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A. B. CONNER, DIRECTOR

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DIVISION OF CHEMISTRY

Losses of Vitamin A and Carotene From Feeds During Storage

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS

T. O. WALTON, President

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Some manufacturers of mixed feeds, having recognized that some of the commercial mixed poultry feeds did not carry enough vitamin A potency, have begun to correct these deficiencies by adding fish liver oils, fish liver oil concentrates or solutions of carotene in oil, or yellow corn or alfalfa leaf meal of high potency. Since vitamin A and carotene are both unstable, it is important to know whether these substances would remain in commercial mixed feeds long enough to have the desired effect.

Cod liver oil, other fish liver oils, concentrates of cod liver oil, and carotene dissolved in oil were added to various feed mixtures, the mixtures stored in several different ways and examined for vitamin A or carotene after definite intervals of time had elapsed. It was found that practically all the vitamin A, added in the form of cod liver oil, other fish oils, or cod liver oil concentrates, was lost after four weeks of storage. When hydroquinone equal to 0.1% of of the feed was used as a stabilizer, the vitamin A did not disappear so quickly, but even then most of it was lost after 3 weeks. The use of fish liver oils in a commercial mixed feed for the purpose of supplying vitamin A appears to be of little or no value, since most of the vitamin A may disappear before the feed is used. If such oils are mixed in a feed to supply vitamin A, the mixture should all be used in 10 days after it is made up, to avoid serious losses of vitamin A.

The carotene in solution in vegetable oil after being added to feeds was more stable than vitamin A in cod liver oil, especially when the mixture was stored at low temperatures. At a temperature of 42-49°F. only 3 to 6% of the carotene was lost in 8 weeks. However, at room temperatures of 77-82°F., from 7 to 27% of the carotene was lost in 4 weeks and from 12 to 53% in 8 weeks.

Carotene in alfalfa products and cryptoxanthin in yellow corn were also found to be unstable, though they were not lost as rapidly as carotene dissolved in oil. At high temperatures there was considerably more loss than at low temperatures. The method of storage had considerable effect on the loss. Large compact samples of the feeds lost carotene at a less rapid rate than small samples loosely packed. Alfalfa leaf meal stored in tightly packed vials at refrigerator temperatures lost only from 0 to 3% carotene per month. Mixtures of feeds with carotene are likely to lose part of the carotene when stored under ordinary conditions, so that when the mixture is fed, the animals will not receive the quantity of carotene originally placed in the feed. Being more stable, carotene is a better source of vitamin A potency than cod liver oil for mixing with feeds. However, most of the carotene may be lost unless the mixture is fed within two or three months after it is made.

CONTENTS

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LOSSES OF VITAMIN A AND CAROTENE FROM FEEDS DURING STORAGE

G. S. FRAPS, Chief, and A. R. KEMMERER, Assistant Chemist,
Division of Chemistry

Vitamin A has been known for some time to be necessary for the life and good health of animals. If the quantity in the diet of young animals is too small, they will stop growing in a short time and then begin to lose weight. With an insufficient quantity of vitamin A, young animals may grow slowly but not make a normal growth. Night blindness is a symptom of a deficiency of vitamin A (15). Animals suffering from this trouble cannot see well at night and may run into all kinds of obstacles. Sore eyes are another symptom of deficiency of vitamin A. An animal having a deficiency of vitamin A becomes weak and susceptible to respiratory troubles and other diseases. A symptom of extreme deficiency in steers and dairy cows is convulsions (16).

An adequate supply of vitamin A is necessary for the growth of young animals (27), for the production of eggs (26), and for a good production of milk (5) or healthy offspring (3). High quantities of vitamin A potency must be fed if cows are to produce milk (5) or if hens are to produce eggs (26) high in vitamin A. Sherwood and Fraps (26) have pointed out that the ration of laying hens should contain about 3 to 7.5 Sherman-Munsell units of vitamin A units per gram. Fraps, Copeland, and Treichler (5) have shown that the requirements of dairy cows for vitamin A are large. The possibility of a deficiency of vitamin A in the diet of range animals has been pointed out by Guilbert and Hart (12), while Converse and Meigs (3) have shown that such deficiencies may occur with dairy cows fed upon low-grade roughages. Vitamin A has a greater importance in animal feeding than it was formerly supposed to have.

Vitamin A is a colorless substance which occurs in fish liver oils, butter, eggs, and the livers of various animals. Carotene is a yellow substance which has vitamin A potency and occurs in alfalfa, carrots, sweet potatoes, and other yellow or green plants or plant products. Cryptoxanthin is a yellow substance having vitamin A potency which occurs with carotene in yellow corn. Other yellow-colored substances occur in plants but have no vitamin A potency. Carotene eaten by animals may be converted into vitamin A which can be stored in the animal body, chiefly in the liver. Animals which receive abundant supplies of carotene (or vitamin A) may store enough vitamin A in the liver to last for several months even though the feed used later is deficient in this vitamin (14).

Since the knowledge of vitamin A has become more and more widespread, and its importance in feeds for livestock more generally recognized, some feed manufacturers have attempted to increase the vitamin A potency of some of their commercial mixed feeds (especially chicken feeds) by additions of carotene dissolved in oil, cod liver oils, fish oil concentrates, or

alfalfa meals high in carotene. Feeds often have to be stored over winter or for even longer periods of time. While it is known that both carotene and vitamin A are unstable, it is not known definitely how long either of them will remain in a feed. Therefore, it is a matter of considerable practical importance to determine how stable carotene and vitamin A are in feeds alone, and when they are added to commercial feeds, and in what way they can best be added to a feed to give the greatest vitamin A potency for the greatest length of time. The work presented here attempts to give some of this needed information.

PREVIOUS WORK

Fraps and Treichler (7) have reported appreciable losses of vitamin A potency from alfalfa, dried milk, yellow corn and other materials during storage. Guilbert (10) has shown that carotene in alfalfa decreased 30 to 50% during storage for 8 weeks at room temperature, while at from –5 to 0° C. there was practically no destruction in the same length of time. It has been shown by others (1, 21, 30) that carotene dissolved in oil is unstable. Holmes, Corbet, and Hartzler (18) have reported that the vitamin A in cod liver oil was completely destroyed in 6 weeks if the oil was stored at room temperature in a bottle one-fourth filled with the oil. When the oil contained 0.1% hydroquinone and 0.1% lecithin, 75% of the vitamin remained after 42 weeks of storage. Marcus (20) found that when a cod liver oil concentrate very rich in vitamin A was added to the U. S. P. basal ration used for vitamin A determinations and stored in the dark, 85% of the vitamin A had been destroyed after 10 days. A summary of the results obtained by the above and other workers is given in Tables 1 and 2.

