon the average effect over the entire visible spectrum.

The samples used in this investigation were all hand-harvested from pastures and cultivated areas in and near Stillwater, Oklahoma. In every case the sample was cut when free of dew or rain, placed in a paper bag, and brought immediately to the laboratory. A weighed sample was dried to determine its moisture content, and the carotene determined at once upon the green material. The moisture content was established so that all analyses of the samples could be referred to the same basis, and the carotene values would thus be comparable. One portion of the sample was then dried

in the sun to prepare a hay comparable to field-cured hay, and another portion dried in the laboratory out of direct sunlight to produce a hay that, when compared with the sun-dried material, would give an indication of the effect of sunlight upon the carotene of forage. These dried samples were ground and placed in sealed glass jars, and stored upon shelves in the laboratory. Portions of several samples were also stored in a dark refrigerated room where the temperature was never in excess of 5° F. in order to determine the effect of storage temperature upon carotene decomposition.

Samples from the same source were taken from time to time throughout the growing season, in order that the relative potencies could be established for the season of the year as well as for the manner of curing. Samples were taken at the growth stages at which feeders would cut the plants for forage purposes.

Samples of silage were taken at the shredder as it was elevated into the silo.

The stored samples were analyzed at intervals in order to ascertain the effect of time of storage upon the destruction of the vitamin.

In summary, carotene determinations were made upon samples as follows:

Green samples.

Green samples stored in alcohol at 0° C.

Samples immediately after drying in the sun.

Samples immediately after drying out of direct sunlight.

Samples dried in the sun and stored at room temperature.

Samples dried in the sun and stored at 5° F.

Samples dried out of direct sunlight and stored at room temperature.

Samples dried out of direct sunlight and stored at 5° F.

Silages.

Samples from the same source were taken at intervals during the growing season. Stored samples were analyzed at intervals during the period of storage.

CAROTENE CONTENT OF OKLAHOMA FEEDS

Effect of Drying and Storage

Table I summarizes most of the data that have been obtained. These data demonstrate that there is less destruction of the carotene of material dried in semi-darkness than of material dried in the sun, and that this advantage continues throughout the storage of the sample. In connection with the method of drying, it has been reported (9, 3) that in machine drying the carotene content is maintained at nearly the value of the green forage. Apparently, carotene destruction in drying is roughly dependent upon the length of time during which hay is exposed to drying conditions, and the main factor determining the relative carotene content of the finished hay is the length of exposure of the hay to the drastic conditions of curing.

In Table II it is shown that the carotene content of dried meals stored for approximately a year at 5° F. is maintained with but little change as compared with the carotene content of meals stored at room temperature for the same length of time. This method of preservation is without practical value for forages, but it does illustrate the effect of storage temperature upon carotene decomposition.

*TABLE I.—The Carotene Content of Oklahoma Feeds.**

Kind of Feed	Date run	Carotene of green feed (p.p.m.)	Method of drying	Carotene of dried feed (p.p.m.)	CAROTENE OF STORED DRIED FEED, IN P. P. M., ON THE DRY BASIS AT APPROXIMATE PERIODS			
					1 mo.	3 mo.	6 mo.	1 yr.
Alfalfa	6/23/37	207.3	Sun	80.9	54.3	38.7	25.5	3.7
Alfalfa			Dark	106.5	72.1	50.3	36.5	5.9
Alfalfa	4/ 6/38	337.3	Sun	141.3	103.5	78.5	47.8	
Alfalfa	5/16/38	252.7	Sun	107.8	89.3	57.0	28.2	
Alfalfa	7/26/38	209.8	Sun	78.5	54.7	38.8	27.6	
Alfalfa	9/26/38	391.4	Sun	143.2	88.9	67.3	36.5	
Bermuda	6/ 1/37	225.5	Sun	101.9		45.3		4.8
Bermuda			Dark	127.5		54.2		6.2
Bermuda	7/28/37	223.8	Sun	93.2		58.7	30.3	4.2
Bermuda			Dark	123.4		73.5	43.4	5.8
Bermuda	4/ 6/38	394.3	Sun	175.3	128.7	81.2	40.7	
Bermuda	5/16/38	373.0	Sun	184.5	147.5	112.5	46.7	
Bermuda	7/26/38	258.5	Sun	121.2	87.3	67.3	24.1	
Bermuda	9/26/38	386.3	Sun	158.4	91.3	70.7	41.2	
White Clover	6/ 1/37	185.6	Sun	61.2	46.4		24.1	3.4
			Dark	88.3	65.4		35.6	4.9
Sweet Clover	6/30/37	190.3	Sun	98.7		66.5		5.3
			Dark	139.8		89.7		7.2
Atlas Sorghum	6/ 9/37	99.5	Sun	55.6			20.3	
			Dark	71.2			38.4	
Sorghum	4/ 6/38	164.8	Sun	57.3	42.0	28.7	17.5	
Sorghum (mature)	9/26/38	76.6	Sun	42.5	38.5	26.3	16.1	
Rye Grass	4/ 6/38	318.3	Sun	135.2	81.9	62.3	31.7	
Rye Grass	5/16/38	213.8	Sun	104.5	77.8	58.4	24.8	
Johnson Grass (seed stage)	6/30/37	74.8	Sun	43.2			4.2	
			Dark	57.5			8.9	
Johnson Grass (seed stage)	5/16/38	117.0	Sun	62.5	43.7	32.7	20.1	
Johnson Grass (seed stage)	9/26/38	69.0	Sun	38.7	27.3	18.7	11.2	
Big Blue Stem	7/21/37	116.6	Sun	48.3			9.6	
			Dark	69.4			17.6	
Little Blue Stem	7/21/37	96.3	Sun	45.2			12.7	
			Dark	63.1			20.8	
Switch Grass	7/21/37	77.3	Sun	39.3			11.6	
			Dark	47.8			13.7	
Soy Beans (pod stage)	8/14/37	106.9	Sun	48.6			23.4	6.2
			Dark	61.2			40.7	9.3
Soy Beans (young)	9/26/38	251.3	Sun	139.3	122.7	84.7	61.6	
Mung Beans (bud stage)	8/ 4/37	192.6	Sun	102.4			47.9	5.3
			Dark	121.1			63.4	7.5
Mung Beans (bud stage)	9/28/38	249.3	Sun	154.3	126.5	82.1	49.7	
Mung Beans (pod stage)	9/28/38	98.7	Sun	64.6	51.3	36.5	24.2	
Wheat	4/ 6/38	298.3	Sun	126.1	86.9	77.8	43.2	
Wheat	5/16/38	181.3	Sun	96.7	79.6	61.3	28.9	