Table 1. Previous work on the stability of vitamin A to storage

Source of vitamin A and treatment	Temperature of storage	Loss per cent	Period of storage	Literature reference
Halibut liver oil. 10 cc. in 40 cc. brown bottle in diffused light	Room	100	21 weeks	18
Halibut liver oil + 1% hydroquinone + 5% lecithin. 10 cc. in 40 cc. brown bottle in diffused light	Room	32	62 weeks	18
Cod liver oil. 10 cc. in 40 cc. brown bottle in diffused light	Room	100	6 weeks	18
Cod liver oil with .1% hydroquinone. 10 cc. in 40 cc. brown bottle in diffused light	Room	54	42 weeks	18
Cod liver oil with .1% hydroquinone and .1% lecithin. 10 cc. in 40 cc. bottle in diffused light	Room	25	42 weeks	18
Vitamin A concentrate added to U.S.P. basal ration for vitamin A assay. Stored in dark	Room	85	10 days	20
Vitamin A concentrate added to hydroquinone. Stored in dark	Room	15	15 days	20
Vitamin A concentrate added to granulated lactose. Stored in dark	Room	90	8 days	20
Vitamin A concentrate added to granulated starch with .1% hydroquinone	Room	5	15 days	20
Unsaponifiable residue of cod liver oil—	Refrigerator			
in peanut oil		100	6 weeks	22
in olive oil		30	6 weeks	22
in coconut oil		0	6 weeks	22
Unsaponifiable residue of cod liver oil with .05% hydroquinone—	Refrigerator			
in peanut oil		0	6 weeks	22
in olive oil		18	6 weeks	22
in coconut oil		5	6 weeks	22
Samples of cod liver oil placed on market for two to four years, not opened	Room	0	.....	4
Cod liver oil irradiated with ultra-violet light	.....	100	8 hours	2
Cod liver oil mixed into a ration containing 20 to 25 per cent very rancid lard	.....	100	Few days	23
Halibut liver oil. 30 cc. in 50 cc. flask exposed to air in diffused light	Room	96	31 days	19
Halibut liver oil with 5% coarsely ground whole oats. 30 cc. in a 50 cc. flask exposed to air in diffused light	Room	94	31 days	19
Salmon oil. 50 cc. in a 125 cc. flask. Exposed to air in diffused light	Room	63	31 days	19
Salmon oil with 5% coarsely ground whole oats. 50 cc. in a 125 cc. flask. Exposed to air in diffused light	Room	0	35 days	19

Table 2. Previous work on the stability of carotene to storage

Source of carotene and treatment	Temperature of storage	Loss per cent	Period of storage	Literatur reference
Alfalfa leaves	− 5 to 0° C	0	8 weeks	10
Dehydrated alfalfa leaf meal	− 5 to 0° C	0	8 weeks	10
Alfalfa leaves in dark	20 to 30° C	30.5	8 weeks	10
Alfalfa leaves in dark	60° C	61.9	9 days	10
Alfalfa leaves in dark	80° C	86.8	9 days	10
Alfalfa leaves exposed to sunlight in the field	.....	69.5	8 weeks	10
Alfalfa cured in the field	.....	70–90	From curing	24
Alfalfa hay artificially dried	.....	0	Drying	25
Alfalfa dried in air	.....	67–88	Air drying	25
Alfalfa hay	Barn.....	50–67	7 months	29
Alfalfa leaf meal	Room.....	50	11 months	7
Baled alfalfa at Mesa, Arizona	Summer....	50	3 months	28
Baled alfalfa at Mesa, Arizona	Winter.....	0.0	3 months	28
Baled alfalfa at Mesa, Arizona	All year....	75	12 months	28
Yellow corn, ground	Room.....	85	30 months	7
Yellow corn, unground	Room.....	60	5 months	7
Carotene dissolved in cottonseed oil	4° C	10	2 months	1
Carotene dissolved in cottonseed oil and hydroquinone	4° C	12	2 months	1
Carotene dissolved in sesame oil	4° C	15	3 months	1
Carotene dissolved in olive oil	4° C	28	2 months	1
Carotene dissolved in olive oil and hydroquinone	4° C	20	2 months	1
Carotene dissolved in corn oil	4° C	20	2 months	1
Carotene dissolved in corn oil and hydroquinone	4° C	23	2 months	1
Carotene dissolved in coconut oil	4° C	24	2 months	1
Carotene dissolved in coconut oil and hydroquinone	4° C	10	2 months	1
Carotene dissolved in ethyl laurate	4° C	72	2 months	1
Carotene dissolved in ethyl laurate and hydroquinone	4° C	11	2 months	1
Carotene dissolved in ethyl sebacate	4° C	63	2 months	1
Carotene dissolved in ethyl sebacate and hydroquinone	4° C	11	2 months	1
Carotene dissolved in cottonseed oil 0.46 mg. carotene per cc	Room.....	31	2 months	1
Carotene dissolved in cottonseed oil 0.01 mg. carotene per cc	Room.....	0.0	2 months	1
Carotene dissolved in olive oil	100°	100	12 days	30
Carotene dissolved in olive oil and hydroquinone	100°	25	6 months	30
Carotene dissolved in olive oil	Room.....	100	30 days	30
Carotene dissolved in peanut oil	Room.....	100	4 weeks	21
Carotene dissolved in corn oil	Room.....	27	8 weeks	21
Carotene dissolved in wesson oil	Room.....	20	8 weeks	21
Carotene dissolved in peanut oil in vacuum	37°	44	8 weeks	21
Carotene dissolved in wesson oil in vacuum	37°	15	8 weeks	21
Carotene dissolved in corn oil in vacuum	37°	15	8 weeks	21

These tables show that vitamin A and carotene will be destroyed if the materials containing them are not kept under proper conditions. Light, exposure to air, and ordinary room or high temperatures destroy both of them. A low temperature, protection from light and air and, in case of vitamin A, the use of an antioxidant such as hydroquinone, appear under some conditions to be favorable to the preservation of vitamin A and carotene.

Method of Procedure

To study the stability of vitamin A in feeds, fish liver oils, which are carriers of this vitamin, were mixed with various feeds and stored under different conditions. The amount of vitamin A was determined by means

of the spectrograph at the beginning of the test and at the end of various intervals. Details are given on subsequent pages.

A similar procedure was followed for feed mixtures that contained carotene. A number of samples containing carotene were stored under various conditions. Details of this procedure are given in connection with the discussion of the work. The method used for carotene is described below. Since the method is colorimetric, the results obtained by it may be too high, because products that have the same color and solubility as carotene may be formed from the carotene during storage. In such case, the loss of carotene would be greater than shown by the analysis.

Method for Carotene in Feeds

The procedure used for the determination of carotene is based on the method of Guilbert (11) and is as follows:

1 to 6 grams feed are refluxed 30 minutes with 20 to 120 cc. of saturated alcoholic potassium hydroxide, free from aldehydes and ketones. Care is exercised to keep portions of the sample from collecting on the sides of the flasks. If any material does collect on the side of the flask, it is washed down with alcohol. After refluxing, the contents of the flask are cooled with water, 50 cc. ethyl ether added, and after shaking for a minute and allowing the sediment to settle, the ether-alcohol mixture is decanted into a liter separatory funnel. This extraction is repeated two more times with 15 cc. portions of ether. Then the residue is broken up by shaking first with 5 cc. of 95% ethyl alcohol and then with 15 cc. of ethyl ether. Usually after 2 or 3 additional extractions with ether, no more color is extracted and the residue is discarded.

To the combined ether-alcohol mixtures in the separatory funnel is added 100 cc. of cold distilled water. The alkaline alcohol water solution containing most of the chlorophyllines and flavines separates is drawn off from the bottom of the funnel and is re-extracted by shaking gently twice with ether in another funnel. If an emulsion is formed, it is cleared by adding 1 cc. of ethyl alcohol. The ether extracts are combined and washed with cold distilled water until free from chlorophyllines and alkali. Washing three or four times by pouring the water through the solution and down the sides of the funnel removes most of the alkali. The remainder is removed by gently shaking the ether solution with 25 cc. portions of water until the wash water no longer gives a color with phenolphthalein.

The ether solution containing the combined carotene and xanthophyll is transferred to a flask and the ether distilled off with diminished pressure. The residue containing both carotene and xanthophyll is dissolved in 30 cc. light petroleum ether, added in 3 portions of 10 cc. each, and transferred to a small separatory funnel. The petroleum ether solution is shaken for 2 minutes with 85% methanol to remove the xanthophyll. This extraction is repeated until the methanol layer is colorless. About 5 or 6 extractions with 85% methanol are usually sufficient. If during the first extraction the lower layer is cloudy, it is drawn off and extracted with 20 cc. petroleum ether. This petroleum ether extract is added to the other petroleum

ether fraction and the shaking with 85% methanol continued. After the petroleum ether solution is shaken with 85% methanol, it is shaken with 90% methanol to remove any traces of xanthophyll which still remain. Usually no color is obtained in the lower layer after 2 or 3 extractions with 90% methanol. The petroleum ether layer is finally washed with water, dried over anhydrous sodium sulphate, concentrated in vacuo and made up to 10, 25, or 50 cc., depending on the amount of color in the solution.

The amount of carotene in the petroleum ether solution is estimated by comparing it with 0.1% potassium dichromate. The carotene solution is placed in the left cup of a micro-colorimeter set at a depth of 0.5, 1.0, 2.0, 3.0, or 4.0 cm. according to the density of the color. The depth of the dichromate solution, contained in the right cup of the colorimeter, is varied until the density of color is equal. An average of 8 independent readings in millimeters is taken. This average should be between 4 and 12 mm. of the dichromate solution. By use of Table 3, the millimeter

Table 3. Carotene dissolved in petroleum ether, equivalent in color to a .1% solution of potassium dichromate

Potassium dichromate, mm.	Carotene, parts per million	Potassium dichromate, mm.	Carotene, parts per million
4.0	2.5	8.0	4.9
4.2	2.6	8.2	5.0
4.4	2.7	8.4	5.2
4.6	2.8	8.6	5.3
4.8	2.9	8.8	5.4
5.0	3.1	9.0	5.6
5.2	3.2	9.2	5.8
5.4	3.4	9.4	5.9
5.6	3.5	9.6	6.0
5.8	3.6	9.8	6.1
6.0	3.8	10.0	6.3
6.2	3.9	10.2	6.5
6.4	4.0	10.4	6.7
6.6	4.1	10.6	6.8
6.8	4.2	10.8	6.9
7.0	4.3	11.2	7.3
7.2	4.5	11.4	7.4
7.4	4.6	11.6	7.5
7.6	4.7	11.8	7.6
7.8	4.8	12.0	7.8

depth of the 0.1% dichromate is transformed into parts per million of carotene. Then the parts per million of carotene (c) in the sample are calculated by use of the following formula No. 1:

$$C=PS/GD.$$

P is parts per million carotene (from Table 3). S is volume of solution in cc. G is grams sample and D is depth of carotene solution in cm.

The parts per million of carotene equivalent to the millimeters of dichromate given in Table 3 were ascertained by comparing 0.1% potassium dichromate in a micro-colorimeter against a petroleum ether solution containing 10 parts per million of purified carotene. To purify the carotene, 0.1 gm. of carotene obtained from the S. M. A. Corporation of Cleveland, Ohio, was dissolved in about 2 cc. of chloroform, precipitated by addition

of 15 cc. absolute methanol, the precipitated carotene filtered off and dried in vacuo over concentrated sulphuric acid in as short a time as possible. Ten mg. of this purified carotene was dissolved in 1 cc. of chloroform and diluted to 1000 cc. with light petroleum ether. This solution was placed in the left cup of a micro-colorimeter and the depth of solution set at 2, 7, 10, 12, 15, 17, 20, and 25 millimeters. The 0.1% potassium dichromate was placed in the right cup and the settings at which its color matched the color for the carotene settings determined.

Four carotene solutions and four potassium dichromate solutions prepared at different times were thus examined. The data are tabulated in Table 4. From the average of these data a curve was plotted with mm. of carotene solution as the abscissa and mm. of .1% potassium dichromate as the ordinate, and the values in Table 3 were finally obtained by reading them directly from the curve. These values are for a petroleum ether solution containing the carotene from 1 gram of sample in 1 cc. with the left cup of the colorimeter set at a depth of 1 cm. Formula No. 1 must be used along with Table 3 for any other concentrations and colorimeter setting.

Table 4. Colorimetric comparison of a 0.1% solution of potassium dichromate with petroleum ether containing 10 parts per million of carotene

Colorimeter setting of carotene solution	Colorimetric readings of bichromate solutions				
	First carotene solution	Second carotene solution	Third carotene solution	Fourth carotene solution	Average
mm.	mm.	mm.	mm.	mm.	mm.
2	2.8	2.6	2.8	2.9	2.8
5	8.0	8.0	8.5	8.7	8.3
7	11.2	11.0	11.1	11.3	11.2
10	15.2	14.1	14.3	15.4	14.8
12	17.0	16.3	16.0	17.2	16.6
15	18.0	19.1	18.6	19.2	18.7
17	.....	21.6	20.3	21.4	21.1
20	.....	23.8	23.3	23.5	23.5
25	.....	25.0	25.3	25.7	25.3

The method for carotene described above does not differentiate between the several vitamin A active pigments in feeds. Alpha carotene, beta carotene, gamma carotene, and cryptoxanthin are all estimated as "carotene."

In order to test the method, known amounts of pure carotene were put through the entire procedure with the result that from 91 to 98% of the added carotene was recovered. The method has also been checked with animals in work already published (9). Weighed amounts of carotene were dissolved in cottonseed oil and fed to rats according to the modified Sherman-Munsell method (8) used in this laboratory. One microgram of this carotene was found to be equal to 1.4 Sherman-Munsell units. The amount of carotene in a number of alfalfa meal samples was determined by this method for carotene. These same alfalfa samples were fed to rats and it was found that one microgram of carotene in the alfalfa was on an average equal to 1.4 Sherman-Munsell units.

Losses of Vitamin A from Cod Liver Oil and Other Fish Liver Oils or Concentrates When Added to Feed Mixtures

Cod liver oil or other fish oils are frequently added to commercial chicken feeds for the purpose of supplying vitamin D, and they are also used at times for supplying vitamin A. Since Marcus (20) has reported that 85% of the vitamin A added to a feed mixture was destroyed in 10 days, a further study of the matter was desirable.

Several different kinds of tests were made upon mixtures of feed with oils containing vitamin A. Some experiments were made to ascertain the effect of temperature, of the antioxidant hydroquinone, and of materials (yeast and soy bean oil meal) which have been claimed to contain substances that delay the destruction of the vitamin A. These experiments were made upon small quantities of material.