* All carotene values presented in the tables in this bulletin are stated in parts per million (p. p. m.) on the dry basis. To convert to U. S. P. XI Units and/or International Units of vitamin A per gram of dried material divide the p. p. m. value by 0.6. To convert to U. S. P. XI Units and/or International Units of vitamin A per pound of dry material multiply the p. p. m. value by 756.

TABLE II.—Stability of the Carotene of Dried Meals Stored at 5° F.

Kind of feed	Date run	Carotene of green sample (p.p.m.)	Method of drying	Carotene of dried sample (p.p.m.)	STORAGE AT ROOM TEMP.		STORAGE AT 5° F.	
					Storage (days)	Carotene (p.p.m.)	Storage (days)	Carotene (p.p.m.)
Alfalfa	6/23/37	207.3	Sun	80.9	347	3.7	342	63.4
			Dark	106.5	347	5.9	342	87.6
Bermuda Grass	6/ 1/37	225.5	Sun	101.9	369	4.8	364	80.2
			Dark	127.5	369	6.2	364	98.7
Bermuda Grass	7/28/37	223.8	Sun	93.2	311	4.2	307	65.6
			Dark	123.4	311	5.8	307	95.2
White Clover	6/ 1/37	185.6	Sun	61.2	369	3.4	364	42.7
			Dark	88.3	369	4.9	364	61.3
Sweet Clover	6/30/37	190.3	Sun	98.7	338	5.3	333	65.2
			Dark	139.8	338	7.2	333	106.5
Soy Beans (pod stage)	8/ 4/37	106.9	Sun	48.6	327	6.2	298	36.3
			Dark	61.2	327	9.3	298	47.5
Mung Beans (bud stage)	8/ 4/37	192.6	Sun	102.4	358	5.3	298	72.3
			Dark	121.1	358	7.5	298	91.5

Effect of Season

It has been observed in this laboratory that the carotene content of a forage varies from high in spring to low in midsummer and back to high in the fall in accordance with the seasonal variation of climatic conditions from warm and moist in the spring to hot and dry in the summer and back to warm and moist in the fall.

This observation is demonstrated in Table III, A and B, and is supported by the findings of other laboratories (1, 8).

TABLE IIIA.—Variation of the Carotene of Alfalfa With the Season.

Date	Stage of growth	Carotene (p.p.m.)	Seasonal condition
4/ 6/38	4″ high	373.3	Spring
5/16/38	First cutting	252.7	Later spring—warmer and drier
7/26/38	Summer cutting	209.6	Summer
9/26/38	Fall growth	391.4	Fall—fall rains begun

TABLE IIIB.—Variation of the Carotene of Bermuda Grass With the Season.

Date	Carotene (p.p.m.)	Seasonal condition
4/ 6/38	394.3	Spring
5/16/38	373.0	Later spring—warmer and drier
7/26/38	258.5	Summer
9/26/38	386.3	Fall—fall rains begun

Effect of Stage of Growth

As shown in Table IV, there is considerable variation of carotene content with the stage of growth, in that the carotene content of a plant parallels the rapidity with which the plant is growing. This parallelism of carotene content and rapidity of growth may be explained by the fact that carotene is formed by a photochemical reaction concurrently with chlorophyll (10), and is thus formed most rapidly when the growth of the plant is most rapid. This correlation of chlorophyll and carotene allows the green chlorophyll color of a plant to be used as a qualitative index of the relative quantity of carotene in the plant; i. e., the greener the plant the higher its carotene value will be, and, conversely, plants which have faded or yellowed will be low in carotene content. The bleaching of the chlorophyll in a hay also appears to approximate rather roughly the decomposition of the carotene in the plant, both in the curing of the hay and in the storage of the dried hay.