The desired quantity of cod liver oil was first mixed with 8 grams of the feed and then diluted to 100 grams with white corn meal. The feeds with which the cod liver oil was mixed were white corn meal alone, white corn meal and hydroquinone, yeast, and soy bean oil meal. Some of these mixtures were stored at refrigerator temperature of 6° C., at room temperature (26° C.) and at 35° C. Mixtures exactly the same, except that no cod liver oil was added, were made up and kept under the same conditions. The estimation of vitamin A was made by the spectrographic method used in this laboratory for vitamin A in butter (6). This method, in brief, consists of measuring the density of absorption of light at 328 millimicrons by the unsaponified residue of the feeds by means of a spectrograph, and calculating the total spectro vitamin A by use of an appropriate factor. Since feeds and other substances contain materials that absorb light at the same wave length as vitamin A but are not vitamin A, the total spectro vitamin A had to be corrected for this pseudo vitamin A. This was done by making the estimation of the pseudo vitamin A in the mixtures that did not contain the cod liver oil, at the same time and under the same conditions as the estimation of the total spectro vitamin A in the mixtures containing the cod liver oil. The amount of pseudo vitamin A was then subtracted from the total amount of spectro vitamin A. If there had been a decrease in the pseudo vitamin A in the sample containing no cod liver oil during the storage, the quantity of vitamin A in the sample with which the vitamin A was mixed would have appeared to be greater than it really was; however, the analyses showed that the pseudo spectro vitamin A was practically unchanged during the period of the experiment, and that no appreciable error could be introduced from this source.

The results of this work are given in Table 5. All the feed mixtures lost vitamin A rapidly. Stored at 6° C., the feeds lost from 34 to 74% in 2 weeks; at room temperature, the loss was 73 to 89% in two weeks and 93 to 100% in 4 weeks; at 35° C., the loss was 73 to 100% in 2 weeks. When hydroquinone had been added, there was no loss the first week, but 71 to 84% of the vitamin A was lost at the end of the third week, and from 83 to 87% had been lost at the end of the fifth week. Storing at 6° C. gave a slightly better stability than storing at room temperature or at

35° C. for the first 2 weeks, but no better stability at the end of 4 weeks. At the end of 4 weeks practically all the vitamin A had disappeared from the samples which did not contain hydroquinone at either the high or low temperature of storage.

The use of hydroquinone, which is a powerful antioxidant, even at the high rate of 0.1% of the feed, prevented the destruction of vitamin A only during the first week. After that the destruction was very rapid and the hydroquinone had lost its effect, for nearly all the vitamin A (83 to 87.0%) had been destroyed at the end of the fifth week. The quantity of hydroquinone used was greatly in excess of that usually added to fish liver oils, which is about .01% of the oil. The yeast and the soy bean oil meal like hydroquinone had no practical effect in delaying the oxidation of the vitamin A (Table 5).

Table 5. Stability of vitamin A in mixed feeds

Constituents of feed	Temperature of storage	Vitamin A lost during storage—per cent				
		One week	Two weeks	Three weeks	Four weeks	Five weeks
Corn meal + cod liver oil	6° C	17.2	34.4	.....	90.0	.....
	26° C	30.0	73.3	.....	93.3	.....
	35° C	43.3	73.3	.....	93.3	.....
Corn meal + 8% yeast + cod liver oil	6° C	74.2	74.2	.....	84.0	.....
	26° C	66.7	88.9	.....	100.0	.....
	35° C	59.2	100.0	.....	100.0	.....
Corn meal + 8% soybean oil meal + cod liver oil	6° C	38.0	70.0	.....	100.0	.....
	26° C	40.0	78.5	.....	100.0	.....
	35° C	29.0	100.0	.....	100.0	.....
Corn meal + 0.1% hydroquinone	6° C	0.0	.....	84.0	.....	86.3
	26° C	0.0	.....	87.0	.....	87.0
	35° C	0.0	.....	71.4	.....	82.9

There are different kinds of cod liver oils and cod liver oil concentrates, and also other liver oils, so that there may be differences in the stability of the vitamin A from different sources when added to mixed feeds. Further tests were made, therefore, upon such different kinds of liver oils or concentrates as were obtainable. The procedure was the same as that described above, and the feed used was corn meal. The results of tests on 12 samples are tabulated in Table 6. The vitamin A lost in 2 weeks ranged from 29 to 100%, while in 4 weeks the loss was from 79 to 100%. The rate of loss of vitamin A was somewhat different with different oils, but not sufficient to be of practical importance or to justify the use of any of them in mixed feeds which are to be stored over one to two weeks. We do not consider it necessary to give the names of these products, but the samples represented the various kinds of such materials on the market.

It was considered possible that vitamin A might be less stable in small samples of 100 grams than in larger packages of 10 kilos. Experiments were made to test this point. The procedure was similar to that with the 100 gram samples, except that larger quantities were used. 200 cc. of

cod liver oil high in vitamin A were mixed well with about 1000 grams of white corn meal. This mixture was then diluted to 10 kilos with corn meal, mixed thoroughly in a mechanical mixer, placed in a small sack and stored at room temperature. A smaller sample of 100 grams of the same mixture was placed in an 8 oz. bottle and stored under the same conditions. A sack of 10 kilos of the corn meal without the cod liver oil was stored under the same condition.

Table 6. Loss of vitamin A at room temperature (26° C.) when 12 different oils were added to corn meal

Number of oil	Liver oil or concentrate added per cent	Specto vitamin A in feeds parts per million			Added vitamin destroyed, per cent	
		Beginning	Stored 14 days	Stored 28 days	In 14 days	In 28 days
61005	2.0	1.5	0.2	.....	86.7	.....
62435	2.0	6.8	0.2	.....	97.1	.....
61946	1.0	7.5	0.0	.....	100.0	.....
59875	2.0	2.9	0.9	.....	70.0	.....
43363	0.5	6.2	2.6	.....	58.1	.....
45551	0.5	3.8	2.7	0.8	28.9	78.9
67017	2.0	5.4	2.3	0.0	57.4	100.0
67092	2.0	0.9	0.0	0.0	100.0	100.0
67057	2.0	5.1	2.8	0.3	45.1	94.1
67261	0.5	5.2	2.6	0.1	50.0	98.1
67333	0.5	4.7	0.9	0.0	80.9	100.0
67132	2.0	12.0	6.8	2.4	43.3	80.0

After definite periods of time had elapsed, samples were taken from the large sample with a fertilizer sampling tube. Parts of all the samples were analyzed at the same time. The results from the two mixtures are tabulated in Table 7. There is only a slight difference in the rate of destruction in the large and small sample after 7, 14, and 28 days. These differences are not sufficient to be of significance. The vitamin A in the large samples was no more stable than in the small ones.

Table 7. The effect of the size of the samples upon the loss of vitamin A from corn meal and cod liver oil stored at room temperatures

Size of sample	Vitamin A destroyed in storage —per cent		
	7 days	14 days	28 days
Mixture A. 10 kilo sacks	54.1	70.6	91.9
100 gram bottle	60.9	81.5	89.2
Mixture B. 10 kilo sacks	12.3	76.7	98.6
100 gram bottle	4.6	70.8	100.0

The results presented here show that vitamin A, when mixed with feed mixtures, is rapidly destroyed and is practically all gone at the end of 4 weeks. Other workers have shown that vitamin A in cod liver oil may be destroyed, sometimes in a short time, when left in bottles partly filled with air (Table 1). When these oils are mixed with feeds, they coat the feed particles so that very large surfaces are exposed to air. It is, there-

fore, reasonable that the vitamin A is rapidly destroyed, and that under such conditions, antioxidants would be of no practical value in retarding the oxidation. It would appear that if cod liver oil is to be fed in a mixed feed, it should be mixed with the feed just before feeding, or not over one week to ten days before feeding. The liver oil itself should be stored in completely filled containers kept at a low temperature.

Losses of Carotene from Yellow Corn, Alfalfa Products, and Other Feeds During Storage

Work done in this and other laboratories already referred to (Table 2) shows that carotene is lost from feeds during storage, especially at elevated temperatures. In order to study the matter further, carotene was estimated from time to time in samples which were stored at different temperatures, in small containers and in large quantities.

The results of the analysis of various feeds stored in pint and quart fruit jars are presented in Tables 8 and 9. At room temperature there were losses of 3 to 4% per month in 2 periods, 10 to 13% in 6 periods, and 15 to 35% per month in 6 periods. Thus the losses may be high at room temperatures.

Table 8. Loss of carotene in feeds during storage in fruit jars at room temperature

Laboratory number	Description	Month sample analyzed	Carotene parts per million	Carotene lost, per cent	Approximate rate of loss per month, per cent of total at beginning
41967	Dehydrated alfalfa leaf meal...	July	53.8		
		Oct.	35.6	34	11
42622	Dehydrated alfalfa leaf meal...	Sept.	66.7		
		Oct.	60.0	10	10
41730	Alfalfa meal.....	Mar.	30.6		
		May	16.3	47	24
		July	10.5	66	10
		Dec.	5.4	82	3
41736	Alfalfa meal.....	May	17.2		
		June	13.9	19	19
		July	7.8	55	36
41745	Alfalfa leaf meal.....	May	53.2		
		Oct.	43.3	19	4
41727	Alfalfa meal.....	May	50.0		
		Dec.	5.4	89	13
60960	Alfalfa meal.....	May	9.7		
		July	7.5	23	12
60750	Alfalfa meal.....	April	17.5		
		May	14.6	17	17
41729	Peanut hay.....	May	7.5		
		July	5.3	29	15
41724	Peanut hay.....	May	11.8		
		Oct.	5.4	54	11
41105	Sorghum hay.....	April	5.0		
		May	3.8	24	24

When stored at refrigerator temperature (Table 9) the losses per month were at the rate of 0 to 4% in 12 periods, 5 to 9% in 2 periods and 10 to 14% in 7 periods. The losses are appreciably lower at refrigerator temperature than at room temperature. Later work shows that the higher losses of 10 to 14% may have been due to exposure to warm air and stirring, when the samples were taken out of the refrigerator from time to time in order to secure portions for analysis.

Table 9. Losses of carotene in feeds during storage in fruit jars at refrigerator temperature

Laboratory number	Description Stored in fruit jars at refrigerator temperature	Carotene parts per million	Carotene lost, per cent	Approximate rate of loss per month, per cent of total at beginning
43280	Dehydrated alfalfa leaf meal	63.5	.....	
	Stored 1 month	55.3	13	13
	Stored 2 months	53.2	16	3
43638	Dehydrated alfalfa leaf meal	70.0	.....	
	Stored 1 month	61.3	12	12
	Stored 2 months	55.6	21	9
	Stored 3 months	52.3	25	4
	Stored 4 months	53.8	23	0
43336	Dehydrated alfalfa leaf meal	65.6	.....	
	Stored 3 months	56.3	14	5
44085	Dehydrated alfalfa leaf meal	60.9	.....	
	Stored 1 month	62.3	0	0
	Stored 2 months	52.5	14	14
44048	Alfalfa meal	36.8	.....	
	Stored 1 month	36.8	0	0
	Stored 2 months	36.9	0	0
	Stored 3 months	36.3	1	1
	Stored 4 months	31.3	15	14
	Stored 5 months	32.5	12	0
43576	Alfalfa meal	18.9	.....	
	Stored 1 month	16.8	11	11
	Stored 3 months	12.9	32	10
43204	Alfalfa meal	18.7	.....	
	Stored 1 month	18.3	2	1
	Stored 2 months	17.9	4	2
43789	Alfalfa meal	12.9	.....	
	Stored 4 months	10.7	17	4
44194	Buffalo grass	49.3	.....	
	Stored 2 months	36.3	26	13
44058	Mesquite grass	42.2	.....	
	Stored 1 month	42.5	0	0

In order to study the stability of the carotene when the air was not changed by opening the container, and stirring was avoided, samples of feeds were tightly packed in vials, of about 10 cc. capacity which were tightly stoppered, and stored at refrigerator temperature. The contents of the vials were discarded after a portion was taken from it for analysis. The results of this work are given in Table 10. The losses of carotene per month were 0 to 3% in 19 cases, 5 to 8% in 5 cases, and 12% in one case. These losses were very low.

Table 10. Losses of carotene during storage in tightly packed vials at refrigerator temperature

Laboratory number	Description	Carotene, parts, per million	Carotene lost, per cent
44397	Alfalfa hay meal	25.3	.....
	Stored 2 months	26.6	0
44276	Alfalfa hay meal	29.8	.....
	Stored 1 month	27.3	8
	Stored 3 months	28.3	5
44194	Buffalo grass	25.0	.....
	Stored 1 month	25.8	0
	Stored 3 months	26.3	0
44184	Buffalo grass	4.5	.....
	Stored 1 month	4.5	0
	Stored 3 months	4.4	2
44188	Buffalo grass	16.2	.....
	Stored 1 month	14.2	12
44065	Feather sage	16.2	.....
	Stored 1 month	15.8	2
	Stored 2 months	16.3	0
44482	Oak leaves	118.8	.....
	Stored 2 months	121.3	0
	Stored 3 months	124.0	0
	Stored 4 months	117.5	1
44386	Yellow corn meal	3.3	.....
	Stored 1 month	3.2	3
	Stored 2 months	3.3	0
	Stored 3 months	3.1	6
44387	Yellow corn meal	5.2	.....
	Stored 1 month	5.2	0
44388	Yellow corn meal	4.6	.....
	Stored 1 month	4.6	0
	Stored 2 months	4.3	7
44395	Dehydrated alfalfa leaf meal	38.8	.....
	Stored 1 month	42.5	0
	Stored 2 months	45.0	0
	Stored 3 months	43.8	0
45223	Alfalfa leaf meal	115.0	.....
	Stored 1 month	117.5	0
	Stored 2 months	107.5	7
	Stored 3 months	112.5	2

The storage at refrigerator temperature of feed firmly packed in homeopathic vials seems to be a very good method of preserving the carotene and is suitable for small quantities of feeds to be used for biological assays for vitamin A.