Carotene of Silages

The values given for silages in Table V indicate that the carotene of silage is relatively stable over long periods of time. This conclusion is supported in the literature. There is evidence that the petroleum ether soluble yellow material used as an index of carotene content in the analysis consists to some extent of substances other than carotene in the case of silage; however, the carotene content of silage is well preserved upon storage, and ensiling a feed is an excellent method of preservation from the vitamin A standpoint (6).

Carotene of Chicken Mashes

In the analysis of several chicken mashes in this laboartory it was observed that in those mashes having an appreciable carotene content there was present some component of rather high carotene value, as alfalfa leaf meal, while the basic cereal mashes had a carotene content of negligible value. The inclusion of some carotene-containing supplement in chicken mashes is therefore advisable.

CONCLUSION

The range of carotene values presented in these tables do not differ appreciably from values found in other states, except for a greater variability; and the conclusion that in order to secure a high carotene content a hay should be cured and removed from exposure to sunlight and weather as rapidly as possible is also supported. The range of carotene values which may be expected in this state are summarized in Table VI.

The high values were found with young, green, fast growing plants, grown in the spring or fall when moisture was plentiful. The low values were for more mature plants, and plants grown during the summer when rainfall was slight and the temperature high. These values were, in all cases, for plants that were still green, and had not been seriously wilted by exposure to sunlight or drought.

TABLE IV.—Variation of Carotene With Stage of Growth.

Kind of feed	Stage of growth	Date run	Carotene content (p.p.m., dry basis)
Sorghum	Young	4/ 6/38	164.8
Sorghum	Mature	9/26/38	76.6
Johnson Grass	Young	5/16/38	117.0
Johnson Grass	Seed Stage	9/26/38	69.0
Johnson Grass	Seed Stage	6/30/37	74.8
Soy Beans	Young	9/26/38	251.3
Soy Beans	Pod Stage	8/ 4/37	106.9
Mung Beans	Young	9/28/38	249.3
Mung Beans	Bud Stage	8/ 4/37	192.6
Mung Beans	Pod Stage	9/28/38	98.7
Wheat	Young	4/ 6/38	298.3
Wheat	Started to Head	5/16/38	181.3

TABLE V.—Carotene Content of Silages.

(p. p. m., Dry Basis)

Kind of silage	Date ensiled	Carotene when ensiled	Time of storage (months)	Carotene of silage
Corn—poor grade, burnt, H_2O added	July 1937	20	22	12.9
Kafir—shocked and headed	Dec. 1938	20	6	8.6
Mung Bean—pod stage	Oct. 1938	98.7	8	83.8
Cane—early maturity	Aug. 1938	108.1	7	46.4

TABLE VI.—Range in Carotene Values of Oklahoma Feeds.

(p. p. m., Dry Basis)

	CAROTENE GREEN		CAROTENE DRIED		CAROTENE 6 MO. STORAGE	
Kind of feed	High	Low	High	Low	High	Low
Bermuda	375	225	185	100	85	30
Alfalfa	390	200	200	80	90	20
Sorghum	165	75	90	35	40	10
Johnson Grass	90	60	50	20	20	7
Soy Beans	250	100	140	45	120	25
Mung Beans	200	100	100	45	70	25
Wheat	300	180	140	70	80	25
White Clover	185	90	65	45	25	15
Sweet Clover	100	80	55	35	30	15
Big Blue Stem	120	70	60	30	20	7
Little Blue Stem	100	65	55	30	20	7
Switch Grass	80	50	45	25	15	5
Rye Grass	320	200	160	105	70	35

APPENDIX:

Storage of Samples in Alcohol

In several cases a weighed sample of green material was placed in aldehyde-free alcohol and stored at 0° C. for later carotene determinations in order to determine whether or not the alcohol-preservation commonly used as a method of storing plant tissue until it can be conveniently analyzed is suitable for use in carotene determinations.

Table VII demonstrates the carotene destruction in green hays stored in alcohol at 0° C., and illustrates that this method of preservation is not suitable for the storage of samples for carotene determinations until the analysis can be conveniently made.

TABLE VII.—Carotene Content of Samples Stored in Alcohol at 0° C.

Sample	Date run	Carotene of green sample (p.p.m.)	Period of storage (days)	Carotene of alcohol-stored sample (p.p.m.)
Alfalfa	6/23/37	207.3	164	60.6
Atlas sorghum	6/ 9/37	79.5	180	49.7
Little blue stem	7/21/37	96.3	165	68.7
Soy beans (pod stage)	8/ 4/37	106.9	160	39.2
Mung beans (bud stage)	8/ 4/37	192.6	160	32.5

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