In order to ascertain further the effect of exclusion of air on the stability of carotene, portions of a sample of dehydrated alfalfa leaf meal high in carotene were placed in homeopathic vials, the air displaced with nitrogen and the vials tightly stoppered and stored at refrigerator temperature (6° C.) and 35° C. Other portions of this same sample were placed in 8 oz. bottles, stirred twice a week with a spatula to allow the feed to come into contact with air, and stored at the same temperatures. All these samples were analyzed for carotene after definite periods of time had elapsed. For each analysis of the samples in the vials, an un-

opened vial was used while the large samples were well mixed and a small portion taken for analysis. The data obtained from this experiment are tabulated in Table 11 and show that samples that are stored over nitrogen and not allowed to come into contact with air lose carotene less rapidly than samples that are stirred and aerated. However, storing the samples in nitrogen is only slightly better than storing them firmly packed in vials (Table 10). The effect of a high temperature is shown in Tables 11 and 12. In Table 12 the loss in the first period of one week at 35° C. was from 22 to 38%, in the second week 2 to 9% and in the third period of 2 weeks, 2 to 6%. The rate of loss during a period of 8 months was then about 4% a month with the alfalfa leaf meal and 1% with the corn. In Table 11, the loss at 35° C. was 50 to 57% the first month, 3 to 5% in the second month, and 13 to 16% in the third month, after which it was 3% or less, except in one period. Thus the carotene is lost rapidly when first exposed to a high temperature, and then much more slowly. This is in accordance with most oxidation-reduction processes. There is usually a short inductive period, then a period of rapid oxidation and a final period of slow oxidation. Also the carotene first destroyed may be on the exterior portion of the material, and the carotene destroyed later may be embedded in such a way as to be partly protected by plant material. It is also possible that fairly stable yellow degradation products of carotene may be formed during storage, and since the loss of carotene was estimated by loss of yellow color, there may appear to be a small loss of carotene where there is really a relatively large loss.

The carotene equivalent (as we term it) in yellow corn was destroyed less rapidly than in alfalfa (Table 12). This may be due to the fact that the active pigment in yellow corn consists for the greater part of cryptoxanthin (40 to 70% according to our analysis), while in alfalfa it is practically all beta carotene (17). However, it is to be noted that the carotene in alfalfa meal No. 43070 was more slowly destroyed than that in Sample 43005. The same factors that caused this difference may have operated to a greater extent in the yellow corn, and the cryptoxanthin content may have had nothing to do with the more rapid rate of destruction.

Because of the high vitamin A potency of some samples of alfalfa leaf meal, they are diluted with corn starch in running biological tests, so as to enable the quantity fed to be weighed more exactly. Since it is possible that a greater loss of carotene might take place with these mixtures than with the unmixed feed, the following experiments were made. One part alfalfa meal was mixed with 9 parts of corn starch that had previously been heated at 105° for 24 hours; portions of the mixture were placed in 8 oz. bottles and stored at refrigerator and at room temperatures. For a control equal amounts of the undiluted alfalfa meal were stored in the same manner. The results of the experiment are given in Table 13. The loss of carotene was considerably more rapid in the first 3 months in the diluted samples than in the undiluted samples. During the fourth month, however, more carotene was lost from the undiluted samples than from the diluted samples. It is possible that the carotene may be

Table 11. The effect of storing dehydrated alfalfa leaf mea l in homeopathic vials with the air displaced by nitrogen upon the stability of carotene in alfalfa meal

Description of sample	Temperature of storage	Carotene at start of experiment parts per million	Carotene lost by storage						
			1 month	2 months	3 months	4 months	5 months	6 months	7 months
Sample stored in an 8 oz. bottle and stirred twice a week, total per cent	6° C	170.0	0	5.9	20.6	38.2	42.6	45.0	39.7
Per cent per month			0	6	15	18	4	2	0
Sample stored in a homeopathic vial in nitrogen, total per cent	6° C	170.0	0	5.9	17.6	23.5	26.4	27.9	26.4
Per cent per month			0	6	12	6	3	2	0
Sample stored in an 8 oz. bottle and stirred twice a week with a spatula, total per cent	35° C	170.0	57.3	61.8	78.5	81.9	85.5	86.4	87.4
Per cent per month			57	5	17	3	4	1	1
Sample stored in a homeopathic vial in nitrogen, total per cent	35° C	170.0	50.0	52.9	65.5	74.5	77.5	78.0	78.0
Per cent per month			50	3	13	9	3	1	0

Table 12. Carotene lost in feeds stored at 35° C.—in per cent

Description	Carotene at beginning parts per million	Percentage of carotene lost										
		1 week	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months	9 months
Dehydrated alfalfa leaf meal	200.0	38	40	46	56	64	69	74	78	81	85	88
Alfalfa meal	13.5	22	30	36	40	44	48	52	56	60	64	63
Yellow corn	5.3	34	43	45	46	47	48	49	50	51	52	53
Per cent loss, per period												
Dehydrated alfalfa leaf meal		38	2	6	10	8	5	5	4	3	4	3
Alfalfa meal		22	8	6	4	4	4	4	4	4	4	0
Yellow corn		34	9	2	1	1	1	1	1	1	1	1

Table 13. Effect of diluting alfalfa with starch upon the rate of destruction of carotene

Laboratory number	Description of sample	Temperature of storage	Carotene originally present, parts per million	Carotene lost by storage, in per cent			
				1 month	2 months	3 months	4 months
44752	Alfalfa meal	Room	165.0	24.2	28.8	39.4	51.5
44753	Alfalfa meal diluted 1:9 with starch	Room	11.7	38.4	44.5	63.2	59.0
44754	Alfalfa meal	Refrigerator (6° C)	165.0	12.1	12.1	18.2	36.4
44755	Alfalfa meal diluted 1:9 with starch	Refrigerator (6° C)	11.7	22.2	31.6	44.4	44.4
	Rate of loss per month (per cent)						
44752	Alfalfa meal	.....	.....	24.2	4.6	10.6	12.1
44753	Alfalfa meal with starch	.....	.....	38.4	6.1	18.7	0
44754	Alfalfa meal	.....	.....	12.1	0	6.1	18.2
44755	Alfalfa meal with starch	.....	.....	22.2	9.4	12.8	0

lost more rapidly from a mixed feed than from the same carrier of carotene when not mixed with another feed.

It appears that samples of feed stored in a compact condition lost less carotene than those stored in a less compact condition and that those stored at a high temperature lost more than those stored at a low temperature. These experiments were all made on small quantities of material.

It seemed possible that the carotene might be more stable when the feed was stored in larger amounts than those used in the work reported above. That such differences might occur was found in an experiment with a sack of alfalfa leaf meal kept for experimental purposes. This sack of meal weighing about 15 pounds was placed in a cold storage (4 to 6° C.), and a small portion of it was placed in a fruit jar and stored in a refrigerator (6°). Samples from both the sack and the jar were taken for analysis from time to time. A large difference in the carotene content of the two samples developed. The results are shown in Table 14, sample 43005. The sample in the fruit jar contained 140 parts carotene per million at the end of 4 months and 108 at the end of 5 months, which were losses of 32 and 47% respectively, while the large sample in the sack had not lost any carotene at the end of six months, and had lost only 27% at the end of 12 months. The difference may be due partly to the stirring of the small sample and partly to exposure to warm air when it was taken from the refrigerator for sampling.

Analyses of portions of large samples of alfalfa meal were made at regular intervals on samples drawn from lots of several hundred pounds used in work on the vitamin A requirements of animals. These samples were stored in unheated rooms; sample 44086 was stored in a large dark bin at the Spur Substation by the Department of Range Animal Husbandry; 43051, 44675, and 45419 were stored in large sacks at the Feeding and Breeding Station by the Division of Poultry Husbandry; 44504 was stored in large sacks at College Station by the Division of Veterinary Science; and 45318 was stored in sacks by the Division of Swine Husbandry. The loss of carotene in these samples is shown in Table 14. The loss of carotene took place slowly in the winter months and proceeded at a relatively rapid rate during the summer months except in sample 44504, in which the rate of destruction was as high in the winter months as it was in the summer months for other samples. The more rapid rate of destruction in this sample must have been caused by something other than the conditions of storage, for the temperature and method of storage were apparently the same for all the samples stored at College Station.

If the data in Table 14 are compared with the data in Table 8, it is seen that the carotene in the large samples was considerably more stable than it was in the smaller samples stored in pint and quart Mason jars. The reason for this is probably that in the large samples the alfalfa on the exterior protects that in the interior from the air. The total amount is, therefore, less exposed to the air, and consequently the oxidation of carotene takes place less rapidly.

Table 14. The effect of storage upon the amount of carotene in large samples of alfalfa

Lab. No.	Description	Month analyzed	Mean monthly temperature	Carotene parts per million	Carotene lost, total per cent	Carotene lost, rate per month per cent
43051	Dehydrated alfalfa leaf meal stored in large sacks at room temperature at College Station by the Division of Poultry Husbandry	Dec., 1935	50.6 F	75.0	0	0
		Jan., 1936	49.0	70.2	6	6
		Feb., 1936	45.8	65.8	12	6
		Mar., 1936	65.2	65.6	13	1
		April, 1936	65.2	64.3	14	1
		May, 1936	73.7	67.1	11	0
		June, 1936	83.0	66.7	11	0
		July, 1936	81.2	60.9	19	8
		Aug., 1936	83.6	44.7	40	21
		Oct., 1936	65.2	40.7	46	6
44675	Dehydrated alfalfa leaf meal stored in large sacks at room temperature at College Station by the Division of Poultry Husbandry	Oct., 1936	65.2	103.8	0	0
		Nov., 1936	54.8	92.5	11	11
		Dec., 1936	55.0	90.0	13	2
		Jan., 1937	50.2	91.7	12	0
		Feb., 1937	54.8	91.3	12	0
44086	Alfalfa hay stored in large dark bin at the Spur Substation by the Division of Range Animal Husbandry	July., 1936	82.2	40.1	0	0
		Aug., 1936	82.4	32.6	19	19
		Sept., 1936	73.2	32.7	19	0
		Oct., 1936	60.0	22.7	43	24
		Nov., 1936	49.6	23.2	42	0
		Dec., 1936	45.7	22.9	43	1
		Feb. 1937	43.5	21.9	45	1
		Mar., 1937	47.6	23.8	41	0
44504	Alfalfa hay stored at room temperature in large sacks at College Station by the Division of Veterinary Science	Sept., 1936	79.4 F	25.0	0	0
		Oct., 1936	65.2	20.9	16	16
		Nov., 1936	54.8	22.1	12	0
		Dec., 1936	55.0	18.5	26	14
		Jan., 1937	50.2	16.6	34	8
		Feb., 1937	54.8	13.3	47	13
		Mar., 1937	56.6	14.1	44	0
		April, 1937	68.2	10.3	59	15
43005	Dehydrated alfalfa leaf meal stored in a 15 lb. sack in a refrigerator at 4°-9° C	At start	.....	205.0	0	0
		5th	.....	210.0	0	0
		6th	.....	211.7	0	0
		7th	.....	195.0	5	5
		9th	.....	160.0	22	9
		10th	.....	150.0	27	5
		11th	.....	145.0	29	2
		12th	.....	150.0	27	0
43005	Dehydrated alfalfa leaf meal stored in quart fruit jar in a refrigerator at 4°-9° C	At start	.....	205.0	.....	.....
		1st	.....	208.0	0	0
		4th	.....	140.4	32	11
		5th	.....	108.3	47	15
45419	Dehydrated alfalfa leaf meal stored in large sacks at room temperature at College Station by the Division of Poultry Husbandry	Feb., 1937	54.8 F	180.0	0	0
		Mar., 1937	56.6	173.8	3	3
		April, 1937	68.2	155.0	14	11
		May, 1937	77.4	137.5	24	10
		June, 1937	83.7	133.3	26	2
45318	Alfalfa leaf meal stored at room temperature at College Station by the Division of Swine Husbandry	Jan., 1937	50.2 F	65.0	0	0
		Feb., 1937	54.8	61.7	5	5
		Mar., 1937	56.6	60.7	7	2
		April, 1937	68.2	56.3	13	6
		May, 1937	77.4	52.2	20	7
		June, 1937	83.7	56.6	13	0
		July, 1937	85.3	46.6	28	15

The results of these tests show that the losses of carotene are lower if the dried material containing carotene is stored in a compact mass than in a loose, mass exposed to the air. They also show that the loss

is greater when the temperature is high than when it is low. When exposed to a high temperature, a considerable portion of the carotene may be lost rapidly, after which the remainder is lost more slowly. During the winter months there may be comparatively slight losses of carotene from yellow corn or alfalfa meal properly stored. In the summer, the losses may be considerable, especially if the storage is in warehouses that are highly heated by exposure to the sun.

The Effect of Storage Upon Losses of Carotene Added in Oil to Feed Mixtures

Since technical advances have made possible the preparation of strong solutions of carotene dissolved in oil, at a price permitting their use in mixed feeds, some manufacturers of mixed feeds have begun to use these preparations in their chicken feeds for the purpose of supplying vitamin A potency. The question of the stability of such preparation when added to mixed feeds has thus become a matter of practical importance.

In order to study the stability of carotene added in oil to mixed feeds, a number of mixtures were tested. The desired quantity of carotene solution was mixed with 8 gm. of feed and then diluted to 100 gm. with white corn meal. The feeds with which the carotene was mixed were white corn meal alone, white corn meal with yeast, dried skim milk and wheat gray shorts. These mixtures were stored both in the refrigerator and at room temperatures and the amount of carotene determined at the end of different periods of time.

Two samples of carotene were used. Sample No. 1 was a commercial preparation of carotene in oil used by a manufacturer of commercial feeds. Sample No. 2 was a commercial crystallized carotene purified and dissolved in a purified cottonseed oil. A very small amount of chloroform was used in sample No. 2 to dissolve the carotene before it was diluted with the oil.

The results are tabulated in Table 15. The experiment continued for 16 weeks with one series and 28 weeks with the other. From the data it is apparent that the carotene in all the mixtures was more stable at 7° C. (45° F.) than at 28° C. (82° F.). The feeds stored at 7° C. (45° F.) lost from 2 to 3% of their carotene in four weeks, 3 to 6% in 8 weeks and 13% in 20 weeks. The feeds stored at 28° C. lost from 7 to 27% of their carotene in four weeks, from 12 to 53% in 8 weeks, 17 to 67% in 12 weeks, and from 24 to 70% in 16 weeks. The losses of carotene from the different samples were different, but the quantities lost in 8 weeks at 28° C. from all the samples was appreciable. The presence of skim milk powder, wheat gray shorts, or yeast which might be assumed to contain stabilizing agents, did not increase the stability of carotene to any practical extent. However, it is of interest to note that yeast decreased the rate of destruction of carotene to a small extent.

Table 15 also shows that the commercial preparation of carotene (No. 1) was more stable in the feed mixtures than our solution of carotene (No. 2). The lower stability of our preparation may have been due to the chloro-

Table 15. Stability of carotene dissolved in oil and added to mixed feeds

Laboratory number	Constituents of feed	Temperature of storage	Carotene at start, parts per million	Carotene destroyed during storage, in per cent							
				2 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	28 weeks
42652	Corn meal + carotene No. 1	7° C	4.6	2.2	2.2	3.3	4.5	6.5	13.4	19.3	27.0
42651	Corn meal + carotene No. 1	28°	4.2	4.6	9.3	16.3	23.3	30.2	37.0	45.2	52.4
42653	Corn meal + 8% yeast + carotene No. 1	28°	5.8	3.5	7.0	12.1	17.2	24.1	34.5	43.5	53.4
42690	Corn meal + 8% skim milk powder + carotene No. 1	28°	4.4	4.4	6.6	15.5	24.4	31.1	40.0	50.0	61.0
42708	Corn meal + 8% wheat gray shorts + carotene No. 1	28°	5.0	5.6	11.3	22.6	34.0	45.2	56.0	62.3	.....
	Average for 28° C (total)	.....	.....	4.5	8.6	16.6	24.7	32.7	41.9	50.3	55.6
	Per cent lost per 2 weeks (average)	.....	.....	4.5	4.1	4.0	4.0	4.0	4.6	4.2	2.8
42992	Corn meal + carotene No. 2	7°	2.9	1.4	3.4	6.0	13.8	27.6	.....	.....	.....
42993	Corn meal + carotene No. 2	28°	2.9	8.6	17.2	34.4	44.8	51.7	.....	.....	.....
42995	Corn meal + 8% yeast + carotene No. 2	28°	2.8	3.4	8.0	17.2	41.4	62.1	.....	.....	.....
42994	Corn meal + 8% skim milk powder + carotene No. 2	28°	3.0	13.3	27.0	53.3	66.7	70.0	.....	.....	.....
42996	Corn meal + 8% wheat gray shorts + carotene No. 2	28°	2.8	7.0	17.2	34.5	55.2	68.9	.....	.....	.....
	Average for 28° C	.....	.....	8.1	17.4	34.9	52.0	63.2	.....	.....	.....
	Rate per 2 weeks	.....	.....	8.1	9.3	8.8	8.6	5.6	.....	.....	.....

form used to help dissolve it in the oil. Tests were made in order to determine whether the lower stability of carotene No. 2 was due to the effect of the chloroform. A saturated solution of carotene in Wesson oil was prepared. The amount of carotene in this solution was then estimated colorimetrically and enough added to 100 grams of corn meal to make the mixture contain approximately 4 parts carotene per million. To a portion of the above carotene solution was added the same amount of chloroform as was added to our carotene preparation No. 2, namely, 0.05 cc. of U. S. P. chloroform for each microgram of carotene. This solution was then added to corn meal in the same proportion as the other. The two feed mixtures were stored at room and at refrigerator temperatures and the carotene determined at monthly intervals. The results are listed in Table 16. In both tests the small amount of chloroform increased the rate of the destruction of the carotene, especially during the first part of the storage at room temperature. Consequently, there is very little doubt that the chloroform in carotene No. 2 was the cause of its being less stable than carotene No. 1. This is in accordance with some previous work in which it was found that a vitamin A preparation, ordinarily very stable in methanol, was rapidly destroyed when dissolved in chloroform (6).

While the losses of the individual mixtures varied, the average rate of loss of carotene at 28° C. for period of 2 weeks as shown in Table 15 was remarkably uniform, being from 4.0 to 4.6% of the carotene at the beginning over a period of 24 weeks, for mixtures with carotene No. 1, and from 8.1 to 9.3% per period for 12 weeks with carotene No. 2. In both cases, the loss is less during the last period. Alfalfa leaf meal stored at 35° C. (Table 12) lost carotene rapidly the first week, and then losses decreased and later were at the rate of 4% a month. The carotene in oil at a lower temperature of 28° was lost at a uniform rate of about 8% a month. This would indicate that some of the carotene in the alfalfa is protected by the plant tissue.

Appreciable amounts of carotene (from 17 to 67%) may be lost from carotene dissolved in oil added to feeds stored at ordinary temperature during a period of 3 months, and larger quantities are lost when stored for longer periods of time. If carotene dissolved in oil is added to commercial mixed feeds to supply vitamin A potency, there should be a liberal allowance for losses during storage.

SUMMARY

1. When fish oils or their concentrates were added to feed mixtures to increase their vitamin A potency, from 79 to 100% of the vitamin A disappeared after 4 weeks at either 7° C. or 28° C. The use of hydroquinone delayed the loss in the first week or two but the loss at the end of 5 weeks was practically the same as if it had not been used.

2. Feeds stored in large amounts lost their vitamin A from fish oils as rapidly as feeds stored in small amounts.

Table 16. Effect of chloroform upon the stability of carotene in feeds

Description of sample	Temperature of storage	Carotene at start, parts per million	Carotene lost, in per cent							
			1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
First test.										
Corn meal and carotene	28° C	4.4	15.9	22.7	31.8	43.2	52.3	61.3	57.0	61.3
Corn meal and carotene	7°	4.6	8.7	6.5	4.4	15.2	17.4	17.4	19.6	22.3
Corn meal and carotene and chloroform	28°	4.4	36.4	38.6	38.6	70.5	65.8	70.5	68.2	68.2
Corn meal and carotene and chloroform	7°	4.6	10.9	8.7	13.0	19.5	.....	26.0	.....	.....
Second test.										
Corn meal and carotene	28°	3.0	0.0	20.0	26.6	40.0	.....	.....	.....	.....
Corn meal and carotene	7°	3.2	0.0	6.3	9.4	21.8	.....	.....	.....	.....
Corn meal, carotene and chloroform	28°	3.3	18.2	48.5	45.5	48.5	.....	.....	.....	.....
Corn meal, carotene and chloroform	7°	3.1	0.0	0.0	12.9	22.6	.....	.....	.....	.....

3. The method used for the determination of carotene in feeds is given.

4. Carotene in alfalfa meal was found to be more stable when the meal was stored at 6° C. than it was when stored at room temperatures. The destruction at room temperature for samples stored in pint or quart jars varied from 6 to 70% in 8 weeks and at refrigerator temperatures from 0 to 26%.

5. The destruction of carotene in samples packed in homeopathic vials at refrigerator temperature was very low, usually from 0 to 3% per month.

6. Alfalfa kept at a temperature of 35° C. lost carotene rapidly at first, and then quite slowly, indicating that some of the carotene may be easily destroyed, while a portion may be so protected that it is much less easily destroyed.

7. A sample of alfalfa leaf meal diluted 1 to 9 with corn starch lost carotene at a more rapid rate than it did when it was not diluted.

8. Large samples of alfalfa meal stored at ordinary temperatures lost carotene slowly during the winter months and comparatively rapidly during some of the summer months. The losses during the summer months were less than for samples stored in pint and quart jars at laboratory temperatures. Losses of as much as 40% were found after storage for three months in the summer.

9. When feed mixtures containing carotene in oil were stored at 7° C., there was a loss of 2 to 3% carotene in 4 weeks of storage, 3 to 6% in 8 weeks, and 5 to 14% in 12 weeks. At 28° C. there was a loss of 7 to 27% carotene in 4 weeks of storage, 12 to 53% in 8 weeks, 17 to 67% in 12 weeks, and 24 to 70% in 16 weeks. If carotene in oil is added to mixed feeds to supply vitamin A potency, there should be liberal allowance for losses in storage.

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